Isolation and Toxicity

by Alkausar Saragih

Submission date: 22-May-2019 03:41PM (UTC+1200)
Submission ID: 1113463482
File name: Isolation_and_Toxicity.pdf (587.12K)
Word count: 3479
Character count: 16779

Asian Journal of Chemistry; Vol. 28, No. 1 (2016), 203-206

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19345

Isolation and Toxicity of Steroidal Alkaloid Glycoside from Fruits of Ranti Hitam (*Solanum blumei* Nees ex Blume)

MURNIATY SIMORANGKIR[1,2,*], TONEL BARUS[2], RIBU SURBAKTI [2] and PARTOMUAN SIMANJUNTAK[3,4]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Jalan Willem Iskandar, Medan, Indonesia
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jalan Padang Bulan, Medan, Indonesia
[3]Research Centre of Biotechnology, Indonesian Institute of Sciences (LIPI), Cibinong, Indonesia
[4]Faculty of Pharmacy, Universitas Pancasila, Srengseng Sawah Jagakarsa, Jakarta, Indonesia

*Corresponding author: Tel: +62 85297841760; E-mail: murni_simor2011@yahoo.co.id

Received: 15 May 2015; *Accepted*: 29 June 2015; *Published online*: 5 October 2015; AJC-17575

One steroidal alkaloid glycoside compound as β2-solanin or solanid-5-ene-(1'→3)-β-D-galactopyranosyl-(1"→3)-β-D-glucopyranoside has been isolated from the fruits of an Indonesian medicinal plant, *Solanum blumei* Nees ex Blume (Solanaceae). The structure of the compound was identified by using spectral data analysis *viz.*, ultraviolet, infrared, 1D NMR (^{1}H-, ^{13}C- and DEPT), 2D NMR (COSY, HMQC and HMBC) and mass spectra. The compound has toxicity with LC_{50} value 17.97 µg/mL by Brine Shrimp Lethality Test (BSLT) method. The compound considered as highly toxic because it has LC_{50} < 30 µg/mL.

Keywords: β2-Solanine, Glycoalkaloid, *Solanum blumei* Nees ex Blume, Indonesian medicinal plant.

INTRODUCTION

Ranti hitam (*Solanum blumei* Nees ex Blume) (Solanaceae) is found in Dairi and Karo, North Sumatera, Indonesia. Traditionally fruits of *S. blumei* have been used as drugs, such as pain medication, fever, abdominal pain, ear pain and anti-inflammatory. To best of our knowledge, the chemical and pharmacological studies on *S. blumei* reports still limited. Simorangkir *et al.* [1] have reported that the phytochemical extracts of leaves and fruits of *S. blumei* conducted in accordance with the method of Harborne [2] show that the ethyl acetate extract of this plant contains alkaloids, steroids and flavonoids while the ethanol extract has many alkaloids, saponins and flavonoids bit, phenols and tannins. The *n*-hexane extract consists of steroids, triterpenoids and little amount of alkaloids. The highest extraction result is obtained in the extracted ethanol of leave and fruit compared with extracts of *n*-hexane and ethylacetate.

The results of toxicity test by Brine Shrimp Lethality Test method (BSLT) [3] for fruit extracts of *S. blumei* showed that the ethanol extract of *S. blumei* fruit had the highest toxicity than ethyl acetate and *n*-hexane extracts with LC_{50} values in a row *i.e.* 21.10; 321.14 and 573.61 µg/mL [4]. According to Atanu *et al.* [5], toxicological properties of the Solanum plant are likely caused by alkaloid content contained in Solanum plants. Based on the results of some tests that have been made to extract *S. blumei*, these plant is potential to become a medicinal plant.

The genus *Solanum* (Solanaceae family) is a very large group of about 1400 species spreaded throughout the temperate and tropical regions of the world. They are rich in steroidal glycosides in the form of glycoalkaloids [6]. *Solanum nigrum* L, one of *Solanum* species is a popular in part due to its toxic content of solanine, a glycoalkaloid found in most parts of the plant, with the highest concentrations in the unripened berries [7]. Although it is considered as rich source of one of the most popular plant poisons, it has also proved to be a reservoir of phytochemicals with pharmacological prospects [8]. Another Solanum is *Solanum chacoense* that has already been studied chemically and found to contain mixture of steroidal alkaloid glycosides as solanidine, leptinidine and acetyl leptidine [9]. Chemotaxonomic analysis for *S. nigrum* plant complex has been carried out by comparison of glyco-alkaloid solasonine, α-solamargine, β-solamargin, α-solanine and their aglycon solasodine and solanidine contained in five taxa of *Solanum nigrum* complex namely *S. americanum* Miil, *S. chenopodioides*, *S. nigrum* L., *S. retroflexum* and *S. villosum* [10].

S. blumei or known as Ranti hitam in Indonesia is not included in the *S. nigrum* taxa complex and there are no reports of research on the content of alkaloids in the plant. In this paper, it is reported the first investigation of the alkaloid of the *Solanum blumei* Nees ex Blume, as an Indonesian medicinal

plant. The compound is toxic with LC_{50} 17.97 µg/mL by Brine Shrimp Lethality Test method. The inhibitory activity against leukemia line cells L_{1210} of reviews these steroidal alkaloid glycoside isolated from *S. blumei* fruits is now in progress.

EXPERIMENTAL

The fruits of *S. blumei* were collected from Kuta Nangka village, sub-district Tanah Pinem, Dairi, North Sumatera Province, Indonesia (Fig. 1). Identification of plant material was carried out at the Herbarium Bogoriensis, Indonesian Institute of Sciences (LIPI) Cibinong, Indonesia, where the herbarium voucher has been kept.

Fig. 1. Plant of *Solanum blumei* Ness ex Blume and section fruits, leaf, and branches flowers

Preparation of plant extracts: The fruits of *S. blumeii* (6.1 kg) were air dried at room temperature and coarsely powdered (520.01 g) [1]. The powder (500.10 g) was macerated with *n*-hexane solvent, followed by ethyl acetate and finished with ethanol solvent. The results of the maceration process were *n*-hexane (10.92 g), ethyl acetate (25.23 g) and ethanol extracts (55,08 g) [1].

Toxicity test: The toxicity of the fractions from the column chromatography analysis were measured by using BSLT method [3]. The concentrations of the sample were 10, 100 and 1000 µg/mL and toxicity measurements were performed in three repetitions. Using this method, the toxicity of a fraction with LC_{50} value is the concentration of the fraction that gives as much as 50 % mortality rate. The toxicity test fractions are presented in Table-1.

Phytochemical test for alkaloids, steroids and sugar: Phytochemical test for alkaloids, steroids and sugar were done using Dragendorff, Liebermann-Burchard and Molisch reagents respectively [2].

Isolation and purification: Isolation and purification of compound **1** was done according to toxicity fraction guide.

Briefly, ethanol extracts (25.02 g) were fractionated by column chromatography technique using silica gel (70-230 mesh, Merck, 200.0 g) as stationary phase and ethylacetate as mobile phase, followed with $CHCl_3$-MeOH (1:1), $CHCl_3$-MeOH-H_2O (5:5:1) and acetic acid-EtOH (1:30) until we got fraction 2-7-3-3 (compound **1**) which showed the highest toxicity with LC_{50} = 17.97 µg/mL (Table-1) as pure alkaloid compound in amorphous white powder form (313 mg).

Chemical structure determination: The chemical structure of the compound **1** was identified by spectral data analysis of ultraviolet (UV), infrared (IR), 1D NMR (^{1}H-, ^{13}C- and DEPT), 2D NMR (COSY, HMQC and HMBC), mass spectra and comparison with literature values.

Spectral data of ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded on JEOL 500 spectrometer (500 MHz for ^{1}H NMR; 125 MHz for ^{13}C NMR) with TMS as internal standard. UV spectra was measured by using a Varian Cary 100 Conc (Shimadzu); IR spectra was recorded as KBr pellets on a Jasco model IR 700 spectrometer. EI-MS mass spectra was taken in the positive mode on HPLC Alliance 2695, Waters Detector Photodiode Array 2996. Thin layer chromatography (TLC) was performed on silica gel GF_{254} percoated plates (0.25 mm, thick, Merck); Column chromatography analysis using silica gel 60 G (70-230 mesh, Merck).

RESULTS AND DISCUSSION

Results of the ethanol extract of *S. blumei* fractionation using silica gel column chromatography (70-230 mesh, Merck) and the results of toxicity BSLT tests of each fraction are presented in Table-1.

TABLE-1
TOXICITY BSLT OF SAMPLE FRACTIONS
COLUMN CHROMATOGRAPHY

No.	Sample	LC_{50} (µg/mL)	No.	Sample	LC_{50} (µg/mL)
1	Fr. 1	37.92	16	Fr. 2-9	29.00
2	**Fr. 2**	**29.00**	17	Fr. 2-7-1	60.66
3	Fr. 3	95.73	18	Fr. 2-7-2	38.28
4	Fr. 4	109.85	19	**Fr. 2-7-3**	**22.81**
5	Fr. 5	60.66	20	Fr. 2-7-4	48.65
6	Fr. 6	66.90	21	Fr. 2-7-5	49.44
7	Fr. 7	31,67	22	Fr. 2-7-6	27.21
8	Fr. 2-1	81,94	23	Fr. 2-7-7	37.28
9	Fr. 2-2	95.73	24	Fr. 2-7-8	49.44
10	Fr. 2-3	37.21	25	Fr. 2-7-3-1	44.19
11	Fr. 2-4	71.92	26	Fr. 2-7-3-2	19.45
12	Fr. 2-5	48.65	27	**Fr. 2-7-3-3**	**17.97**
13	Fr. 2-6	36.22		**(Comp. 1)**	
14	**Fr. 2-7**	**27.21**	28	Fr. 2-7-3-4	39.43
15	Fr. 2-8	60.66	29	Fr. 2-7-3-5	54.21

Results fractionation of the ethanol extract *S.blumei* through silica gel column chromatography (70-230 mesh, Merck) was obtained pure fraction Fr 2-7-3-3 (compound **1**) (Table-1). The obtained white powder pure compound **1** has shown positive reaction to Dragendorff and Liebermann-Burchard reagents that indicates characteristic for steroidal alkaloid compound. Additionally, compound **1** presents positive reaction to Molisch's test which supports reviews assessment of their presence in a glycosidic form. The compound **1** isolated from *S. blumei* fruits had toxicity with LC_{50} 17.97 µg/mL based on BSLT method (Table-1). The compound considered as highly toxic because it has LC_{50} < 30 µg/mL [11].

Ultraviolet spectra for compound **1** indicates maximum wavelength at λ 191.8 nm which is characteristic for chromo-

phore alkene group (double bond). Infrared spectra has shown functional group at wave number 3100 cm^{-1} (OH); 1064.71 cm^{-1} (C-N-C); 1560.41 cm^{-1} (C=C aliphatic) and 2505.53 cm^{-1} (C-H strech).

^1H NMR spectra has exhibited 4 methyl groups (CH_3) at δ_H 1.10; 1.26; 0.85 and 1.05 ppm (s). Seven methine protons (CH) have been found at δ_H 3.51 (d); 1.26 (d); 0.94 (d); 2.01 (m); 1.99 (s); 3.40 (t) and 1.47 ppm (m). The presence of eight methines (CH) in high magnetic field indicate sugar group (carbohydrate) in the chemical structure at δ_H 4.39 (d); 3.45 (t); 3.97 (m); 3.30 (d); 3.42 (t); 3.79 (d); 3.61 (d); 3.64 ppm (d) and two anomeric protons at δ_H 4.71 (q) and 4.80 ppm (q) with β-anomer position [12]. Twelve methylene (CH_2) protons have been located at δ_H 1.10 (d); 1.91 (s); 1.79 (s); 1.170 (q); 2.41 (d); 2.23 (t); 1.88 (t); 2.41 (d); 3.41 (t); 1.99 (s); 1.91 (s) and 3.65 ppm (d), while alken group (-CH=C-) has been found at δ_H 5.37 ppm (d).

^{13}C NMR spectra and distortionless enhancement by polarization transfer (DEPT) analysis exhibit 39 carbons. Two olifinic carbons are found at δ_C 142.14 (s) and 122.49 ppm (d). Two methine carbons as C-anomeric are obtained at δ_C 102.47 (d) and 103.00 ppm (d), while four methyl carbons (CH_3) are located at δ_C 16.69 (q); 19.94 (q); 17.95 (q) and 18.97 ppm (q).

2D NMR hetero multiple quantum coherence (HMQC) spectra analysis for compound **1** indicate correlation between proton and carbon in one bond (*geminal*). For example, proton at δ_C 40 (C-6) are correlated with δ_H 5.37 (H-6); δ_C 22.03 with δ_H 2.41 (C-11 and H-11), *etc*. Correlation between proton and proton is confirmed by correlation between H-2 with H-3, H-3 with H-4, H-6 with H-7 and H-15 with H-16 result analysis spectra of 2D NMR correlation spectroscopy (COSY) for compound **1**. Hetero multiple bond connectivity (HMBC) spectra analysis has showed correlation between proton and carbon from more than one bond distance. Proton at δ_H 4.70 ppm (H-1') is correlated with 2 carbons at δ_C 79.93 ppm (C-3) and δ_C 75.35 (C-2'). Proton at δ_H 3.30 ppm (H-5') is correlated with carbons at δ_C 102.47 ppm (C-1'); 76.88 ppm (C-3') and 62.08 ppm (C-6'). Proton at δ_H 1.99 ppm (H-15) is associated with carbons at δ_C 42.86 (C-20) and δ_C 17.95ppm (C-21), *etc*. Ilustration for long-range correlation from HMBC spectra of compound **1** is shown in Fig. 2.

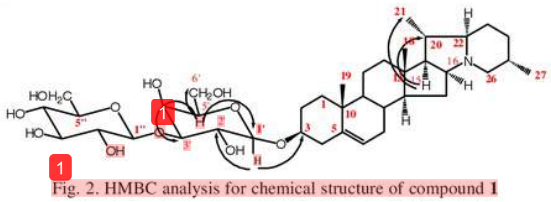

Fig. 2. HMBC analysis for chemical structure of compound **1**

Mass spectra (MS) data analysis shows molecule ion at *m/z* 722 $(M+H)^+$. This indicates that chemical structure of the compound **1** has molecular weight 721 for molecular formula $C_{39}H_{63}NO_{11}$. This is caused by addition of one H atom with molecular weight 1 but slipped out in order to get stable structure with molecular weight 721. Further, this molecule is fragmented by releasing two sugar molecules from glycoside molecule and leaded to glycon with molecular ion peak and fragmented ion at *m/z*: 560, 396, 380 and 150. Fragmentation pattern of fraction F2-7-3-3 (compound **1**) chemical structure can be seen in Fig. 3.

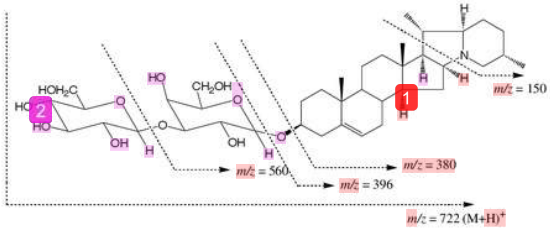

Fig. 3. Fragmentation pattern analysis result for compound **1**

Confirmation for chemical structure of compound **1** has been made by literature investigation. The chemical shift comparison between compound **1** and α-solanine can be seen in Table-2. Based on the comparison, compound **1** has similarity with α-solanine, however, compound **1** has 2 units of hexose sugars (galactocyl and glucocyl) with β-anomer position as glycoside alkaloid.

TABLE-2
COMPARISON CHEMICAL SHIFT DATA (ppm) BETWEEN COMPOUND **1** AND STANDARD β-SOLANINE

Carbon	Compound (CD_3OD)	α-Solanin (DMSO-d) [Ref. 9]	Carbon	Compound (CD_3OD)	α-Solanin (DMSO-d) [Ref. 9]
1	38.60 (t)	37.52	24	30.80 (t)	30.69
2	30.84 (t)	31.54	25	29.87 (d)	30.98
3	79.93 (d)	76.90	26	62.08(t)	59.58
4	40.59 (t)	39.91	27	18.97 (q)	19.40
5	142.14 (s)	140.33	1'	102.47 (d)	98.42 gal
6	122.49 (d)	121.39	2'	75.35 (d)	73.43
7	33.20 (t)	36.16	3'	76.88 (d)	83.58
8	32.89 (d)	31.26	4'	70.76 (d)	67.81
9	51.66 (d)	49.70	5'	76.84 (d)	72.93
10	38.10 (s)	36.91	6'	62.08 (t)	68.41
11	22.03 (t)	20.44	1"	103.00(d)	103.95 glu
12	39.77 (t)	39.91	2"	73.85 (d)	72.05
13	42.16 (s)	39.78	3"	72.55 (d)	74.06
14	57.77 (d)	56.99	4"	72.31 (d)	69.96
15	33.20 (t)	36.16	5"	76.84 (d)	76.00
16	79,80 (d)	68.41	6"	68.08 (t)	62.52 (t)
17	63.31 (d)	60.58	1"'	–	100.41 rha
18	16.69 (q)	16.68	2"'	–	69.76
19	19.94 (q)	18.95	3"'	–	70.43
20	42.86 (d)	36.41	4"'	–	72.52
21	17.95 (q)	17.81	5"'	–	60.91
22	73.85 (d)	74.65	6"'	–	18.32
23	33.20 (t)	28.97	–	–	–

Based on ultraviolet (UV), FTIR, 1D ^1H and ^{13}C NMR, 2D NMR (HMQC, COSY and HMBC), mass spectra and chemical shift (proton and carbon) spectral data comparison, compound **1** can be determined as glycoside steroidal alkaloid β2-solanine which contains 2 units of hexose sugars (galactocyl and glucocyl) with molecular formula $C_{39}H_{63}NO_{11}$. According to IUPAC, compound **1** is called as β2-solanin or [solanid-5-ene-(1'→3)-β-D-galactopyranosyl-(1"→3')-β-D-glucopyra-

noside] with molecular formula $C_{39}H_{63}NO_{11}$. LCMS/MS spectral data shows $m/z = 722$ $(M+H)^+$ to give molecular weight 721 for compound **1**.

It has been isolated one steroidal alkaloid glycoside, β2-solanine (compound **1**) from the ethanol extract of fruits of *S. blumei* Nees ex Blume. The steroidal alkaloid glycoside compounds are toxic with LC_{50} 17.97 µg/mL (Table-1). Active compound provides high mortality. Based on brine shrimp toxicity test method, a sample is considered as highly toxic if it has LC_{50} < 30 µg/mL and it is considered as toxic if it has LC_{50} 30-1000 µg/mL and less toxic if it has LC_{50} >1000 µg/mL [11]. According to Atanu *et al*. [5], toxicological properties of the *Solanum* plants are likely caused by alkaloid content contained in *Solanum* plants.

Eltayeb *et al*. [13] reported that a high concentration of glycoalkaloid solanine was also found in *S. nigrum*, but the highest was obtained in unripe berries. Small unripe fruits of *S. nigrum* had a high concentration of solasodine, but both concentration and absolute amount of alkaloids per fruit were decreased along with fruit maturation. Ikeda *et al*. [14] reported that two new steroidal oligoglycosides (nigrum I and II) were isolated from of *S. nigrum*. Hu *et al*. [15] had isolated three anti-neoplastic steroidal glycosides (β-2-solamargine, solamargine and degalactotigonin) from *S. nigrum* and all of them exhibited cytotoxicity in six cultured human solid tumor cell lines (HT-29, HCT-15, LNCaP, PC-3, T47D and MDA-MB-231).

Shabana *et al*. [6] have also isolated three steroidal alkaloids (solasodine, solamargine and solasonine) from ethanol extract of *Solanum melongena* fruit peels and showed potency as anticancer against hepatocellular carcinoma. Kartika *et al*. [16] have also been isolated alkaloid from fruit of *Scorodocarpus borneensis* Becc which exhibits bioactivity.

The steroidal alkaloid glycoside (compound **1**) isolated from *S. blumei* fruits had toxicity with LC_{50} 17.97 µg/mL based on Brine Shrimp Toxicity Test method (Table-1). The compound considered as highly toxic because it has LC_{50} < 30 µg/mL [11]. Based on the toxicity test results of steroidal alkaloid glycoside isolated from *S. blumei* fruits, this plant is potential to be Indonesian medicinal plant. The inhibitory activity against leukimia cell line L_{1210} of these steroidal alkaloid glycoside isolated from *S. blumei* fruits research is now in progress.

Conclusion

The results presented that the pure compound isolated from fruit of *S. blumei* is a steroidal alkaloid glycoside. Based on the spectral data analysis of ultra violet, infrared, 1D NMR (^{1}H-, ^{13}C- and DEPT), 2 D NMR (COSY, HMQC and HMBC), mass spectra and comparison with literature values, the structure of the compound **1** was established as β2-solanin or [solanid-5-ene-(1'→3)-β-D-galactopyranosyl-(1"→3')-β-D-glucopyranoside]. The compound had toxicity with LC_{50} 17.97 µg/mL based on Brine Shrimp Toxicity Test method. The compound considered as highly toxic because it has LC_{50} < 30 µg/mL.

ACKNOWLEDGEMENTS

This work was supported by Directorate of Research and Community Services, Directorate General of Higher Education, Ministry of Education and Culture Indonesian, in accordance with the Implementation Agreement Assignment Doctoral Dissertation Research, number of 064/SP2H/PL/Dit.Litabmas/II/2015, dated 5 February 2015.

REFERENCES

1. M. Simorangkir, R. Surbakti, T. Barus and P. Simanjuntak, Proceeding of National Seminar on Chemistry: Sumatera Utara University, ISBN: 979-458-692-7, pp. 303-311 (2013) (in Indonesian).
2. J.B. Harborne, Phytochemical Methods, A Guide to Modern Methods of Plants Analysis, Chapman & Hall, New York, pp. 7-43 (1973).
3. B. Meyer, N. Ferrigni, J. Putnam, L. Jacobsen, D. Nichols and J. McLaughlin, *Planta Med.*, **45**, 31 (1982).
4. M. Simorangkir, R. Surbakti, T. Barus and P. Simanjuntak, Proceeding of National Seminar on Chemistry, Post Graduated of Sumatera Utara University, ISBN: 979-458-746, pp. 226-229 (2014) (in Indonesian).
5. F.O. Atanu, U.G. Ebiloma, and E.I. Ajayi, *Biotechnol. Mol. Biol. Rev.*, **6,** 001 (2011).
6. M.M. Shabana, M.M. Salama, S.M. Ezzat and L.R. Ismail, *J. Carcinogene Mutagene*, **4**, 149 (2013).
7. M.R. Cooper and A.W. Johnson, Poisonous Plants in Britain and other Effects on Animals and Man, Ministry of Agriculture, Fisheries Food, Ref. Book No. 161, pp. 219-220 (1984).
8. S.J. Lee and K.T. Lim, *Toxicol. In Vitro*, **20**, 1088 (2006).
9. D.R. Lawson, T.P. Green, L.W. Haynes and A.R. Miller, *J. Agric. Food Chem.*, **45**, 4122 (1997).
10. A. Mohy-Ud-Din, K. Zaheer-Ud-Din, M. Ahmad and M.A. Kashmiri, *Pak. J. Bot.*, **42**, 653 (2010).
11. M. Steven and R. Molyneux, Bioactive Natural Products, CRC Press Boca Raton Ann Arbor, London, Tokyo, pp. 3-14 (1993).
12. R.M. Silverstein, F. Webster and D. Kiemle, Spectrometric Identification of Organic Compounds, John Wiley & Son Inc., New York, edn 7, pp. 5/267-5/270 (2005).
13. E.A. Eltayeb, A.S. Al-Ansari and J.G. Roddick, *Phytochemistry*, **46**, 489 (1997).
14. T. Ikeda, H. Tsumagari and T. Nohara, *Chem. Pharm. Bull. (Tokyo)*, **48**, 1062 (2000).
15. K. Hu, H. Kobayashi, A. Dong, Y. Jing, S. Iwasaki and X. Yao, *Planta Med.*, **65**, 35 (1999).
16. R. Kartika, T. Barus, R. Surbakti and P. Simanjuntak, *Asian J. Chem.*, **26**, 6047 (2014).

Isolation and Toxicity

ORIGINALITY REPORT

16%	3%	16%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Murniaty Simorangkir, Tonel Barus, Ribu Surbakti, Partomuan Simanjuntak. "Isolation and Toxicity of Steroidal Alkaloid Glycoside from Fruits of Ranti Hitam (Solanum blumei Nees ex Blume)", Asian Journal of Chemistry, 2016 Publication	16%
2	"Spectroscopic Data of Steroid Glycosides: Spirostanes, Bufanolides, Cardenolides", Springer Nature, 2007 Publication	<1%

Exclude quotes	On	Exclude matches	Off
Exclude bibliography	On		

Isolation and Toxicity