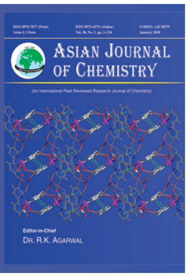
Asian Journal of Chemistry; Vol. 30, No. 8 (2018), 1751-1754

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2018.21285



Isolation of Steroid Compounds from Suruhan (*Peperomia pellucida* L. Kunth) and Their Antimalarial Activity

NURHAYATI BIALANGI[1], ADAM MUSTAPA[2], YUSDA SALIMI[1], ARI WIDIANTORO[3] and BOIMA SITUMEANG[4,*]

[1]Department of Chemistry, Faculty of Mathematic and Natural Sains, State University of Gorontalo, Gorontalo 96128, Indonesia
[2]Department of Pharmacy, Faculty of Sport and Healthy, State University of Gorontalo, Gorontalo 96128, Indonesia
[3]Department of Chemistry, Faculty of Mathematics and Natural Sains, Tanjungpura University, Kota Pontianak, Kalimantan Barat 78115, Indonesia
[4]Department of Chemistry, Sekolah Tinggi Analis Kimia Cilegon, Banten 42411, Indonesia

*Corresponding author: E-mail: boimatumeang@gmail.com

Received: 14 February 2018; *Accepted*: 27 March 2018; *Published online*: 30 June 2018; AJC-18968

Suruhan (*Peperomia pellucida*) is easily found in Indonesia and has the potential of being a herb medicine. General phytochemical screening revealed the presence of flavonoid, steroid, triterpenoid, alkaloid and tannin compounds. Two steroid compounds were isolated from ethyl acetate fraction of the *Peperomia pellucida.* The compounds were isolated with chromatography method and antimalarial activity test with Desjardin method. Based on the spectral evidence IR, ^{1}H NMR, ^{13}C NMR, 2D NMR and MS spectroscopies, structures were determined to be stigmasterol (**1**) and fucosterol (**2**). The results of antimalarial activity test showed IC_{50} value of stigmasterol is 5.24 ppm and fucosterol is 0.85 ppm. This is the first report so far of isolation of steroid compounds from *Peperomia pellucida* plant from Gorontalo, Indonesia and their antimalarial activity test.

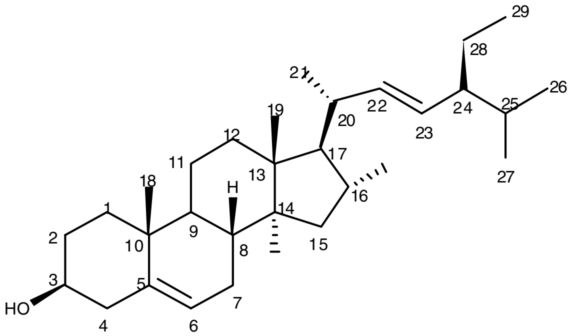
Keywords: Suruhan, Steroid, Antimalarial, *Peperomia pellucida*.

INTRODUCTION

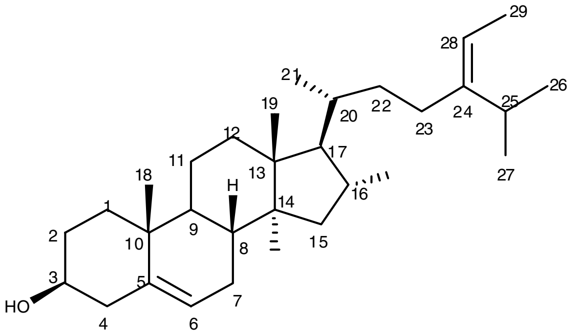
Peperomia pellucida is known as Suruhan, easy found from northeast to southeast, Indonesia. *Peperomia pellucida* is a small tree belonging to the family *Peperomia* [1]. The genus *Peperomia* is the second largest genus in the *Piperaceae* family and comprises more than 600 species widely distributed in Indonesia. The plant is widely distributed in Gorontalo, Sumatera and Java Islands and also many tropical Asian and South American countries [1,2]. Traditionally the suruhan plant (*Peperomia pellucida*) are used as an acne pimples, skin diseases, headaches, pain relief in rheumatism and gout rheumatism drugs [3]. The results of phytochemical screening revealed the presence of flavonoids, alkaloids, steroids, saponins and tannins [4]. The results of the proximate analysis showed ash content, high crude fiber content, while carbohydrate content was observed to be the highest. Suruhan plants are usually grown in the wet rock crevices [4,5].

Infectious diseases and parasites are one of the major diseases in the world. According to World Health Organization (WHO) data in 2011, infectious diseases and parasites are the third leading cause of death in the world [6]. In Indonesia, malarial diseases is one of the major cause of death especially in north Indonesia. Malarial diseases cause by infection of *Plasmodium falciparum*. Natural products have been a source of medicinal agents and traditional medicine system that have been used for thousand of years in countries. The major classes of plant photochemicals include steroid, phenolic, terpenoid and essential oils, alkaloids and polypeptides. Among these, steroid compounds one of the most numerous and structurally diverse plant phitochemicals that exist in nature. World Health Organization also has been estimated that approximately 80 % of the world's inhabitans rely mainly on traditional medicines for their primary health care [7].

As the plant is being used extensively in Indonesia as an herbal medicine, it is necessary to have knowledge of the constituents of the plant of our native species. Previous phytochemical investigation reported in the isolation of pyran compound and antipyretic, gastroprotector, antimalarial and hypotensive activity from extract *P. pellucida* [8-11]. In this research, the ethyl acetate fraction subjected to chromatographic separation to afford two steroid, including stigmasterol (1) and fucosterol (2) (Fig. 1). Both compound were isolated for the first time from this species and tested as antimalarial.



Stigmasterol (**1**)



Fucosterol (**2**)

Fig. 1. Structure of sigmasterol (**1**) and fucosterol (**2**)

EXPERIMENTAL

The experimental specimen is suruhan (*Peperomia pellucida)* were collected from north Gorontalo, Gorontalo province, Indonesia in June 2016. It was identified in Herbarium biology laboratorium, Faculty of mathematics and natural sciences Gorontalo State University. The chemicals used in this research were ethyl acetate, *n*-hexane, methanol, distilled water, silica gel G60 (70-320 mesh), thin layer chromatography (TLC) silica plate, octadecylsilane (ODS) RP-18 TLC, 10 % H_2SO_4 in ethanol, ethanol 70 %, whole blood and erythrocyte.

Spectrum measurements were performed using a various of spectroscopies tools. Infrared spectra were measured with Shimadzu FTIR, ^{1}H and ^{13}C NMR spectra were measured using JEOL JNMA-500 which works at 500 MHz (for ^{1}H NMR spectrum) and at 125 MHz (for ^{13}C NMR spectrum), 1-D and 2-D NMR with TMS as an internal standard and ES-MS spectrometry (UPLC MS/MS TQD type Waters), Laminar air flow, Memmert incubator, Hirayama HVE-50 autoclave, microscope, micro pipets and evaporator.

Extraction and isolation: Dried *P. pellucida* plant (204 g) were soaked in 2 L methanol 96 % for 2 days. The shole mixture was then filtered through filter paper and the filtrate was then evaporated under reduce pressure at 45 °C using a Buchi Rorary Evaporator to provide 90 g of a gummy concentrate of the crude extract. A portion of the methanol extract (70 g) was dissolve in 96 % methanol. It was partitioned with *n*-hexane and then with ethyl acetate. All the extracts were filtered through a cotton plug followed by Whatman filter paper and then concentrated by using a rotary vacuum evaporator to provide *n*-hexana (5 g), then with ethyl acetate (3 g) extractives.

Chromatographic separation: The column was packed with fine TLC grade siliga gel G60 was used as the packing material. A column having 50 cm height and 5 cm in diameter was packed with the silica gel G60 under reduced pressure. The coloumn was washed with methanol and then with *n*-hexane to facilitate compact packing. The ethyl acetate fraction was subjected to column chromatography. The column was then eluted using *n*-hexane (150 mL) followed by mixture of *n*-hexane-ethyl acetate (10:0-0:10). A total of 11 fractions (A-K) were collected each in 30 mL beakers.

The fraction E and F (0.27 g) was subjected to column chromatography over silica gel (Kieselgel G60, mesh 70-230) using a mixture of *n*-hexane:ethyl acetate (9:1) as an eluting solvent, affording 40 fractions (EF01–EF40). Fraction EF9 was found to yield crystal on the wall of the beakers. The crystals were washed with *n*-hexane carefully. As a result mother solution was obtained leaving back the needle shape crystals, which were isolate as compound **1**.

The column fractions EF13-15 of crude ethyl acetate fraction were bulked together as they showed similar TLC feature with different R_f value. The mixed fraction was found to yield crystal on the wall of the beaker. The crystals were washed with *n*-hexane carefully. As the result mother solution was obtained leaving back the colourless sharp crystal. These sharp crystal provide the compound **2**. The purification results of compound were determined by TLC on silica gel and ODS with several solvent systems and showed a single spot (> 95 % pure).

Test for steroid with Liebermann-Burchard reaction: A few crystals of compounds **1** and **2** were dissolve in chloroform and a few drops of concentrated sulfuric acid were added to it followed by the addition of 2-3 drops of acetic anhydride. In this case both compounds **1** and **2** turned to violet blue and finally formed green colour, which indicates the presence of steroids [12].

Antimalarial activity test: The antimalarial activity of the compound was tested using the Desjardins method. The test was performed using *P. falciparum* 3D7 strain. The *P. falciparum* culture was blended in RPHS media containg ± 1 % paracitemia and placed in 1 mL 24 wells. The RPHS media was replaced by sample contained RPHS media with different sample concentration. Culture was then incubated for 48 h and followed by paracite harvesting. The paracite was then swapped with Giemsa coloured blood. The paracitemia percentage was calculated by comparing the number of infencted erythrocites to 500 erithrocites [13,14]. The formula is as follows:

$$\text{Paracitemia (\%)} = \frac{\sum \text{infected erytrosite}}{500 \text{ erytrosite}} \times 100$$

The growth and growth inhibition percentage were calculated using the following formula:

$$\text{Growth (\%)} = \frac{\text{Tested paracitemia}}{\text{Control paracitemia}} \times 100$$

$$\text{Growth inhibition (\%)} = \frac{A - B}{A} \times 100$$

where, A = control paracitemia, B = tested paracitemia.

The growth inhibition percentage for each concentration were used in SPSS program to calculate the IC_{50} in ppm unit.

Characterization compounds 1 and 2: Different spectroscopic methods were used to elucidate the structure of isolated compounds **1** and **2**. Among the spectroscopic techniques IR, ^{1}H and ^{13}C NMR, HMQC, HMBC and H-H COSY were carried out. The infrared spectrum was recorded on Shimadzu affinity-1, ^{1}H and ^{13}C NMR spectra were recorded using $CDCl_3$ as solvent on JEOUL NMR 500 MHz spectrometer, Lembaga Ilmu Pengetahuan (LIPI), Indonesia.

Compound 1: White crystalline needle. IR (KBr, ν_{max}, cm^{-1}): 3373.6, 1641.7, 1457.3, 1381.6, 1247, 1038.7, 881.6. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.90 (1H, d, *J* 5.1 Hz, H6), 5.17 (1H, dd, *J* 15.0, 8.4 Hz, H22), 5.30 (1H, dd, *J* 15.0, 8.4 Hz, H23), 3.17 (1H, dd, *J* 9.6, 4.8 Hz, H3), 1.00, 0.68 (3H, s, H19 and H18), 0.92 (3H, d, *J* 6.0 Hz, H21), 0.98 (3H, d, *J* 8.0 Hz, H29), 1.77 (1H, m, H25), 0.84 (3H, d, *J* 7.2 Hz, H26) and 1.17 (3H, d, *J* 7.2 Hz, H27). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 37.4 (CH_2, C1), 32.1 (CH_2, C2), 71.9 (CH, C3), 42.1 (CH_2, C4), 140.5 (CH, C5), 121.9 (CH, C6), 31.8 (CH_2, C7), 41.8 (CH, C8), 50.2 (CH, C9), 36.6 (Cq, C-10), 21.2 (CH_2, C11), 39.8 (CH_2, C12), 42.4 (Cq, C13), 56.9 (CH, C14), 24.4 (CH_2, C15), 29.4 (CH_2, C16), 56.0 (CH, C17), 18.4 (CH_3, C18), 12.4 (CH_3, C19), 19.5 (CH, C20), 20.5 (CH_3, C21), 138.7 (CH, C22), 129.4 (CH, C23), 52.9 (CH, C24), 49.1 (CH, C25), 20.4 (CH_3, C26), 19.3 (CH_3, C27), 25.5 (CH_2, C28), 12.9 (CH_3, C29).

Compound 2: White crystal. IR (KBr, ν_{max}, cm^{-1}): 3430.2, 2937.4, 1625.8, 1450.6, 1366.7, 923.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.30 (1H, br. d, *J* 5.2 Hz, H-6), 5.18 (1H, q, *J* 6.7 Hz, H-28), 3.43 (1H, m, H-3), 1.59 (3H, br s, H-21), 1.01 (3H, s, H-19), 1.03 (3H, br s, H-21), 1.02 (3H, d, *J* 1.2 Hz, H-27), 0.96 (3H, d, *J* 1.2 Hz, H-26), 0.68 (3H, s, H-18). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 36.2 (CH_2, C1), 30.5 (C-2), 71.5 (CH, C3), 42.7 (CH_2, C4), 140.2 (CH, C5), 122.3 (CH, C6), 30.9 (CH_2, C7), 31.6 (CH, C8), 50.3 (CH, C9), 36.2 (Cq, C10), 21.0 (CH_2, C11), 39.3 (CH_2, C12), 42.1 (Cq, C13), 56.5 (CH, C14), 24.5 (CH_2, C15), 28.7 (CH_2, C16), 55.4 (CH, C17), 11.8 (CH, C18), 19.4 (CH_3-C19), 36.4 (CH, C20), 18.7 (CH_3, C21), 35.2 (CH_2, C22), 25.6 (CH_2, C23), 146.9 (CH, C24), 34.7 (CH, C25), 22.2 (CH_3, C26), 22.1 (CH_3, C27), 115.5 (CH, C28), 31.1 (CH_3, C29).

RESULTS AND DISCUSSION

Characterization compounds: Pure compound was isolated as a white powder. The mass spectral data of the compound **1** gave a molecular formula $C_{29}H_{48}O$ and compound **2** $C_{29}H_{48}O$, which was supported by the ^{13}C NMR, ^{1}H NMR and DEPT 135 spectral data.

The compound **1** is a white needle shapes crystal, which gave positive Lieberman-Burchard test for steroid. The IR spectrum (KBr) of isolated showed characteristic absorption frequencies at 3373.6 cm^{-1} (O-H stretching); and 1247 cm^{-1} indicated C-O bond vibrations. The C-C vibrations was at 1641 cm^{-1}; other absorption peaks includes 1457.3 cm^{-1} (CH_2); vibration at 1381.6 cm^{-1} (O-H), 1038 cm^{-1} (cycloalkane) and 881.6 cm^{-1} [15-17]. The H NMR spectra of compound showed the presence of two methyl singlets at δ 0.71 and 0.98 ppm (H-18, H-19); three methyl doublets that appeared at δ 1.10, 0.84 and 1.17 ppm; and a methyl triplet at δ 1.08 ppm. Isolated compound also showed protons at δ 4.91, 5.17 and 5.30 suggesting the presence of three protons corresponding to that of a trisubstituted and a disubstituted olefinic [16-18]. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublets at δ 3.17 ppm. The ^{13}C NMR spectrum showed which six methyl groups at δ: 12.4 (C-29), 18.4 (C-28), 19.3 (C-27), 20.4 (C-26), 12.9 (C-24) and 40.5 (C-18); the signals due to an olefenic group at δ: 140.5 (C-5), 121.9 (C-6), 138.7 (C-20) and 129.4 (C-21) indicated of stigmasterol compound [19,20]. The deshielded signal at δ 71.9 ppm was due to C3 with a hydroxyl group attached to it. The confirmation of the structure of isolated was accomplished through the 2D NMR experiments (COSY and HMBC). The above spectral data supported the presence of sterol skeleton having a hydroxyl group at C3 position with two double bonds at C5/C6 and C22/C23 with six methyl groups which was supported by the key COSY and HMBC. Thus, the structure of isolated compound was assigned as stigmasterol. The physical and spectral data are consistent to the reported literature values of stigmasterol (Fig. 1) [20-23].

The compound **2** is a white crystal which gave positive Lieberman-Burchard test for steroid. The IR spectrum (KBr) of isolated showed characteristic absorption frequencies at 3430.2 cm^{-1} (O-H stretching). Absorption at 2937.4 cm^{-1} is due to aliphatic C-H stretching. Other frequencies include 1625.8 cm^{-1} as a result C=C stretching however this band was weak at 1450.6 cm^{-1} was a bending frequency for cyclic (CH_2) and 1366.7 cm^{-1} for C-H bending. The vibration at 923.5 cm^{-1} due to C-O stretching [15-18]. Compound **2** was identified as fucosterol by comparison the ^{1}H NMR and ^{13}C NMR spectra with those of compound **1**. The NMR and MS data were very similar to those of compound **1** except the position of olefenic proton and quaternary carbon while one methylene signal were present for H28 and quaternary carbon at C24. The olefenic proton for H22 and H23 in compound **2** were absent. The ^{13}C NMR spectrum showed 29 carbons including an oxymethine carbon signal at δ 71.5 and two olefenic carbon at δ 122.3 and δ 115.5. the double bonded unsaturation at δ 146.9 and δ 115.5 was characteristics of fucosterol [15,16] and two methylene carbon signals were present at δ 35.2 and 25.6 for C22 and C23 confirmed by DEPT 135. If we compared with DEPT 135 for compound **2** then we confirmed that this compound was having six methyl (CH_3) groups, ten methylene (CH_2) groups, nine methane (CH) groups and four quaternary carbons (Cq) groups. These assignment are in good agreement for the structure of fucosterol [15,24,25].

Antimalarial activity: Paracitemia and growth inhibition percentage shown by the sample compounds **1** and **2** were calculated (Table-1). The positive control in this test was quinoline and artemisin 10^{-3} M was used as comparison. All data of paracitemia, growth and growth inhibition percentage was used to calculate the IC_{50} and its liniar regression curve. The regression equation was used to calculate the IC_{50}. The IC_{50} of the compound **1** is 5.24 ppm and compound **2** 0.85 ppm. Compound **2** has good antimalarial activity than compound **1**. This is the first time reported steroid from *P. pellucida* and antimalarial activity test against *Plasmodium falcivarum.*

TABLE-1
P. falciparum 3D7 STRAIN AVERAGE PARACITEMIA, GROWTH AND GROWTH INHIBITION PERCENTAGE BY COMPOUNDS **1** and **2**

Concentration (μg/mL)	Compound **1** (Stigmasterol)			Compound **2** (Fucosterol)		
	Paracitemia (%)	Growth (%)	Inhibition (%)	Paracitemia (%)	Growth (%)	Inhibition (%)
Control (quinolin)	2.3	0	0	5.8	100.00	0
Artemicyn 10^{-3} M	0.3	13.04	86.96	0.4	6.89	93.11
1×10^{-9}	2.3	100.00	0	3.2	55.86	44.14
1×10^{-8}	2.2	95.65	4.35	2.1	36.21	63.79
1×10^{-7}	1.7	73.91	26.09	1.9	32.76	67.24
1×10^{-6}	1.3	56.52	43.48	1.6	27.59	72.41
1×10^{-5}	1.1	47.82	52.18	1.4	24.14	75.86
1×10^{-4}	1.0	43.47	56.53	0.8	13.79	86.21
1×10^{-3}	0.7	30.43	69.57	0.6	10.34	89.66
1×10^{-2}	0.5	21.73	78.27	0.1	1.72	98.28

Conclusion

In this research we successfully isolated two steroid compounds from ethyl acetate fraction of *Peperomia pellucida.* Compund **1** were stigmasterol and compound **2** were fucosterol. The IC_{50} of the compound **1** is 5.24 ppm and compound **2** 0.85 ppm. This is the first report of isolation steroid compounds of *Peperomia pellucida* from North Gorontalo district, Indonesia.

ACKNOWLEDGEMENTS

The authors thank The Ministry of Research and Higher Education of Indonesia Republic for funding this collaboration (RISTEKDIKTI) and Mrs. Fajriah, M.Sci. as well as Dr. Achmad, for their help in conducting the NMR measurements.

REFERENCES

1. M.S. Yusuf, I. Wulandari, L. Amelia and A. Katrin, *J. Pharmacogn.*, **9**, 239 (2017); https://doi.org/10.5530/pj.2017.2.40.
2. M. Habsah, A. Yosie, K. Baker, C.C. Siang, D.F. Syamsumir, A. Alias and S.A.M. Radzi, *J. Chem. Pharm. Res.*, **7**, 578 (2015).
3. G.M.D.P. Cera, N.R.B. Ramos and N.I.T. Siazon, *J. Pharmacol.*, 143 (2011).
4. P. Majumder, P. Abraham and V. Satya, *Res. J. Pharm. Biol. Chem. Sci.*, **2**, 358 (2011).
5. A.K. Rahman and S. Islam, *Turk. J. Biol.*, **32**, 37 (2008).
6. G.S. Singh and S. Pandeya, *Nat. Prod. Med. Chem.*, **63**, 101 (2011).
7. N.R. Farnsworth, O. Akerele and A. Bingel, *Bull. World Health Organ.*, **63**, 965 (1985).
8. C.R. Nwokocha, D.U. Owu, K. Kinlocke, J. Murray, R. Delgoda, K. Thaxter, G. McCalla and L. Young, *Med. Aromat. Plants*, **1**, 105 (2012); https://doi.org/10.4172/2167-0412.1000105.
9. R. Rojas-Martínez, J. Arrieta, L. Cruz-Antonio, D. Arrieta-Baez, A.M. Velázquez-Méndez and M.E. Sánchez-Mendoza, *Molecules*, **18**, 11327 (2013); https://doi.org/10.3390/molecules180911327.
10. Y. Susilawati, R. Nugraha, A. Muhtadi, S. Soetardjo and U. Supratman, *Molbank*, **2015**, M855 (2015); https://doi.org/10.3390/M855.
11. N. Bialangi, M. Mustapa, Y. Salimi, A. Widiantoro and B. Situmeang, *J. Pendidikan Kimia*, **8**, 33 (2016).
12. J.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, Chapman and Hall, London, edn 3, pp. 129-138 (1998).
13. M.S. Zaridah, M.A. Azah and A. Rohani, *J. Trop. For. Sci.*, **18**, 74 (2006).
14. H. Overgaard, P. Sirisopa, B. Mikolo, K. Malterud, H. Wangensteen, Y.-F. Zou, B. Paulsen, D. Massamba, S. Duchon, V. Corbel and F. Chandre, *Molecules*, **19**, 21276 (2014); https://doi.org/10.3390/molecules191221276.
15. S.H. Hwang, J.M. Jang and S.S. Lim, *Fish. Aquat. Sci.*, **15**, 191 (2012); https://doi.org/10.5657/FAS.2012.0191.
16. Y. Ahmed, S. Rahman, P. Akhtar, F. Islam, M. Rahman and Z. Yakoob, *Int. Food Res. J.*, **20**, 2939 (2013).
17. R. Nasution, T. Barus, P. Nasution and N. Saidi, *Int. J. Pharm. Tech. Res.*, **6**, 1279 (2014).
18. L.L. Pierre and M.N.J Moses, *J. Innov. Pharm. Biol. Sci.*, **2**, 88 (2015).
19. U. Kolak, G. Topcu, S. Birteqsiz, G. Otuk and A. Ulubele, *Turk. J. Chem.*, **209**, 177 (2005).
20. A. Kamboj and A.K. Saluja, *Int. J. Pharm. Sci.*, **3**, 94 (2014).
21. P.S. Jain and S.B. Bari, *Asian J. Plant Sci.*, **9**, 163 (2010); https://doi.org/10.3923/ajps.2010.163.167.
22. V.S.P. Chaturvedula and I. Prakash, *Int. Curr. Pharm. J.*, **1**, 239 (2012); https://doi.org/10.3329/icpj.v1i9.11613.
23. G. Prasanna and M. Chitra, *Int. J. Pharmacog. Phytochem. Res.*, **7**, 6 (2015); https://doi.org/10.5958/0975-4385.2015.00002.3.
24. P.C. Trivedi and N. Choudrey, *J. Pharm. Res.*, **4**, 4252 (2001).
25. I.I.-W. Kim, C.-K. Lee, H.S. Kim and S.-H. Jung, *Arch. Pharm. Res.*, **26**, 9 (2003); https://doi.org/10.1007/BF03179923.