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Alex Grumezescu <al.grumezescu@gmail.com> Oct 19, 2020, 10:08 AM

to me

Dear Author,

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Alexandru Mihai GRUMEZESCU
Editor in Chief
http://grumezescu.com/
http://biointerfaceresearch.com/
Biointerface Research in Applied Chemistry
review@BiointerfaceResearch.com
briac@amgtranscend.org

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Review Of Scopoletin: Isolation, Analysis Process and Pharmacological Activity

Adang Firmansyah [1]https://orcid.org/0000-0002-6834-3320, **Wiwin Winingsih** [2]https://orcid.org/0000-0003-3830-4504, **Jesicha Dian Yenbyeebe Manobi** [3,*] https://orcid.org/0000-0001-9378-9159

[1] Sekolah Tinggi Farmasi Indonesia 1; adangfirmansyah@stfi.ac.id
[2] Sekolah Tinggi Farmasi Indonesia 2; winingsih341@gmail.com
[3] Sekolah Tinggi Farmasi Indonesia 3; jesichadymanobi@gmail.com
* Correspondence: e-mail@e-mail.com; if there are multiple corresponding authors, add author initials) (F.L.);
Scopus Author ID 57216094882

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Abstract: Background: Scopoletin (7-hydroxy-6-methoxy coumarin) is a coumarin phenolic compound found in many plants and includes coumarin derivatives which are superior in several types of plants. The article was created to provide information regarding the isolation process, analysis of pharmacological activity. Methods: The method used is to study and analyze scopoletin articles from national and international journals. Results: scopoletin has several pharmacological activities, Isolation of scopoletin 0.93% in Morinda citrifolia L, 0.17% in Convolvulus pluricaulis, 0.3% Artemisia annua, 0.027% Lasianthus lucidus Blume, 1,933mg / 100g Helichrysum italicum. Scopoletin analysis can be identified using Thin Layer Chromatography (TLC), high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), Nuclear Magnetic Resonance, and Mass spectrometry. Conclusions: In conclusion, Scopoletin extract content of 0.93% in noni fruit using the Soxhlet method. The highest scopoletin content was 0.3% in the stems of artemisia annua. Scopoletin has several pharmacological activities. Scopoletin compounds were identified using thin layer chromatography with the eluent C4H8O2: CH3OH: H2O (100: 6: 4) with an Rf value of 0.91. Analysis using high performance liquid chromatography obtained a retention time of 19.583 minutes. The ^{1}H NMR spectrum of Scopoletin shows two doublets with a coupling constant of 9.2 Hz at δ 6.22 and 7.88 ppm, defined as H-3 and H-4, a single methoxyl group at δ 3.93 ppm and two aromatic singlets respectively. -At 7,13 & 679 respectively. The mass spectrum of isolated Scopoletin showed an M-1 peak at 191.10.

Keywords: scopoletin, isolation, analysis, pharmacological activity

1. Introduction

Scopoletin is widespread in the plant kingdom and can be isolated from various parts (roots, fruit, leaves, stems, etc.) of plants [1]. Scopoletin is a coumarin phenolic originating from the phenylpropanoid pathway, and can be isolated from various types of plants [2]. The presence of scopoletin was initially detected using thin layer chromatography (TLC) and its various pharmacological activities were further explained through a number of investigations [3]. Further identification or isolation of scopoletin using several methods, namely the Soxhlet method, reflux, percolation, maceration, and supercritical CO2 and can be analyzed using high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), Nuclear Magnetic Resonance, and Mass spectrometry.

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From the background presented, this article was intended to provide a collection of information about a more efficient way to isolate, analyze and determine the pharmacological activity of the compound scopoletin.

Source of Scopoletin

Scopoletin is widespread in the plant kingdom and can be isolated from different parts (roots, fruit, leaves, stems, etc.) of a plant [1]. Such as namely *Sinomonium acutum* [4], *Solanum lyratum* [5], *roots of Brunfelsia hopeana* [6], *Artemisia feddei* [7], *Helichrysum italicum* [8], *Manihot esculenta* [9], *Canscora decussate* [10], *Chenopodium murale* [11], *Erycibe obtusifolia Benth* [12], *Hypochaeris radicata* [13], *Cirsium setidens* [14], *Aleurites moluccana (L.)* [15], *Lasianthus lucidus Blume* [16], *Morinda citrifolia* [17], *Nicotiana tabacum* [18], *Ipomoea digitata* [19], *Aegle marmelos* [20], *Ipomoea reniformis* [21], *Artemisia iwayomogi* [22], *Macaranga gigantifolia Merr* [23], *Artemisia annua* [24], *Tetrapleura tetraptera* [25], *Tilia cordata Mill.* [26], *Melia azedarach L.* [27] *Acer saccharum Marsh.* [28], *Hymenodictyon obovatum* [29], *Fagraea ceilanica* [30], *Magnolia fargesii* [31], *Morus alba* L. (Po-sa) [68], *Clausena excavate* Burm.f. (Pyin-daw-thein) [70].

Pharmacological Activity Of Scopoletin

Scopoletin has several pharmacological activities, namely antihepatotoxicity [5], antibacterial [16], antithyroid [20], antifungal [27,71,85], antitubercular [32], antimigratory [33], antihypertensive [34,35], antioxidant [36], antiproliferative [37], antiinflamation [17,38,39,66,69,77,79], neurological [40,41,42,72,74,82,83], antidopaminergic and antiadrenergic [43], Antidiabetic [67], Antihyperuricemic [73]. Pharmacological Activities Of Scopoletin were tabulated in table 1.

Table 1. Pharmacological Activity Of Scopoletin

Pharmacologycalact	Dose	Reference
Antihepatotoxicity	Scopoletin significantly decreased the release of glutamate pyruvate transaminase and sorbitol dehydrogenase from cultured rat hepatocyte carbon tetrachloride primary intoxication by 53 and 58% at 1 to 50 M doses, respectively.	5
Antibacterial	Scopoletin 1.4 µmol / g of stem bark (200 g dry weight) shows activity as an anti-pseudomonas agent. exhibits antibacterial.	16
Antithyroid	Scopoletin (1.00 mg / kg, p.o.) given daily for 7 days decreased serum thyroid hormone levels and glucose and liver glucose-6-phosphatase activity.	20

	Scopoletin showed very good inhibitory activity on the formation of AGEs with an IC50 value of 2.93 µM and showed an inhibitory activity of RLAR with an IC50 value of 22.5 µM.	27
Antifungal	The minimum inhibitory concentrations ranged from 0.07 ± 0.00 µg / ml and 0.15 ± 0.00 µg / ml. The antifungal activity of scopoletin extracted on some of the damaging fungi in foodstuffs.	71
	The characteristic minimum inhibitory concentration (MIC90) of Scopoletin against Candida species ranged from 67.22 and 119 µg / mL is effective against Candida spp. and antifungal activity.	85
Antitubercular	Scopoletin at a dose of 40 mg / ml significantly acts as an antitubercular agent against Mycobacterium tuberculosis strain H37Rv.	32
Antimigratory	Scopoletin 0.58% (w / w) and can inhibit viability, migration in MCF-7 cells and has the potential to be developed as an anticancer agent for breast cancer.	33
Antihypertensive	Scopoletin 0.46 + 0.05% significantly reduced blood pressure in hypertensive rats.	34
	Scopoletin at doses of 1, 3 and 10 mg / kg reduced levels of IL-4 type I and Scopoletin at a dose of 10 mg / kg decreased serum levels.	35
Antioxidant	Scopoletin (17.4 µg / mL) showed potential antioxidant activity.	36
Antiproliferative	Scopoletin showed a reduced anti-proliferative effect on all cancer cell lines (IC50 103 and above 600µg / ml).	37
Antiinflamation	Scopoletin 0.62 µmol / g inhibited nitric oxide (NO) production in a manner dependent on the concentration of lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cells and exhibited induced quinone reductase (QR) in Hepa 1c1c7 cell culture.	17
	Scopoletin 100 mg / kg showed anti-inflammatory activity by croton oil-induced rat ear edema.	38
	Scopoletin (0.63–2.50 g / kg) as a potential preventive and therapeutic agent for gastro-esophageal inflammatory disease, primarily through its antisecretory and prokinetic activities including inhibitory activity on serotonin, free radicals, and cytokine-mediated inflammation.	39
	Scopoletin showed remarkable activity on LDL oxidation (IC50 = 10.2 µM) and exerted a vascular anti-inflammatory effect on human endothelial cells EA.hy926 activated by TNF-α.	66
	Scopoletin (2.0, 10.0, 50.0 mg / kg) showed that inhibition of nuclear factor-kappa B and a mitogen-activated protein kinase signaling pathway involving anti-inflammatory activity and regulation of the excitatory / inhibitory balance could be associated with anxiolytic effects.	69
	Demonstrated an inhibitory effect on HNE (human neutrophil elastase) activity, with IC50 values ranging from 3.6–74.3 µM.	77
	Scopoletin prevented oxidative stress and apoptosis and activated	79

	Nrf2 signaling.	
Neurological	Scopoletin (10 mg / kg, p.o.) shows that antidepressant-like effects are dependent on serotonergics (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenoceptors) and dopaminergics (dopamine D1 and D2 receptors).	40
	Scopoletin has a memory enhancement property, which is based on the direct agonistic activity of nAChR.	41
	Scopoletin which was identified as the main constituent of the extract was found to be an inhibitor of GABA-T (IC50 = 10.57 M).	42
	Downregulation of Bid, Bax, and interrupted expression of caspase-9 induced by scopoletin down regulates cleaved caspase-3 expression, inhibits cleaved PARP expression, and ultimately, inhibits the mitochondrial apoptotic pathway.	72
	Scopoletin 100 mg / kg indicates that Scopoletin provides neuroprotection, relieves neuronal apoptosis, and improves neuronal autophagy.	74
	The IC50 concentrations for the inhibition of AChE and BuChE enzymes by scopoletin were 5.34 and 9.11μM, respectively.	82
	Pretreatment of SH-SY5Y cells with 5 mM of scopoletin protected against the cell death induced by H2O2, and decreased the levels of apoptotic cells and ROS.	83
Antidopaminergic and Antiadrenergic	The dose of scopoletin (<200 μg / mL) in mice, shows antidopaminergic and antiadrenergic activity.	43
Antidiabetic	Scopoletin has an anti-diabetic effect by stimulating translocation of GLUT4 through plasma membrane activation of PI3K and AMPK pathways in 3T3-L1 adipocytes, thereby regulating glucose uptake.	67
Antihyperuricemic	Scopoletin exhibits a weak urate reduction effect after sustained oral administration. Scopoletin exhibits an inhibitory effect on both serum and liver XOD activity.	73

References numbered in the table are listed below; 5. Kang et al., 1998; 16. Napiroon et al., 2018; 17. Nitteranon et al., 2011; 20. Panda And Kar, 2006; 27. Carpinella et al., 2005; 32. Mauliku et al., 2017; 33. Noor et al., 2018; 34. Wigati et al., 2017; 35. Aldi et al., 2015; 36. Gwak et al., 2011; 37. Thani et al., 2010; 38. Ding et al., 2008; 39. Moon et al., 2007; 40. Capra et al., 2010; 41. Hornick et al., 2011; 42. Mishra et al., 2010; 43. Pandy et al., 2014; 66. Kang et al., 2020; 67. Jang et al., 2020; 69. Luo et al., 2020; 71. Njankouo et al., 2020; 72. Lee et al., 2020; 73. Zeng et al., 2020; 74. Zhou et al., 2020; 77. Lee et al., 2020; 79. Narasimhan et al., 2020; 82. Kashyap et al., 2020; 83. Gay et al., 2020; 85. Das et al., 2020.

Physicochemical Properties Of Scopoletin

Scopoletin atau 7-hydroxy-6-methoxychromen-2-one (Fig. 1) belongs to the simple coumarin group and is derived from 1,2-benzopyrones found in higher plants derived from the common phenylpropanoid pathway [3]. Synonyms for scopoletin are Gelseminic acid,

Chrysatropic acid, Scopoletine, 6-Methylesculetin, Murrayetin, Scopoletol, Escopoletin, Methylesculetin, 6-O-Methylesculetin, Esculetin-6-methyl ether, 7-Hydroxy-5-methoxycoumarin, 6-Methoxyumbelliferone [44]. Research conducted [21] states that scopoletin has a melting point of 202-204° C, has the chemical formula $C_{10}H_8O_4$ with a molecular weight of 192.17 g/mol, boiling point: 413.5° C, and flash point: 172.4° C, scopoletin light-yellow amorphous powder [75]. Scopoletin dissolves in organic solvents such as ethanol, Dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF), which must be cleaned with an inert gas. The solubility of scopoletin in this solvent was about 2, 30, and 50 mg / ml, respectively [44]. Scopoletin is soluble in acetonitrile [1], methanol, ethyl acetate and N-hexane [45]. Scopoletin is slightly soluble in water and slightly soluble in aqueous buffers. For maximum solubility in buffered water, scopoletin must first be dissolved in dimethyl formamide (DMF) and then diluted with an aqueous buffer of choice. Scopoletin has a solubility of about 0.2 mg / ml in DMF: PBS (pH 7.2) 1: 4 [44]. FT-Infrared, Nuclear Magnetic Resonance, and Mass Spectrometry (Figures 2, 3, 4) [10,21,30,59,63,64,65,66,67].

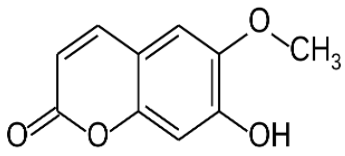

Figure 1. Chemical Structure of Scopoletin

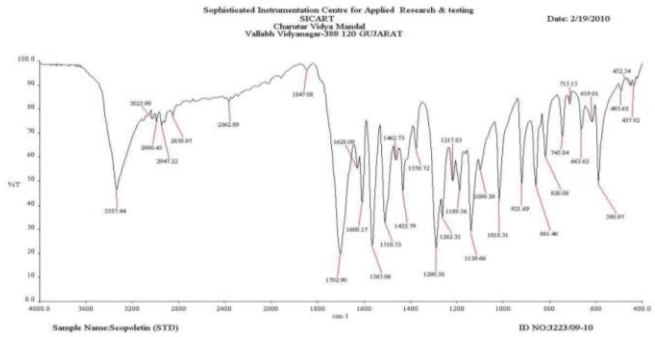

Figure 2. IR spectrum of standard Scopoletin

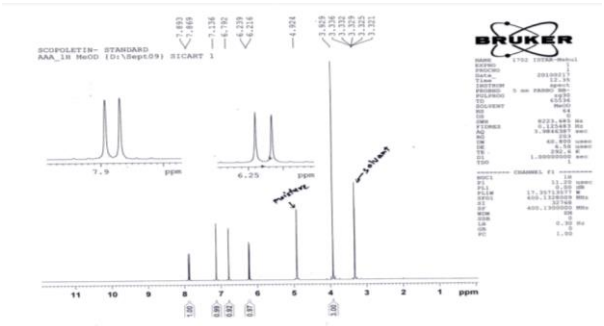

Figure 3. Nmr of standard Scopoletin

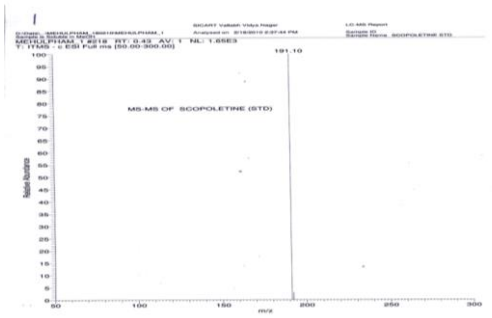

Figure 4. Mass spectrum of standard Scopoletin

Isolation and Analysis Of Scopoletin

Scopoletin compounds can be obtained by an isolation process. Generally, traditional isolation procedures rely on successive extraction of fresh or dry plant material with a solvent of increasing polarity. For this purpose, solvents methanol, acetonitrile, ethanol, hexane, have been commonly used. This is because these compounds are relatively easy to dissolve in these compounds. Recently, many studies have used methanol [16,46], aqueous acetonitrile (1: 5 v / v) [1] or various types of solvents (hexane, ethanol and methanol) [47]. A detailed description of the multistep method for extraction and purification of scopoletin has recently been presented from several plants (Table 3), including the stem of *Artemisia annua* [1], *Helichrysum italicum* [8], *Lasianthus lucidus* Blume [16], *Convolvulus pluricaulis* [46], *Morinda citrifolia* L [47], *Morus alba* L. (Po-sa) [68], *Clausena excavate* Burm.f. (Pyin-daw-thein) [70].

Tabel 2. The Isolation Process of Scopoletin

Isolation method	Solvent	% Yield	Reference
Percolation for 6-8 hours. Column Chromatography. Elution of scopoletin in a methanol chloroform mixture.	(acetonitrile: water) dilute acetonitrite in the ratio 1: 5	0,3%	1
Supercritical fluid extraction (SFE). Dried immortelle flowers were weighed at 1.5 MPa and 25 ° C. CO_2 flow rate (1.94 kg / hr). Each extraction process lasts for 90 min. The extract was stored at 4–6 ° C until HPLC analysis.	CO_2	1,744 mg / 100 g from the H. italicum flower	8

Maceration. The extract MeOH, applied to a glass column filled with 25 to 40 µm silica gel. The fraction was checked for purity by thin layer chromatography (TLC) and UV detection (wavelength 365 nm).	Methanol	0,027%	16
Extract at reflux 80-85C for 1-2 hours. The extract was examined with hplc.	Methanol (99%), 50% alcohol and water.	0,1738%	46
Soxhlet. The extract was examined by gas chromatography-mass spectrometry (GC-MS) technique.	various types of solvents (hexane, ethanol and methanol) at 90 ° C for ethanol, 75 ° C for methanol and hexane, for 4 hours.	0.93%	47
Pet-Ether is obtained and defatted then divided into ethyl acetate and water. The ethyl acetate soluble extract was obtained, separated by gradient elution column chromatography. From this separation, F2 is collected. From the condensed fraction F2, the scopoletin compound was collected by paper chromatography with a solvent formate / A: H2O (2:98).	The 95% ethanol extract was extracted with pet-ether (60C-80 ° C).	0,0009%	68
Extracted using a separating funnel. the pet-ether layer is evaporated, partitioned between ethyl acetate and water. The ethyl acetate was separated by column chromatography. Elution gradient with PE: EtOAc. Scopoletin was isolated from the F IV fraction. The scopoletin compound was washed with pet ether followed by ethyl acetate and then purified by recrystallization from methanol.	250 mL pet-eter (60-80 C)	0.32% Pyin-daw-thein ethyl acetate extract	70

References numbered in the table are listed below; 1. Jain et al., 2002; 8. Jokić et al., 2016; 16. Napiroon et al., 2018; 46. Upadhyay et al., 2013; 47. Muenmuang et al., 2017; 68. Sann et al., 2020; 70. Nu, 2012.

Scopoletin has been studied for decades. Identification and quantification were carried out in various ways including Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as well as Gas Chromatography Mass Spectrometry (GC-MS) [3]. The most common method used to screen fractions from a separation procedure is Thin Layer Chromatography (Table 3) [10,16,21,27,28,30,48,49,60]**.** Scopoletin emits blue fluorescence when the developed Thin Layer Chromatography (TLC) plates are examined under ultraviolet illumination at a wavelength of 365 nm [50]. High Performance Liquid Chromatography (HPLC), with different detection methods, has been used for purification (preparative) and is widely used for the identification and quantification (analytical) of scopoletin (Table 4) [17,25,33,46,53,55,56,57,58,61,62,65,70].

Table 3. Identification Method of Scopoletin Identification by TLC

Mobile phase	Retention Factor (Rf)	Reference
$C_4H_8O_2$: CH_3COOH : CH_2O_2 : H_2O (10: 0,5: 0,5: 1,5)	0,78	10
C_6H_{14} : $C_4H_8O_2$ (7: 3)	0,5	16
C_7H_8 : $C_4H_8O_2$: CH_2O_2 (5: 4: 1)	0,47	21
$CHCl_3$: C_2H_3N (2: 1)	0,75	27
C_6H_6 : C_2H_5OH (100 : 22)	0.42	28
C_6H_{14} : $C_4H_8O_2$ (7: 3)	0,42	30
CH_2Cl_2 : CH_3OH (19: 1)	0,5	48
CHCl3 : CH_3OH (9: 1) or CHCl3 : CH_3OH : H_2O (65: 30: 5)	0,53	49
$C_4H_8O_2$: CH_3OH : H2O (100: 6: 4)	0.91	60
Petroleum Ether: Ethyl Acetate: Formic Acid (3: 7: 0.3)	0,5	65
PE: EtOAc (1: 2)	0,5	70

References numbered in the table are listed below; 10. Sethiya et al., 2015; 16. Napiroon et al., 2018; 21. Mehul et al., 2011; 27. Carpinella et al., 2005; 28. Miller et al., 1990; 30. Ferdinal et al., 2015; 48. West et al., 2010; 49. Potterat et al., 2007; 60. Nandhasri et al., 2005; 65. Vyas et al., 2020; 70. Nu, 2012.

Table 4. Analytical Method of Scopoletin by HPLC

UV Detection	Mobil phase	Flow Rate (ml/min)	Rt (min)	Reference
254 nm	0,1% formic acid in water (eluant A) and ACN (eluant B)	1,0	12.5	17
350 nm	CH3COONa: C_2H_3N (80:20)	1.0	5.497	25
254 nm	0,01M acetic acid: acetonitrile: methanol (60:20:20)	1,0	4.3	33
366 nm	CH_3OH: H_2O (0.1 %v/v HCOOH); 3:7	1.0	19.579	46
345 nm	Glacial acetic acid (0.5%) : Methanol (26%) : Deionised	1.0	16.35	53

	water (55%)			
345 nm	CH_3OH and H_2O (49:51, v/v); 0.05% (v/v) H_3PO_4	1.0	5.1	55
300 nm	55: 45 (% v/v) CH_3OH: H_2O (0.1% CH_3COOH)	1.0	4.6	56
220 nm	CH_3OH: H_2O (0.05% HCOOH)	1.0	7.87	57
345 nm	(A) H_2O: H_3PO_4 (99.7:0.3 % v/v) (B) ACN: H_2O: H_3PO_4 (79.9:20:0.3% v/v) Method-A:B (75:25 %v/v)	1.0	6.03	58
350 nm	0,01 M sodium acetate: acetronitrile (80:20, v / v); isokratik	1,0	5.585	61
280 nm	0,1% (v / v) Trifluoroacetic acid in water (A) dan 100% acetronitrile (B); gradien	1,0	11.812	62
230 nm	water (A) and methanol (B) both contain 0.1% TFA: 30-65% B; gradien	1,0	13.28	76

References numbered in the table are listed below; 17. Nitteranon et al., 2011; 25. Ojewole, 1984; 33. Noor et al., 2018; 46. Upadhyay et al., 2013; 53. Nahata et al., 2018; 55. Xia et al., 2007; 56. Shinde et al., 2014; 57. Deivi et al., 2015; 58. Deep et al., 2014; 61. Mahattanadul et al., 2010; 62. Vidya et al., 2012; 76. Guetchueng et al., 2020.

2. Methods

Searches for this review were conducted online at Google Scholar, and Google Patent. The search keywords used were: "Scopoletin" and "Isolation" and "Analysis" and "Pharmacological Effects". Literature reviews were obtained from journals, and research reports in March-August 2020. The number of the reviewed literatures were 105 journals related to their relevance and fulfilling the search keywords, determined independently by three authors, only those selected by at least two authors to be reviewed and put in the script.

3. Results and Discussion

Scopoletin (Fig. 1) belongs to the simple coumarin group and is derived from 1,2-benzopyrones found in higher plants which originate from the common phenylpropanoid pathway. The coumarin structure (2H-1-benzopyran-2-one) is formed by ortho-hydroxylation of cinnamates, trans / cis side chain isomerization, and lactonization. Ortho-hydroxylation is a key step in coumarin biosynthesis as a branch point of lignin biosynthesis [3].

Scopoletin has several pharmacological activities namely antihepatotoxicity [5], antibacterial [16], antithyroid [20], antifungal [27], antitubercular [32], antimigratory [33], antihypertensive [34,35], antioxidant [36], antiproliferative [37], antiinflamation [17,38,39], neurological [40,41,42], antidopaminergic and antiadrenergic [43]. In this research [5] scopoletin significantly decreased the release of glutamate pyruvate transaminase and sorbitol dehydrogenase from cultured rat hepatocyte carbon tetrachloride primary intoxication by 53

and 58% at 1 to 50 M doses, respectively. Scopoletin 1.4 µmol / g of stem bark (200 g dry weight) shows activity as an anti-pseudomonas agent exhibits antibacterial [16]. Scopoletin (1.00 mg / kg, p.o.) given daily for 7 days decreased serum thyroid hormone levels and glucose and liver glucose-6-phosphatase activity [20]. Scopoletin showed very good inhibitory activity on the formation of AGEs with an IC50 value of 2.93 µM and showed an inhibitory activity of RLAR with an IC50 value of 22.5 µM [27]. Scopoletin at a dose of 40 mg / ml significantly acts as an antitubercular agent against Mycobacterium tuberculosis strain H37Rv [32]. Scopoletin 0.58% (w / w) and can inhibit viability, migration in MCF-7 cells and has the potential to be developed as an anticancer agent for breast cancer [33]. Scopoletin 0.46 + 0.05% significantly decreased the blood pressure of hypertensive rats [34]. Scopoletin at doses of 1, 3 and 10 mg / kg decreased IL-4 type I and Scopoletin at a dose of 10 mg / kg decreased serum levels [35]. Scopoletin (17.4 μg / mL) showed potential antioxidant activity [36]. Scopoletin showed reduced anti-proliferative effect on all cancer cell lines (IC50 103 and above 600µg / ml) [37]. Scopoletin 0.62 µmol / g inhibited nitric oxide (NO) production in a manner dependent on the concentration of lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cells and demonstrated quinone reductase (QR) induction in Hepa 1c1c7 cell culture [17]. Scopoletin 100 mg / kg showed anti-inflammatory activity by croton oil-induced rat ear edema [38]. Scopoletin (0.63–2.50 g / kg) as a potential preventive and therapeutic agent for gastro-esophageal inflammatory disease, mainly through its antisecretory and prokinetic activities including inhibitory activity on serotonin, free radicals, and cytokine-mediated inflammation [39]. Scopoletin (10 mg / kg, p.o.) demonstrated that antidepressant-like effects were dependent on serotonergics (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenoceptors) and dopaminergics (dopamine D1 and D2 receptors) [40]. Scopoletin has a memory enhancement property, which is based on the direct agonistic activity of nAChR [41]. Scopoletin which was identified as the main constituent of the extract was found to be a GABA-T inhibitor (IC50 = 10.57 M) [42]. The dose of scopoletin (<200 μg / mL) in mice, showed antidopaminergic and antiadrenergic activity [43].

The isolation process begins with an extraction process that aims to attract scopoletin compounds in plants. From several studies, the most widely used solvent is methanol. This is because these compounds are relatively easy to dissolve in these compounds. The first-stage extraction process in conventional isolation is mostly carried out by the maceration method for ± 72 hours so that the solvent used will enter the cell through the cell wall of the simplicia containing scopoletin so that the cell contents will dissolve due to the difference in concentration between the solution inside the cell and outside. cell. High concentration solution will be pushed out and replaced by low concentration solvent (diffusion process). Other extraction methods such as using Soxhlet have been carried out at temperatures of 40ºC-60ºC and the reflux method. The extraction method with the help of heating will speed up the dissolving process, because when heating is done, the particles at high temperatures will move faster than at low temperatures so that the contact between the solute and the solvent becomes more effective. Other extraction methods such as using the supercritical CO_2 method at a temperature of 80ºC-85°C for 1-2 hours. From the extraction process of the scopoletin compound, the extraction results were obtained using the Soxhlet method 0.93% [47]. From the results of the isolation process of scopoletin in table 2, the number of isolates was significantly different, the isolates produced from the isolation process were 0.3% on artemisia annua stems with the Percolation method for 6-8 hours, then the extracts were

obtained in Column Chromatography and dissolution of scopoletin in a methanol chloroform mixture [1].

Analytical methods that can assist the identification process of scopoletin compounds include using Thin Layer Chromatography as initial identification. Further identification can be done using a UV-Vis spectrophotometer. Further identification can also be carried out using High Performance Liquid Chromatography under various conditions resulting in different retention times. High Performance Liquid Chromatography identifies target compounds on the principle that when the sample moves through the stationary phase (it can be solid or liquid), it is carried away by the mobile phase (it can be a liquid or a gas). The various components in the sample will be separated based on their different affinities for the stationary phase. Components that can interact strongly with the stationary phase will move more slowly so that they can be separated from other components with weak interactions. In addition, several complementary identifications must also be carried out to identify the structure of the compounds obtained from the isolation process, such as using FT-Infra Red, Nuclear Magnetic Resonance, and Mass spectrometry.

TLC (Thin Layer Chromatography) is a qualitative test to determine the purity of a compound. Based on several studies (table 2), good thin layer chromatography results were obtained, namely the ratio of C4H8O2: CH3OH: H2O (100: 6: 4) with an Rf value of 0.91 [60]. The value of Rf is very characteristic for certain compounds in certain eluents. The Rf value is a comparison of the distance traveled by the eluent and the mobile phase on the TLC plate. Compounds that have a greater Rf means that they have low polarity, and vice versa. This is because the stationary phase is polar. A more polar compound is held firmly in the stationary phase, resulting in a lower Rf value. A good TLC Rf ranges from 0.2 - 0.8. if Rf is too high, all you have to do is reduce the polarity of the eluent, and vice versa. The value of Rf can be used as evidence in the identification of compounds. If the Rf value has the same value, then the compound can be said to have the same or similar characteristics as its comparison. The success of the separation depends on the difference in the solubility of the components to be separated in the solvent [69]. Most coumarin compounds are active against UV rays, this is because coumarin has a conjugated double bond, and it is known that UV absorption rays are able to absorb a conjugated bond or have a chromophore group. Scopoletin is a coumarin compound so that scopoletin will have a fluorescent blue color when exposed to a UV lamp with a wavelength of 365 nm [63]. The appearance of stains under 365 nm UV light indicates that the compound has at least two conjugated double bonds. The appearance is due to the interaction power between UV rays and the chromophore groups bound by the auxochromes present in the stain. Visible light fluorescence is the emission of light emitted by these components when the excited electrons from the base energy level to a higher energy level then return to their original state while releasing energy, so that the visible stain on the UV lamp looks bright because the silica gel used is not fluorescent to 365 nm UV light [64].

HPLC stands for High Performance Liquid, which can be interpreted as a method of separating molecules from liquid media that are given high pressure. The function of HPLC is to determine or measure or analyze the levels of active ingredients in a sample (medicine, food or herbs). Based on several research results (table 4), the results of scopoletin analysis by Upadhyay et el., 2013 show that the retention time of scopoletin 19,583 shows that the

compound scopoletin [46] is the same as the scopoletin standard (Figure 5), namely the retention time of 19,579 minutes.

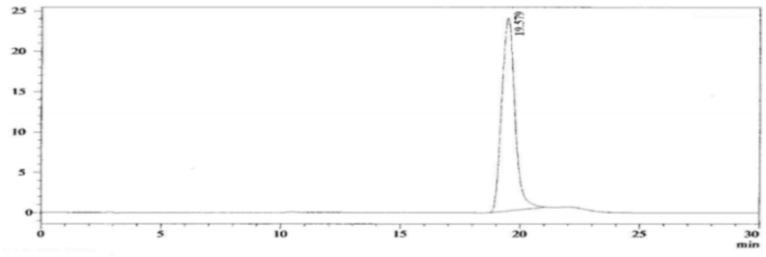

Figure 5 HPLC Chromatogram of Reference Standart (Scopoletin)

Table 6 The data from FTIR spectrum of standard Scopoletin

Peaks (cm-1)	Functional group
3337.44	O-H Alcohol group present
2850.97	C-H group present
1702.90	Carbonyl C=O group present
1628.09	CH=CH group present
1565.06	Benzene ring present
1510.53	Benzene ring present
861.46	Due to disubstitution of benzene

Table 6 shows the data obtained from FTIR Spectroscopy and possible functional groups present. In the IR spectral analysis, the Peak at 3337.44, 3318 & 3341.44 cm-1 , a broad band is most probably the result of O-H stretching vibrations of phenol OH group. The peak at 2850.97, 2926 - 2990 & 2875.05 cm-1 showed C-H Streching due to –CH3. The peak at 1702.90, 1698 & 1703.42 indicates the presence of -C=O, Carbonyl group. The peak at 1628.09, 1602 & 1606.75 showed the presence of -CH=CH group. The peak at 1565.06, 1517.01, 1510.53 & 1568.83, 1567.68, 1511.16 indicates the presence of benzene ring. The peak at 861.46 & 861.50 showed the presence of disubstitution of benzene ring in both standard Scopoletin and isolated compound V respectively. The above Comparision with standard confirms that isolated compound V and isolated compound Y is Scopoletin (Figures 2, 6&7) [21]. The Peak at 3325 cm-1, a broad band is most probably the result of O-H stretching vibrations of phenol OH group. The peak at 3055 of C=CH. The peak at 2920 of –CH3, CH2. The peak at 2850 of –OCH3. The peak at 1705 of δ lactone. The peak at 1604 of aromatic ring) (Figure 8) [68]. Spektrum FTIR pada gambar 9 menunjukkan pita pada 3325 cm-1 karena getaran peregangan OH alkohol atau fenolik-OH kelompok. Pita pada 1705 cm-1 muncul karena gugus lakton karbonil (C = O). Pita pada 1612, 1566 dan 1504 cm-1 menunjukkan getaran regangan C=C dari kelompok aromatik. Pita pada 1288 dan 1134 cm-1 muncul karena peregangan getaran C-O-C dalam kelompok Ar-O dan peregangan C-OH [70].

Table 7 The data from NMR spectrum of standard Scopoletin and isolated compound V.

	Standard Scopoletin		Isolated Compound V		Isolated Compound Z	
No. of H atom	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**
3	6.23	1H, d(9.2)	6.22	1H, d(9.2)	6.2	1H, d(9.2)
4	7.88	1H, d(9.6)	7.88	1H, d(9.2)	7.6	1H, d(9.2)
5	7.14	1H, S	7.13	1H, S	6.8	1H, S
8	6.79	1H, S	6.79	1H, S	6.9	1H, S
C-6-OMe	3.93	3H, S	3.93	3H, S	3.7	3H, S

Table 7 shows the data obtained from NMR Spectroscopy. The ^{1}H NMR spectrum of standard Scopoletin and Isolated Compound V showed two doublets with coupling constant of 9.2 Hz at δ 6.23, 6.22 and 7.88 ppm, which were assigned as H-3 and H-4, respectively in standard Scopoletin and isolated compound V, characteristic for coumarins. The ^{1}H NMR spectrum of Scopoletin showed a methoxyl group singlet at δ 3.93 ppm and two aromatic singlets at δ 7.14, 7.13 & 6.79 ppm respectively in standard Scopoletin and isolated compound V which were explained by 6,7-disubstitution. So it confirms that isolated compound V is Scopoletin (Figures 3&8). Figure 9 also illustrates that ^{1}H NMR spectrum showed two doublets with coupling constant of 9.2 Hz at δ 6.2, 7.6 ppm which were assigned as H-3 and H-4. The ^{1}H NMR spectrum of Scopoletin showed a methoxyl group singlet at δ 3.7 ppm and two aromatic singlets at δ 6.8 and 6.9 ppm [68]. On research [78] the 1H-NMR spectrum of the compound showed signals of coumarin compound with two signals of cis-o efi ic proto t δH 7.88 (H-4) and 6.22 (H-3), two aromatic protons t δH 7.12 (H-5); 6.79 (H-8). In addition, a methoxy group signal at δH 3.97 (OCH3) was found. On research [80] ^{1}H NMR spectrum of compound showed two characteristic olefinic protons of coumarin moiety at 7.83 and 6.09, two aromatic protons at δ 7.10 (s) and 6.65 (s), and a methoxy groups at δ 3.77 (s), respectively. On research [81] the ^{1}H-NMR spectrum of the compound showed signals of 7.69 (H4), 6.86 (H-5), 6,61 (H-8), 6,01 (H-3), 3.81 (OCH3). On research [84] the ^{1}H-NMR spectrum of the compound showed of (500.13 MHz, CDCl3), δ (ppm): 3.88 (3H, s, OMe), 6.18 (1H, d, J= 9.2 Hz, H-3), 6.77 (1H, s, H-5), 6.84 (1H, s, H-8), 7.50 (1H, d, J = 9.2 Hz, H-4).

Table 8 The molecular weight data from mass spectrum of standard Scopoletin and isolated compound V.

No. Sample	M-1 Peak	Molecular weight
Standard Scopoletin	191.10	192.10
Isolated Compound V	191.10	192.10

Mass spectra of both standard Scopoletin and isolated Compound V shows M-1 peak at 191.10 which indicate that their molecular weight is same, 192.10. So it confirms that isolated compound V is Scopoletin (Figures 4&9).

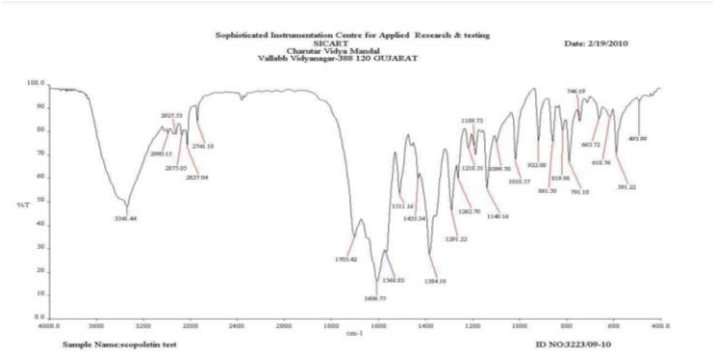

Figure 6. IR spectrum of Isolated V Compound

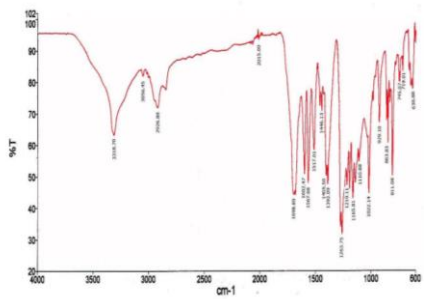

Figure 7. IR spectrum of Isolated Y Compound

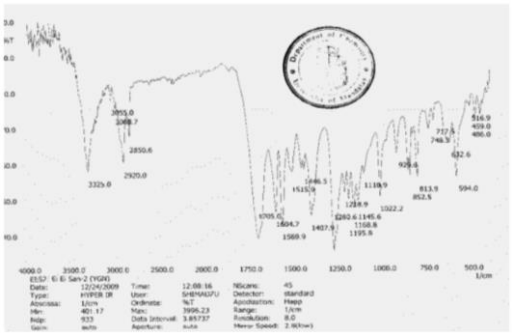

Figure 8. IR spectrum of Isolated Compound Z

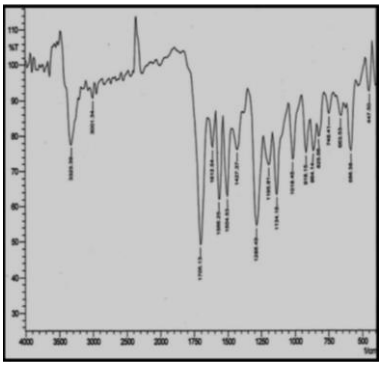

Figure 9. IR spectrum of Isolated Compound

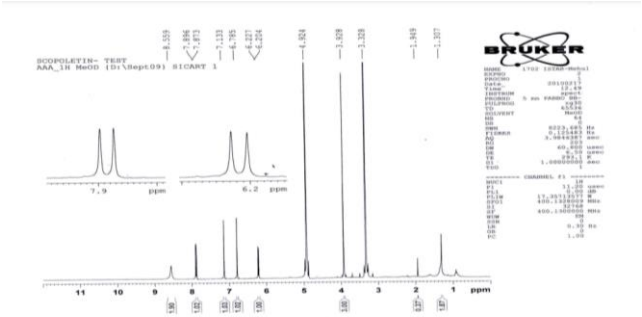

Figure 10. Nmr spectrum of Isolated Compound V

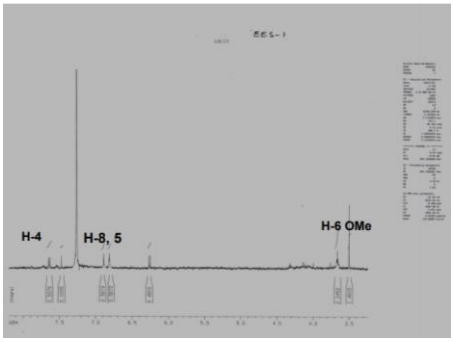

Figure 11. Nmr spectrum of Isolated Compound Z

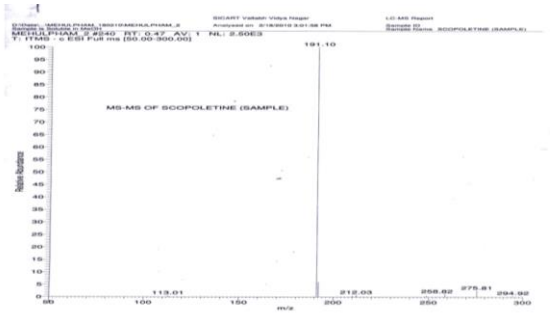

Figure 12. Mass spectrum of Isolated Compound V

4. Conclusions

The highest scopoletin extract content was found in noni fruit by 0.93% using the Soxhlet method. The highest isolation process of scopoletin compound was 0.3% on artemisia annua stems. Scopoletin has several pharmacological activities. Scopoletin compounds were identified using thin layer chromatography with $C_4H_8O_2$: CH_3OH: H_2O (100: 6: 4) eluent with an Rf value of 0.91, analysis using high performance liquid chromatography obtained a retention time of 19.583 minutes, the ^1H NMR spectrum of Scopoletin showed two doublets with a constant Coupling 9.2 Hz at δ 6.22 and 7.88 ppm, designated as H-3 and H-4. The ^1H NMR spectrum of Scopoletin showed a single methoxyl group at δ 3.93 ppm and two aromatic singlets at δ 7.13 & 679 respectively. The mass spectrum of isolated Scopoletin showed an M-1 peak at 191.10 indicating that its molecular weight the same, 192.10.

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Review Of Scopoletin: Isolation, Analysis Process and Pharmacological Activity

Adang Firmansyah [1]https://orcid.org/0000-0002-6834-3320, **Wiwin Winingsih** [2]https://orcid.org/0000-0003-3830-4504, **Jesicha Dian Yenbyeebe Manobi** [3,*] https://orcid.org/0000-0001-9378-9159

[1] Sekolah Tinggi Farmasi Indonesia 1; adangfirmansyah@stfi.ac.id
[2] Sekolah Tinggi Farmasi Indonesia 2; winingsih341@gmail.com
[3] Sekolah Tinggi Farmasi Indonesia 3; jesichadymanobi@gmail.com
* Correspondence: e-mail@e-mail.com; if there are multiple corresponding authors, add author initials) (F.L.);
Scopus Author ID 57216094882

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Abstract: Background: Scopoletin (7-hydroxy-6-methoxy coumarin) is a coumarin phenolic compound found in many plants and includes coumarin derivatives which are superior in several types of plants. The article was created to provide information regarding the isolation process, analysis of pharmacological activity. Methods: The method used is to study and analyze scopoletin articles from national and international journals. Results: scopoletin has several pharmacological activities, Isolation of scopoletin 0.93% in Morinda citrifolia L, 0.17% in Convolvulus pluricaulis, 0.3% Artemisia annua, 0.027% Lasianthus lucidus Blume, 1,933mg / 100g Helichrysum italicum. Scopoletin analysis can be identified using Thin Layer Chromatography (TLC), high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), Nuclear Magnetic Resonance, and Mass spectrometry. Conclusions: In conclusion, Scopoletin extract content of 0.93% in noni fruit using the Soxhlet method. The highest scopoletin content was 0.3% in the stems of artemisia annua. Scopoletin has several pharmacological activities. Scopoletin compounds were identified using thin layer chromatography with the eluent C4H8O2: CH3OH: H2O (100: 6: 4) with an Rf value of 0.91. Analysis using high performance liquid chromatography obtained a retention time of 19.583 minutes. The ^{1}H NMR spectrum of Scopoletin shows two doublets with a coupling constant of 9.2 Hz at δ 6.22 and 7.88 ppm, defined as H-3 and H-4, a single methoxyl group at δ 3.93 ppm and two aromatic singlets respectively. - At 7,13 & 679 respectively. The mass spectrum of isolated Scopoletin showed an M-1 peak at 191.10.

Keywords: scopoletin, isolation, analysis, pharmacological activity

1. Introduction

Scopoletin is widespread in the plant kingdom and can be isolated from various parts (roots, fruit, leaves, stems, etc.) of plants [1]. Scopoletin is a coumarin phenolic originating from the phenylpropanoid pathway, and can be isolated from various types of plants [2]. The presence of scopoletin was initially detected using thin layer chromatography (TLC) and its various pharmacological activities were further explained through a number of investigations [3]. Further identification or isolation of scopoletin using several methods, namely the Soxhlet method, reflux, percolation, maceration, and supercritical CO2 and can be analyzed using high performance liquid chromatography (HPLC), Fourier-transform infrared spectroscopy (FTIR), Nuclear Magnetic Resonance, and Mass spectrometry.

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From the background presented, this article was intended to provide a collection of information about a more efficient way to isolate, analyze and determine the pharmacological activity of the compound scopoletin.

Source of Scopoletin

Scopoletin is widespread in the plant kingdom and can be isolated from different parts (roots, fruit, leaves, stems, etc.) of a plant [1]. Such as namely *Sinomonium acutum* [4], *Solanum lyratum* [5], *roots of Brunfelsia hopeana* [6], *Artemisia feddei* [7], *Helichrysum italicum* [8], *Manihot esculenta* [9], *Canscora decussate* [10], *Chenopodium murale* [11], *Erycibe obtusifolia Benth* [12], *Hypochaeris radicata* [13], *Cirsium setidens* [14], *Aleurites moluccana (L.)* [15], *Lasianthus lucidus Blume* [16], *Morinda citrifolia* [17], *Nicotiana tabacum* [18], *Ipomoea digitata* [19], *Aegle marmelos* [20], *Ipomoea reniformis* [21], *Artemisia iwayomogi* [22], *Macaranga gigantifolia Merr* [23], *Artemisia annua* [24], *Tetrapleura tetraptera* [25], *Tilia cordata Mill.* [26], *Melia azedarach L.* [27] *Acer saccharum Marsh.* [28], *Hymenodictyon obovatum* [29], *Fagraea ceilanica* [30], *Magnolia fargesii* [31].

Pharmacological Activity Of Scopoletin

Scopoletin has several pharmacological activities, namely antihepatotoxicity [5], antibacterial [16], antithyroid [20], antifungal [27], antitubercular [32], antimigratory [33], antihypertensive [34,35], antioxidant [36], antiproliferative [37], antiinflamation [17,38,39], neurological [40,41,42], antidopaminergic and antiadrenergic [43]. Pharmacological Activities Of Scopoletin were tabulated in table 1.

Table 1. Pharmacological Activity Of Scopoletin

Pharmacology activity	Dose	Reference
Antihepatotoxicity	Scopoletin significantly decreased the release of glutamate pyruvate transaminase and sorbitol dehydrogenase from cultured rat hepatocyte carbon tetrachloride primary intoxication by 53 and 58% at 1 to 50 M doses, respectively.	5
Antibacterial	Scopoletin 1.4 µmol / g of stem bark (200 g dry weight) shows activity as an anti-pseudomonas agent. exhibits antibacterial.	16
Antithyroid	Scopoletin (1.00 mg / kg, p.o.) given daily for 7 days decreased serum thyroid hormone levels and glucose and liver glucose-6-phosphatase activity.	20
Antifungal	Scopoletin showed very good inhibitory activity on the formation of AGEs with an IC50 value of 2.93 µM and showed an inhibitory activity of RLAR with an IC50 value of 22.5 µM.	27

Antitubercular	Scopoletin at a dose of 40 mg / ml significantly acts as an antitubercular agent against Mycobacterium tuberculosis strain H37Rv.	32
Antimigratory	Scopoletin 0.58% (w / w) and can inhibit viability, migration in MCF-7 cells and has the potential to be developed as an anticancer agent for breast cancer.	33
Antihypertensive	Scopoletin 0.46 + 0.05% significantly reduced blood pressure in hypertensive rats.	34
	Scopoletin at doses of 1, 3 and 10 mg / kg reduced levels of IL-4 type I and Scopoletin at a dose of 10 mg / kg decreased serum levels.	35
Antioxidant	Scopoletin (17.4 μg / mL) showed potential antioxidant activity.	36
Antiproliferative	Scopoletin showed a reduced anti-proliferative effect on all cancer cell lines (IC50 103 and above 600µg / ml).	37
Antiinflamation	Scopoletin 0.62 µmol / g inhibited nitric oxide (NO) production in a manner dependent on the concentration of lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cells and exhibited induced quinone reductase (QR) in Hepa 1c1c7 cell culture.	17
	Scopoletin 100 mg / kg showed anti-inflammatory activity by croton oil-induced rat ear edema.	38
	Scopoletin (0.63–2.50 g / kg) as a potential preventive and therapeutic agent for gastro-esophageal inflammatory disease, primarily through its antisecretory and prokinetic activities including inhibitory activity on serotonin, free radicals, and cytokine-mediated inflammation.	39
Neurological	Scopoletin (10 mg / kg, p.o.) shows that antidepressant-like effects are dependent on serotonergics (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenoceptors) and dopaminergics (dopamine D1 and D2 receptors).	40
	Scopoletin has a memory enhancement property, which is based on the direct agonistic activity of nAChR.	41
	Scopoletin which was identified as the main constituent of the extract was found to be an inhibitor of GABA-T (IC50 = 10.57 M).	42
Antidopaminergic and Antiadrenergic	The dose of scopoletin (<200 μg / mL) in mice, shows antidopaminergic and antiadrenergic activity.	43

References numbered in the table are listed below; 5. Kang et al., 1998; 16. Napiroon et al., 2018; 17. Nitteranon et al., 2011; 20. Panda And Kar, 2006; 27. Carpinella et al., 2005; 32. Mauliku et al., 2017; 33. Noor et al., 2018; 34. Wigati et al., 2017; 35. Aldi et al., 2015; 36. Gwak et al., 2011; 37. Thani et al., 2010; 38. Ding et al., 2008; 39. Moon et al., 2007; 40. Capra et al., 2010; 41. Hornick et al., 2011; 42. Mishra et al., 2010; 43. Pandy et al., 2014.

Physicochemical Properties Of Scopoletin

Scopoletin atau 7-hydroxy-6-methoxychromen-2-one (Fig. 1) belongs to the simple coumarin group and is derived from 1,2-benzopyrones found in higher plants derived from the common phenylpropanoid pathway [3]. Synonyms for scopoletin are Gelseminic acid, Chrysatropic acid, Scopoletine, 6-Methylesculetin, Murrayetin, Scopoletol, Escopoletin, Methylesculetin, 6-O-Methylesculetin, Esculetin-6-methyl ether, 7-Hydroxy-5-methoxycoumarin, 6-Methoxyumbelliferone [44]. Research conducted [21] states that scopoletin has a melting point of 202-204° C, has the chemical formula $C_{10}H_8O_4$ with a molecular weight of 192.17 g/mol, boiling point: 413.5° C, and flash point: 172.4° C. Scopoletin dissolves in organic solvents such as ethanol, Dimethyl sulfoxide (DMSO), and dimethyl formamide (DMF), which must be cleaned with an inert gas. The solubility of scopoletin in this solvent was about 2, 30, and 50 mg / ml, respectively [44]. Scopoletin is soluble in acetonitrile [1], methanol, ethyl acetate and N-hexane [45]. Scopoletin is slightly soluble in water and slightly soluble in aqueous buffers. For maximum solubility in buffered water, scopoletin must first be dissolved in dimethyl formamide (DMF) and then diluted with an aqueous buffer of choice. Scopoletin has a solubility of about 0.2 mg / ml in DMF: PBS (pH 7.2) 1: 4 [44]. FT-Infrared, Nuclear Magnetic Resonance, and Mass Spectrometry (Figures 2, 3, 4) [10,21,30,59,63,64,65,66,67].

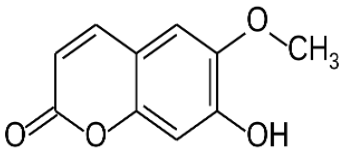

Figure 1. Chemical Structure of Scopoletin

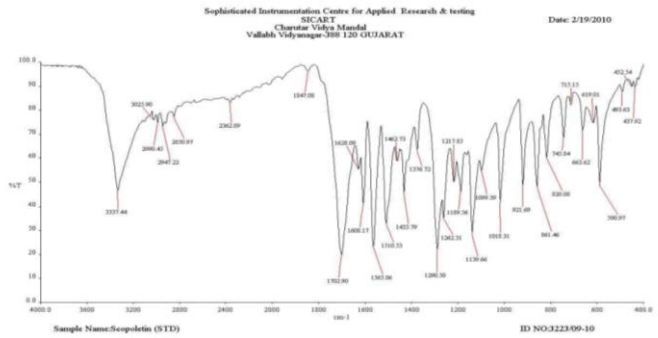

Figure 2. IR spectrum of standard Scopoletin

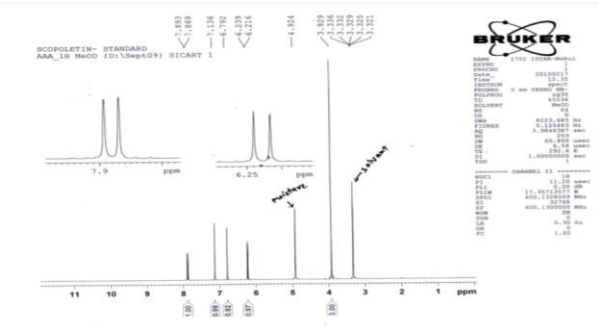

Figure 3. Nmr of standard Scopoletin

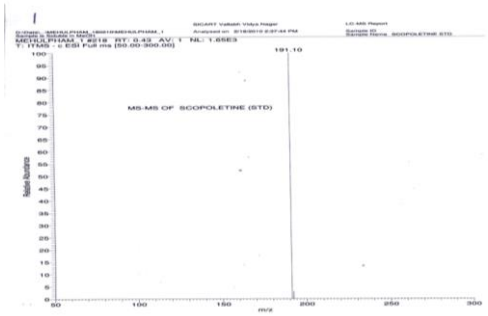

Figure 4. Mass spectrum of standard Scopoletin

Isolation and Analysis Of Scopoletin

Scopoletin compounds can be obtained by an isolation process. Generally, traditional isolation procedures rely on successive extraction of fresh or dry plant material with a solvent of increasing polarity. For this purpose, solvents methanol, acetonitrile, ethanol, hexane, have been commonly used. This is because these compounds are relatively easy to dissolve in these compounds. Recently, many studies have used methanol [16,46], aqueous acetonitrile (1: 5 v / v) [1] or various types of solvents (hexane, ethanol and methanol) [47]. A detailed description of the multistep method for extraction and purification of scopoletin has recently been presented from several plants (Table 3), including the stem of *Artemisia annua* [1], *Helichrysum italicum* [8], *Lasianthus lucidus* Blume [16], *Convolvulus pluricaulis* [46], *Morinda citrifolia* L [47].

Tabel 2. The Isolation Process of Scopoletin

Isolation method	Solvent	% Yield	Reference
Percolation for 6-8 hours. Column Chromatography. Elution of scopoletin in a methanol chloroform mixture.	(acetonitrile: water) dilute acetonitrite in the ratio 1: 5	0,3%	1
Supercritical fluid extraction (SFE). Dried immortelle flowers were weighed at 1.5 MPa and 25 ° C. CO_2 flow rate (1.94 kg / hr). Each extraction process lasts for 90 min. The extract was stored at 4–6 ° C until HPLC analysis.	CO_2	1,744 mg / 100 g from the H. italicum flower	8
Maceration. The extract MeOH, applied to a glass column filled with 25 to 40 µm silica gel. The fraction was checked for purity by thin layer chromatography (TLC) and UV detection (wavelength 365 nm).	Methanol	0,027%	16
Extract at reflux 80-85C for 1-2 hours. The extract was examined with hplc.	Methanol (99%), 50% alcohol and water.	0,1738%	46
Soxhlet. The extract was examined by gas chromatography-mass spectrometry (GC-MS) technique.	various types of solvents (hexane, ethanol and methanol) at 90 ° C for ethanol, 75 ° C for methanol and hexane, for 4 hours.	0.93%	47

References numbered in the table are listed below; 1. Jain et al., 2002; 8. Jokić et al., 2016; 16. Napiroon et al., 2018; 46. Upadhyay et al., 2013; 47. Muenmuang et al., 2017.

Scopoletin has been studied for decades. Identification and quantification were carried out in various ways including Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) as well as Gas Chromatography Mass Spectrometry (GC-MS) [3]. The most common method used to screen fractions from a separation procedure is Thin Layer Chromatography (Table 3) [10,16,21,27,28,30,48,49,60]**.** Scopoletin emits blue fluorescence when the developed Thin Layer Chromatography (TLC) plates are examined under ultraviolet illumination at a wavelength of 365 nm [50]. High Performance Liquid Chromatography (HPLC), with different detection methods, has been used for purification (preparative) and is widely used for the identification and quantification (analytical) of scopoletin (Table 4) [17,25,33,46,53,55,56,57,58,61,62].

Table 3. Identification Method of Scopoletin Identification by TLC

Mobile phase	Retention Factor (Rf)	Reference
$C_4H_8O_2$: CH_3COOH : CH_2O_2 : H_2O (10: 0,5: 0,5: 1,5)	0,78	10
C_6H_{14} : $C_4H_8O_2$ (7: 3)	0,5	16
C_7H_8 : $C_4H_8O_2$: CH_2O_2 (5: 4: 1)	0,47	21

$CHCl_3$: C_2H_3N (2: 1)	0,75	27
C_6H_6 : C_2H_5OH (100 : 22)	0.42	28
C_6H_{14} : $C_4H_8O_2$ (7: 3)	0,42	30
CH_2Cl_2 : CH_3OH (19: 1)	0,5	48
CHCl3 : CH_3OH (9: 1) or CHCl3 : CH_3OH : H_2O (65: 30: 5)	0,53	49
$C_4H_8O_2$: CH_3OH : H2O (100:6:4)	0.91	60

References numbered in the table are listed below; 10. Sethiya et al., 2015; 16. Napiroon et al., 2018; 21. Mehul et al., 2011; 27. Carpinella et al., 2005; 28. Miller et al., 1990; 30. Ferdinal et al., 2015; 48. West et al., 2010; 49. Potterat et al., 2007; 60. Nandhasri et al., 2005.

Table 4. Analytical Method of Scopoletin by HPLC

UV Detection	Mobil phase	Flow Rate (ml/min)	Rt (min)	Reference
254 nm	0,1% formic acid in water (eluant A) and ACN (eluant B)	1,0	12.5	17
350 nm	CH3COONa: C_2H_3N (80:20)	1.0	5.497	25
254 nm	0,01M acetic acid: acetonitrile: methanol (60:20:20)	1,0	4.3	33
366 nm	CH_3OH: H_2O (0.1 %v/v HCOOH); 3:7	1.0	19.579	46
345 nm	Glacial acetic acid (0.5%) : Methanol (26%) : Deionised water (55%)	1.0	16.35	53
345 nm	CH_3OH and H_2O (49:51, v/v); 0.05% (v/v) H_3PO_4	1.0	5.1	55
300 nm	55: 45 (% v/v) CH_3OH: H_2O (0.1% CH_3COOH)	1.0	4.6	56
220 nm	CH_3OH: H_2O (0.05% HCOOH)	1.0	7.87	57
345 nm	(A) H_2O: H_3PO_4 (99.7:0.3 % v/v) (B) ACN: H_2O: H_3PO_4 (79.9:20:0.3% v/v) Method-A:B (75:25 %v/v)	1.0	6.03	58
350 nm	0,01 M sodium acetate: acetronitrile (80:20, v / v); isokratik	1,0	5.585	61

280 nm	0,1% (v / v) Trifluoroacetic acid in water (A) dan 100% acetronitrile (B); gradien	1,0	11.812	62

References numbered in the table are listed below; 17. Nitteranon et al., 2011; 25. Ojewole, 1984; 33. Noor et al., 2018; 46. Upadhyay et al., 2013; 53. Nahata et al., 2018; 55. Xia et al., 2007; 56. Shinde et al., 2014; 57. Deivi et al., 2015; 58. Deep et al., 2014; 61. Mahattanadul et al., 2010; 62. Vidya et al., 2012.

2. Methods

Searches for this review were conducted online at Google Scholar, and Google Patent. The search keywords used were: "Scopoletin" and "Isolation" and "Analysis" and "Pharmacological Effects". Literature reviews were obtained from journals, and research reports in March-August 2020. The number of the reviewed literatures were 105 journals related to their relevance and fulfilling the search keywords, determined independently by three authors, only those selected by at least two authors to be reviewed and put in the script.

3. Results and Discussion

Scopoletin (Fig. 1) belongs to the simple coumarin group and is derived from 1,2-benzopyrones found in higher plants which originate from the common phenylpropanoid pathway. The coumarin structure (2H-1-benzopyran-2-one) is formed by ortho-hydroxylation of cinnamates, trans / cis side chain isomerization, and lactonization. Ortho-hydroxylation is a key step in coumarin biosynthesis as a branch point of lignin biosynthesis [3].

Scopoletin has several pharmacological activities namely antihepatotoxicity [5], antibacterial [16], antithyroid [20], antifungal [27], antitubercular [32], antimigratory [33], antihypertensive [34,35], antioxidant [36], antiproliferative [37], antiinflamation [17,38,39], neurological [40,41,42], antidopaminergic and antiadrenergic [43]. In this research [5] scopoletin significantly decreased the release of glutamate pyruvate transaminase and sorbitol dehydrogenase from cultured rat hepatocyte carbon tetrachloride primary intoxication by 53 and 58% at 1 to 50 M doses, respectively. Scopoletin 1.4 µmol / g of stem bark (200 g dry weight) shows activity as an anti-pseudomonas agent exhibits antibacterial [16]. Scopoletin (1.00 mg / kg, p.o.) given daily for 7 days decreased serum thyroid hormone levels and glucose and liver glucose-6-phosphatase activity [20]. Scopoletin showed very good inhibitory activity on the formation of AGEs with an IC50 value of 2.93 µM and showed an inhibitory activity of RLAR with an IC50 value of 22.5 µM [27]. Scopoletin at a dose of 40 mg / ml significantly acts as an antitubercular agent against Mycobacterium tuberculosis strain H37Rv [32]. Scopoletin 0.58% (w / w) and can inhibit viability, migration in MCF-7 cells and has the potential to be developed as an anticancer agent for breast cancer [33]. Scopoletin 0.46 + 0.05% significantly decreased the blood pressure of hypertensive rats [34]. Scopoletin at doses of 1, 3 and 10 mg / kg decreased IL-4 type I and Scopoletin at a dose of 10 mg / kg decreased serum levels [35]. Scopoletin (17.4 µg / mL) showed potential antioxidant activity [36]. Scopoletin showed reduced anti-proliferative effect on all cancer cell lines (IC50 103 and above 600µg / ml) [37]. Scopoletin 0.62 µmol / g inhibited nitric oxide (NO) production in a manner dependent on the concentration of lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cells and demonstrated quinone reductase (QR) induction in Hepa 1c1c7 cell culture [17]. Scopoletin 100 mg / kg showed anti-inflammatory activity by croton oil-induced rat ear edema

[38]. Scopoletin (0.63–2.50 g / kg) as a potential preventive and therapeutic agent for gastro-esophageal inflammatory disease, mainly through its antisecretory and prokinetic activities including inhibitory activity on serotonin, free radicals, and cytokine-mediated inflammation [39]. Scopoletin (10 mg / kg, p.o.) demonstrated that antidepressant-like effects were dependent on serotonergics (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenoceptors) and dopaminergics (dopamine D1 and D2 receptors) [40]. Scopoletin has a memory enhancement property, which is based on the direct agonistic activity of nAChR [41]. Scopoletin which was identified as the main constituent of the extract was found to be a GABA-T inhibitor (IC50 = 10.57 M) [42]. The dose of scopoletin (<200 μg / mL) in mice, showed antidopaminergic and antiadrenergic activity [43].

The isolation process begins with an extraction process that aims to attract scopoletin compounds in plants. From several studies, the most widely used solvent is methanol. This is because these compounds are relatively easy to dissolve in these compounds. The first-stage extraction process in conventional isolation is mostly carried out by the maceration method for ± 72 hours so that the solvent used will enter the cell through the cell wall of the simplicia containing scopoletin so that the cell contents will dissolve due to the difference in concentration between the solution inside the cell and outside. cell. High concentration solution will be pushed out and replaced by low concentration solvent (diffusion process). Other extraction methods such as using Soxhlet have been carried out at temperatures of 40ºC-60ºC and the reflux method. The extraction method with the help of heating will speed up the dissolving process, because when heating is done, the particles at high temperatures will move faster than at low temperatures so that the contact between the solute and the solvent becomes more effective. Other extraction methods such as using the supercritical CO_2 method at a temperature of 80ºC-85°C for 1-2 hours. From the extraction process of the scopoletin compound, the extraction results were obtained using the Soxhlet method 0.93% [47]. From the results of the isolation process of scopoletin in table 2, the number of isolates was significantly different, the isolates produced from the isolation process were 0.3% on artemisia annua stems with the Percolation method for 6-8 hours, then the extracts were obtained in Column Chromatography and dissolution of scopoletin in a methanol chloroform mixture [1].

Analytical methods that can assist the identification process of scopoletin compounds include using Thin Layer Chromatography as initial identification. Further identification can be done using a UV-Vis spectrophotometer. Further identification can also be carried out using High Performance Liquid Chromatography under various conditions resulting in different retention times. High Performance Liquid Chromatography identifies target compounds on the principle that when the sample moves through the stationary phase (it can be solid or liquid), it is carried away by the mobile phase (it can be a liquid or a gas). The various components in the sample will be separated based on their different affinities for the stationary phase. Components that can interact strongly with the stationary phase will move more slowly so that they can be separated from other components with weak interactions. In addition, several complementary identifications must also be carried out to identify the structure of the compounds obtained from the isolation process, such as using FT-Infra Red, Nuclear Magnetic Resonance, and Mass spectrometry.

TLC (Thin Layer Chromatography) is a qualitative test to determine the purity of a compound. Based on several studies (table 2), good thin layer chromatography results were obtained, namely the ratio of C4H8O2: CH3OH: H2O (100: 6: 4) with an Rf value of 0.91 [60]. The value of Rf is very characteristic for certain compounds in certain eluents. The Rf value is

a comparison of the distance traveled by the eluent and the mobile phase on the TLC plate. Compounds that have a greater Rf means that they have low polarity, and vice versa. This is because the stationary phase is polar. A more polar compound is held firmly in the stationary phase, resulting in a lower Rf value. A good TLC Rf ranges from 0.2 - 0.8. if Rf is too high, all you have to do is reduce the polarity of the eluent, and vice versa. The value of Rf can be used as evidence in the identification of compounds. If the Rf value has the same value, then the compound can be said to have the same or similar characteristics as its comparison. The success of the separation depends on the difference in the solubility of the components to be separated in the solvent [69]. Most coumarin compounds are active against UV rays, this is because coumarin has a conjugated double bond, and it is known that UV absorption rays are able to absorb a conjugated bond or have a chromophore group. Scopoletin is a coumarin compound so that scopoletin will have a fluorescent blue color when exposed to a UV lamp with a wavelength of 365 nm [63]. The appearance of stains under 365 nm UV light indicates that the compound has at least two conjugated double bonds. The appearance is due to the interaction power between UV rays and the chromophore groups bound by the auxochromes present in the stain. Visible light fluorescence is the emission of light emitted by these components when the excited electrons from the base energy level to a higher energy level then return to their original state while releasing energy, so that the visible stain on the UV lamp looks bright because the silica gel used is not fluorescent to 365 nm UV light [64].

HPLC stands for High Performance Liquid, which can be interpreted as a method of separating molecules from liquid media that are given high pressure. The function of HPLC is to determine or measure or analyze the levels of active ingredients in a sample (medicine, food or herbs). Based on several research results (table 4), the results of scopoletin analysis by Upadhyay et el., 2013 show that the retention time of scopoletin 19,583 shows that the compound scopoletin [46] is the same as the scopoletin standard (Figure 5), namely the retention time of 19,579 minutes.

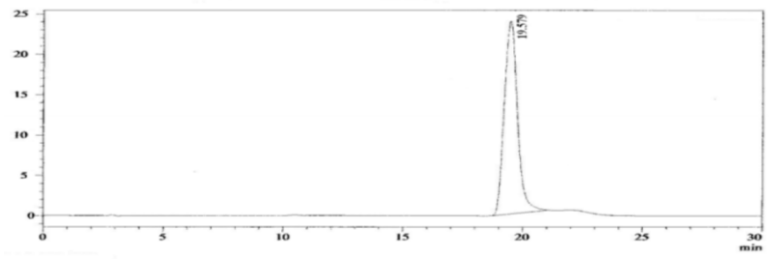

Figure 5 HPLC Chromatogram of Reference Standart (Scopoletin)

Table 6 The data from FTIR spectrum of standard Scopoletin, isolated compound V, and isolated compound Y.

Peaks (cm-1)			Functional group
Standard Scopoletin	Isolated Compound V	Isolated Compound Y	
3337.44	3341.44	3318	O-H Alcohol group present
2850.97	2875.05	2926 - 2990	C-H group present
1702.90	1703.42	1698	Carbonyl C=O group present

1628.09	1606.75	1602	CH=CH group present
1565.06	1568.83	1567.68	Benzene ring present
1510.53	1511.16	1517.01	Benzene ring present
861.46	861.50		Due to disubstitution of benzene

Table 6 shows the data obtained from FTIR Spectroscopy and possible functional groups present. In the IR spectral analysis, the Peak at 3337.44, 3318 & 3341.44 cm-1 , a broad band is most probably the result of O-H stretching vibrations of phenol OH group. The peak at 2850.97, 2926 - 2990 & 2875.05 cm-1 showed C-H Streching due to –CH3. The peak at 1702.90, 1698 & 1703.42 indicates the presence of -C=O, Carbonyl group. The peak at 1628.09, 1602 & 1606.75 showed the presence of -CH=CH group. The peak at 1565.06, 1517.01, 1510.53 & 1568.83, 1567.68, 1511.16 indicates the presence of benzene ring. The peak at 861.46 & 861.50 showed the presence of disubstitution of benzene ring in both standard Scopoletin and isolated compound V respectively. The above Comparision with standard confirms that isolated compound V and isolated compound Y is Scopoletin (Figures 2, 6&7) [21].

Table 7 The data from NMR spectrum of standard Scopoletin and isolated compound V.

	Standard Scopoletin		**Isolated Compound V**	
No. of H atom	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**
3	6.23	1H, d(9.2)	6.22	1H, d(9.2)
4	7.88	1H, d(9.6)	7.88	1H, d(9.2)
5	7.14	1H, S	7.13	1H, S
8	6.79	1H, S	6.79	1H, S
C-6-OMe	3.93	3H, S	3.93	3H, S

Table 7 shows the data obtained from NMR Spectroscopy. The ^{1}H NMR spectrum of standard Scopoletin and Isolated Compound V showed two doublets with coupling constant of 9.2 Hz at δ 6.23, 6.22 and 7.88 ppm, which were assigned as H-3 and H-4, respectively in standard Scopoletin and isolated compound V, characteristic for coumarins. The ^{1}H NMR spectrum of Scopoletin showed a methoxyl group singlet at δ 3.93 ppm and two aromatic singlets at δ 7.14, 7.13 & 6.79 respectively in standard Scopoletin and isolated compound V which were explained by 6,7-disubstitution. So it confirms that isolated compound V is Scopoletin (Figures 3&8).

Table 8 The molecular weight data from mass spectrum of standard Scopoletin and isolated compound V.

No. Sample	**M-1 Peak**	**Molecular weight**
Standard Scopoletin	191.10	192.10
Isolated Compound V	191.10	192.10

Mass spectra of both standard Scopoletin and isolated Compound V shows M-1 peak at 191.10 which indicate that their molecular weight is same, 192.10. So it confirms that isolated compound V is Scopoletin (Figures 4&9).

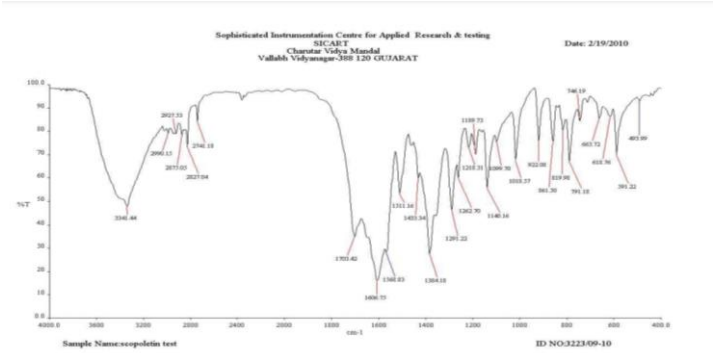

Figure 6. IR spectrum of Isolated V Compound [21]

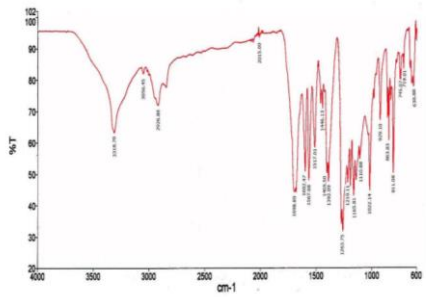

Figure 7. IR spectrum of Isolated Y Compound [30]

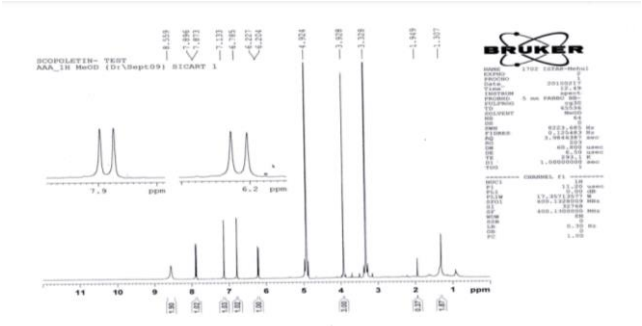

Figure 8. Nmr spectrum of Isolated Compound V [21]

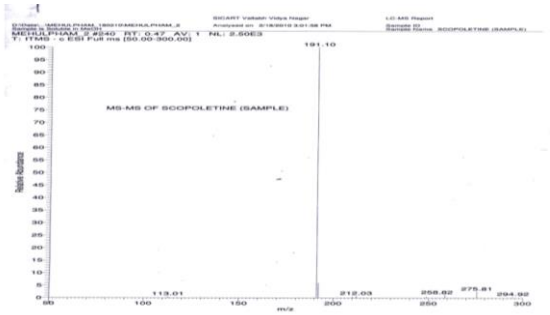

Figure 9. Mass spectrum of Isolated Compound V [21]

4. Conclusions

The highest scopoletin extract content was found in noni fruit by 0.93% using the Soxhlet method. The highest isolation process of scopoletin compound was 0.3% on artemisia annua stems. Scopoletin has several pharmacological activities. Scopoletin compounds were identified using thin layer chromatography with C4H8O2: CH3OH: H2O (100: 6: 4) eluent with an Rf value of 0.91, analysis using high performance liquid chromatography obtained a retention time of 19.583 minutes, the ^{1}H NMR spectrum of Scopoletin showed two doublets with a constant Coupling 9.2 Hz at δ 6.22 and 7.88 ppm, designated as H-3 and H-4. The ^{1}H NMR spectrum of Scopoletin showed a single methoxyl group at δ 3.93 ppm and two aromatic singlets at δ 7.13 & 679 respectively. The mass spectrum of isolated Scopoletin showed an M-1 peak at 191.10 indicating that its molecular weight the same, 192.10.

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Review Of Scopoletin: Isolation, Analysis Process and Pharmacological Activity

Adang Firmansyah [1], Wiwin Winingsih [2], Jesicha Dian Yenbyeebe Manobi [3]

[1]Sekolah Tinggi Farmasi Indonesia, adangfirmansyah@stfi.ac.id
[2]Sekolah Tinggi Farmasi Indonesia, winingsih341@gmail.com
[3]Sekolah Tinggi Farmasi Indonesia 3; jesichadymanobi@gmail.com

*corresponding author e-mail address: adangfirmansyah@stfi.ac.id

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