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Review
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Review of Scopoletin: Isolation, Analysis Process, and Pharmacological Activity

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Abstract: Scopoletin (7-hydroxy-6-methoxy coumarin) is a coumarin phenolic compound widely found in plants and includes coumarin derivatives that are superior in several types of plants. This article was created to provide information regarding the isolation process, analysis, and pharmacological activity. The method used is to study and analyze scopoletin articles from national and international journals. From the data sources studied, the yield of scopoletin extract in *Morinda citrifolia* L was 0.93%, *Helichrysum italicum* was 1.933mg / 100g. The scopoletin content in *Convolvulus pluricaulis* is 0.1738%, *Artemisia annua* is 0.3%, Lasianthus lucidus is 54 mg, and *Morus alba* L. (Po-sa) is 0.0009%. The highest yield of scopoletin extract was 0.93% found in noni (*Morinda citrifolia* L) using the Soxhlet method. The highest scopoletin content was 0.3% in *Artemisia annua* using column chromatography and recrystallization. Scopoletin identification can be done using Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC), Fourier Transform Infrared Spectrophotometer (FTIR), Nuclear Magnetic Resonance, and Mass Spectrometry. Based on in vitro studies, scopoletin has pharmacological activities, including as an antihepatotoxicity, antibacterial, antifungal, antitubercular, and antioxidant. Pharmacological activities that have been proven *in vivo* are antithyroid, antihypertensive, anti-proliferative, anti-inflammatory, neurological, anti-dopaminergic and anti-adrenergic, antidiabetic, and antihyperuricemic activities. From the various pharmacological activities of scopoletin, it has the potential to be further developed.

Keywords: scopoletin; isolation; analysis; pharmacological activity.

1. Introduction

Scopoletin is phenolic coumarin derived from the phenylpropanoid pathway and can be isolated from various plants [1,2]. The presence of scopoletin was initially detected by thin-layer chromatography (TLC). Its various pharmacological activities have been further described in several studies [3]. Further identification or isolation of scopoletin using several methods, namely the Soxhlet method, reflux, percolation, maceration, and supercritical CO_2 and can be analyzed using Mass spectrometry, high-performance liquid chromatography (HPLC), Nuclear Magnetic Resonance, and Fourier-transform infrared spectroscopy (FTIR).

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 compound scopoletin's pharmacological activity.

2. Source of Scopoletin

Scopoletin is very common in the plant kingdom and can be isolated from various parts of the plant (roots, fruits, leaves, stems, etc.) [1], such as *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) [32], *Clausena excavate* Burm.f. (Pyin-daw-thein) [33].

3. Pharmacological Activity Of Scopoletin

Scopoletin has several pharmacological activities, namely antihepatotoxicity [5], antibacterial [16], antithyroid [20], antifungal [27,34,35], antitubercular [36], antimigratory [37], antihypertensive [38,39], antioxidant [40], antiproliferative [41], antiinflamation [17,42-47], neurological [48-53], antidopaminergic and antiadrenergic [54], Antidiabetic [55], Antihyperuricemic [56]. Pharmacological activities of Scopoletin were tabulated in table 1.

Table 1. Pharmacological Activity Of Scopoletin.

Pharmacologycal act	Dose	Reference
Antihepatotoxicity	Scopoletin can significantly reduce the release of glutamate pyruvate transaminase and sorbitol dehydrogenase by 53% and 58% in the initial carbon tetrachloride poisoning rat liver cells at doses of 1 to 50 M.	[5]
Antibacterial	scopoletin 1,4 µmol / g in bark (200 g dry weight) showed activity as an anti-pseudomonas preparation.	[16]
Antithyroid	Taking Scopoletin (1.00 mg/kg, p.o.) daily for 7 consecutive days can reduce serum thyroid hormone levels and glucose 6 phosphatase and sugar activity.	[20]
Antifungal	Scopoletin has excellent inhibitory activity on ordering AGEs, with an IC50 value of 2.93 µM and an RLAR inhibitory activity with an IC50 value of 22.5 µM.	[27]
	The minimum concentration inhibitory ranges were 0.07 ± 0.00 µg / ml and 0.15 ± 0.00 µg / ml. The antifungal activity of scopoletin can extract some destructive fungi in food.	[34]
	The minimum inhibitory concentration (MIC90) of scopoletin against Candida was 67.22, and 119 µg / mL was effective against Candida. And antifungal activity.	[35]
Antitubercular	Scopoletin at a dose of 40 mg/ml can be used as an anti-tuberculosis drug for Mycobacterium tuberculosis strain H37Rv.	[36]
Antimigratory	Scopoletin 0.58% (w / w) and can inhibit viability, move to MCF-7 cells, and can be developed as an anticancer drug for breast cancer.	[37]
Antihypertensive	Scopoletin 0.46 + 0.05% can significantly reduce blood pressure in hypertensive rats.	[38]
	Scopoletin at doses of 1, 3, and 10 mg/kg decreased IL-4 type I levels, and Scopoletin at doses of 10 mg/kg decreased serum levels.	[39]
Antioxidant	Scopoletin (17.4 µg / mL) showed potential antioxidant activity.	[40]
Anti-proliferative	The anti-proliferative effect of scopoletin on all cancer cell lines (IC50 103 and above 600µg / ml) was reduced.	[41]
Antiinflammation	Scopoletin 0.62µmol / g inhibited nitric oxide (NO) production in a manner dependent on lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cell concentration. It was shown that quinones were induced in 1c1c7 Reductase (QR) hepatocyte culture.	[17]
	Scopoletin 100 mg/kg showed anti-inflammatory activity in rat ear edema caused by croton oil.	[42]

Pharmacologycal act	Dose	Reference
	Scopoletin (0.63-2.50 g / kg) is a potential preventive and therapeutic agent for inflammatory gastroesophageal diseases, mainly through its antisecretory and promoter activities, including serotonin inhibition, free radicals, and inflammation-mediated cytokine effects.	[43]
	Scopoletin showed significant activity on LDL oxidation (IC50 = 10.2μM), and exerted a vascular anti-inflammatory effect on human endothelial cells EA.hy926 activated by TNF-α.	[44]
	Scopoletin (2.0, 10.0, 50.0 mg/kg) demonstrated that inhibition of nuclear factor-κB and mitogen-activated protein kinase signaling pathways involved in anti-inflammatory activity and regulation of the excitatory/inhibitory balance may be associated with anxiolytic effects.	[45]
	It has an inhibitory effect on HNE (human neutrophil elastase) activity, with IC50 values ranging from 3.6 to 74.3 μM.	[46]
	Scopoletin can prevent oxidative stress and apoptosis, as well as activate Nrf2 signaling.	[47]
Neurological	Scopoletin (10 mg/kg, po) exhibits effects such as antidepressant depending on serotonergic drugs (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenergic receptors), and dopaminergic drugs (dopamine D1 and D2 receptors).	[48]
	Scopoletin, which is confirmed to be the main component of the extract, is a GABA-T inhibitor (IC50 = 10.57 M).	[49]
	The decrease in Bid, Bax, and caspase-9 expression induced by scopoletin will decrease cleaved caspase-3 expression, inhibit cleaved PARP expression, and ultimately inhibit the mitochondrial apoptotic pathway.	[50]
	Scopoletin 100 mg/kg indicates that Scopoletin provides neuroprotection, reduces neuronal apoptosis, and improves neuronal autophagy.	[51]
	The IC50 concentrations of scopoletin that inhibited the AChE and BuChE enzymes were 5.34 and 9.11μM, respectively.	[52]
	Initial treatment of SH-SY5Y cells with 5 mM scopoletin can prevent H_2O_2 induced cell death and reduce levels of apoptotic cells and ROS.	[53]
Antidopaminergic and Antiadrenergic	The baseline dose for Eastern mice (<200 μg / ml) showed anti-dopaminergic and anti-adrenergic activity in mice.	[54]
Antidiabetic	Scopoletin has a diabetes-stimulating effect, can stimulate GLUT4 transport, and regulate glucose uptake via plasma membrane activation of PI3K and AMPK pathways in 3T3-L1 adipocytes.	[55]
Antihyperuricemic	After continuous oral administration, Scopoletin exhibits a weak urate reduction effect. Sphingolipids have an inhibitory effect on XOD activity in serum and liver.	[56]

4. Physicochemical Properties Of Scopoletin

Scopoletin is also known as the pigment 7-hydroxy-6-methoxychromen-2-one (Figure 1), is a simple coumarin group derived from 1,2-benzopyrones found in higher plants, synthesized from a common phenylpropanoid pathway [3]. Scopoletin has several synonyms: Gelseminic acid, Chrysatropic acid, Scopoletine, 6-Methylesculetin, Murrayetin, and 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 [57]. Scopoletin's solubility study found that it can be miscible in organic solvents such as ethanol, Dimethyl sulfoxide (DMSO), and dimethylformamide (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 [58]. Scopoletin is soluble in acetonitrile [1], methanol, ethyl acetate, and N-hexane [59]. Scopoletin is slightly soluble in water and slightly soluble in aqueous buffers. DMF should be added to the scopoletin

aqueous solution to enhance its solubility in the buffered solution. Scopoletin has a solubility of about 0.2 mg / ml in DMF: PBS (pH 7.2) 1: 4 [58].

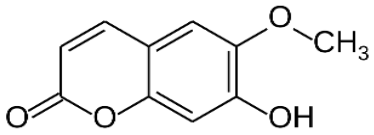

Figure 1. Chemical Structure of Scopoletin.

5. Isolation and Analysis Of Scopoletin

Scopoletin compounds can be obtained by the isolation method. A system, a traditional separation procedure for the continuous extraction of fresh or dry plant material with increasing polarity solvents. For this, the solvents methanol, acetonitrile, ethanol, hexane are usually used. This is because these compounds are relatively easy to dissolve in these compounds. Recently, many studies have used methanol [16,61], aqueous acetonitrile (1: 5 v / v) [1] or various types of solvents (hexane, ethanol and methanol) [62]. A detailed description of the multistep method for extraction and purification of scopoletin has recently been presented from several plants (Table 2), including the stem of *Artemisia annua* [1], *Helichrysum italicum* [8], *Lasianthus lucidus* Blume [16], *Morus alba* L. (Po-sa) [32], *Morinda citrifolia* L [62], *Convolvulus pluricaulis* [81].

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	1.933 mg / 100 g from the H. italicum flower	[8]
Maceration. The extract MeOH was 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	54mg	[16]
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: H_2O (2:98).	The 95% ethanol extract was extracted with pet-ether (60C-80 ° C).	0,0009%	[32]
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%	[62]
Extract at reflux 80-85C for 1-2 hours. The extract was examined with HPLC.	Methanol (99%), 50% alcohol, and water.	0,1738%	[81]

Scopoletin has been observed for decades. Identification and quantification are carried out in a variety of ways, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GC-MS) [3]. The most

common method used to filter fractions from the separation process is thin layer chromatography (Table 3) [10,16,21,27,28,30,33,63,64,65]. When a thin layer chromatography (TLC) plate was developed under ultraviolet irradiation with a wavelength of 365 nm, Scopoletin emitted blue fluorescence [66].

Table 3. Identification method of Scopoletin identification by TLC.

Mobile Phase	Stationary Phase	Retention Factor (Rf)	Reference
$C_4H_8O_2$: CH_3COOH : CH_2O_2 : H_2O (10: 0,5: 0,5: 1,5)	Silica Gel 60F_{254} (Merck, Darmstadt, Germany)	0,78	[10]
C_6H_{14} : $C_4H_8O_2$ (7: 3)	Silica Gel 60 F_{254} (20 × 20 cm; Merck)	0,5	[16]
C_7H_8 : $C_4H_8O_2$: CH_2O_2 (5: 4: 1)	Silica Gel G as adsorbant	0,47	[21]
$CHCl_3$: C_2H_3N (2: 1)	Silica Gel 60F_{254} (Merck, Darmstadt, Germany)	0,75	[27]
C_6H_6 : C_2H_5OH (100 : 22)	Silica Gel impregnated with a fluorescent dye.	0.42	[28]
C_6H_{14} : $C_4H_8O_2$ (7: 3)	Silica Gel 60F_{254}	0,42	[30]
PE: EtOAc (1: 2)	Silica Gel (40-60 µm, Wakogel)	0,5	[33]
CH_2Cl_2 : CH_3OH (19: 1)	Silica Gel 60F_{254} (Merck, Darmstadt, Germany)	0,5	[63]
$CHCl_3$: CH_3OH (9: 1) or $CHCl_3$: CH_3OH : H_2O (65: 30: 5)	Silica Gel 60F_{254} (Merck, Darmstadt, Germany)	0,53	[64]
$C_4H_8O_2$: CH_3OH : H_2O (100: 6: 4)	Silica Gel 60 G and water at the ratio of 1:1	0.91	[65]

High-performance chromatography (HPLC) with different detection methods has been used for purification (preparative). It has been widely used to measure and quantify (analysis) Scopoletin (Table 4) [17,25,37,61,67-74].

Table 4. Analytical Method of Scopoletin by HPLC**.**

UV Detection	Mobil phase	Flow Rate (ml/min)	Rt (min)	Column	Reference
254 nm	0,1% formic acid in water (eluant A) and ACN (eluant B)	1,0	12.5	C18 (250 × 4.6 mm, 5 µm)	[17]
350 nm	CH3COONa: C_2H_3N (80:20)	1.0	5.497	C18 (4,6 mm × 150 mm, 5 µm)	[25]
254 nm	0,01M acetic acid: acetonitrile: methanol (60:20:20)	1,0	4.3	C18 (250 × 4.6 mm, 5 µm)	[37]
366 nm	CH_3OH: H_2O (0.1 %v/v HCOOH); 3:7	1.0	19.579	C18 (8 mm × 4 mm, 5 µm)	[61]
345 nm	Glacial acetic acid (0.5%) : Methanol (26%) : Deionised water (55%)	1.0	16.35	(C18) (250 mm × 4.60 mm, 5 µl)	[67]
345 nm	CH_3OH and H_2O (49:51, v/v); 0.05% (v/v) H_3PO_4	1.0	5.1	C18 (4,6 mm × 150 mm, 5 µm)	[68]
300 nm	55: 45 (% v/v) CH_3OH: H_2O (0.1% CH_3COOH)	1.0	4.6	C18 (250 mm × 4.6 mm, 5 µm)	[69]
220 nm	CH_3OH: H_2O (0.05% HCOOH)	1.0	7.87	C18 (250 mm × 4.6 mm, 5 µm)	[70]
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	C18 (250 × 212 mm, 15 µm)	[71]
350 nm	0,01 M sodium acetate: acetronitrile (80:20, v / v); isokratik	1,0	5.585	C18 (4.6 mm × 150 mm, 5.0 µm)	[72]
280 nm	0,1% (v / v) Trifluoroacetic acid in water (A) dan 100% acetronitrile (B); gradien	1,0	11.812	C18 (250 × 4.6 mm, 5 µm)	[73]

UV Detection	Mobil phase	Flow Rate (ml/min)	Rt (min)	Column	Reference
230 nm	water (A) and methanol (B) both contain 0.1% TFA: 30-65% B; gradien	1,0	13.28	ACE prep-column (150 × 21.2 mm, 10 µm)	[74]

6. 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 literature was 103 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.

7. Results and Discussion

Scopoletin (Figure 1) includes simple coumarin, which is derived from 1,2-benzopyrone in plants high in the common plant phenylpropane pathway. The coumarin structure (2H-1-benzopyran-2-one) is formed by ortho-hydroxylation of cinnamic acid, trans/cis side-chain isomerization, and lactonization. O-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 [27,34,35], antitubercular [36], anti-migratory [37], antihypertensive [38,39], antioxidant [40], antiproliferative [41], antiinflamation [17,42,43,44,45,46,47], neurological [48,49,50,51,52,53], antidopaminergic and antiadrenergic [54], antidiabetic [55], antihyperuricemic [56]. In this research [5] scopoletin can significantly reduce the release of glutamate pyruvate transaminase and sorbitol dehydrogenase by 53% and 58% in the initial carbon tetrachloride poisoning of rat liver cells at doses of 1 to 50 M. Scopoletin 1,4 µmol / g in bark (200 g dry weight) showed activity as an anti-pseudomonas preparation [16]. Taking Scopoletin (1.00 mg/kg, p.o.) daily for 7 consecutive days can reduce serum thyroid hormone levels and glucose 6 phosphatase and sugar activity [20]. Scopoletin has excellent inhibitory activity on ordering AGEs, with an IC50 value of 2.93 µM and an RLAR inhibitory activity with an IC50 value of 22.5 µM [27]. The minimum concentration inhibitory ranges were 0.07 ± 0.00 µg / ml and 0.15 ± 0.00 µg / ml. The antifungal activity of scopoletin can extract some destructive fungi in food [34]. The minimum inhibitory concentration (MIC90) of scopoletin against Candida was 67.22, and 119 µg / mL was effective against Candida. And antifungal activity [35]. At a dose of 40 mg / ml, scopoletin can be used as an anti-tuberculosis drug for Mycobacterium tuberculosis strain H37Rv [36]. Scopoletin 0.58% (w / w) can inhibit viability, move to MCF-7 cells, and be developed as an anticancer drug for breast cancer [37]. Scopoletin 0.46 + 0.05% can significantly reduce blood pressure in hypertensive rats [38]. Scopoletin at doses of 1, 3, and 10 mg/kg decreased IL-4 type I levels, and Scopoletin at doses of 10 mg/kg decreased serum levels [39]. Scopoletin (17.4 µg/mL) showed potential antioxidant activity [40]. The anti-proliferative effect of scopoletin on all cancer cell lines (IC50 103 and above 600µg / ml) was reduced [41]. Scopoletin 0.62µmol / g inhibited nitric oxide (NO) production in a manner dependent on lipopolysaccharide-induced RAW 264.7 (LPS) macrophage cell concentration. It was shown that quinones were induced in 1c1c7 Reductase (QR) hepatocyte culture [17]. Scopoletin 100 mg/kg showed anti-inflammatory activity in rat ear edema caused by croton oil

[42]. Scopoletin (0.63-2.50 g/kg) is a potential preventive and therapeutic agent for inflammatory gastroesophageal diseases, mainly through its antisecretory and promoter activities, including serotonin inhibition, free radicals, and inflammation-mediated cytokine effects [43]. Scopoletin showed significant activity on LDL oxidation (IC50 = 10.2μM), and exerted a vascular anti-inflammatory effect on human endothelial cells EA.hy926 activated by TNF-α [44]. Scopoletin (2.0, 10.0, 50.0 mg/kg) demonstrated that inhibition of nuclear factor-κB and mitogen-activated protein kinase signaling pathways involved in anti-inflammatory activity and regulation of the excitatory/inhibitory balance may be associated with anxiolytic effects [45]. It has an inhibitory effect on HNE (human neutrophil elastase) activity, with IC50 values ranging from 3.6 to 74.3 μM [46]. Scopoletin can prevent oxidative stress and apoptosis, as well as activate Nrf2 signaling [47]. Scopoletin (10 mg/kg, po) exhibits effects such as antidepressant depending on serotonergic drugs (5-HT2A receptors), noradrenergic systems (α1- and α2-adrenergic receptors), and dopaminergic drugs (dopamine D1 and D2 receptors) [48]. Scopoletin, which is confirmed to be the extract's main component, is a GABA-T inhibitor (IC50 = 10.57 M) [49]. The decrease in Bid, Bax, and caspase-9 expression induced by scopoletin will decrease cleaved caspase-3 expression, inhibit cleaved PARP expression, and ultimately inhibit the mitochondrial apoptotic pathway [50]. Scopoletin 100 mg/kg indicates that Scopoletin provides neuroprotection, reduces neuronal apoptosis, and improves neuronal autophagy [51]. The IC50 concentrations of scopoletin inhibited the AChE and BuChE enzymes were 5.34 and 9.11μM, respectively [52]. Initial treatment of SH-SY5Y cells with 5 mM scopoletin can prevent H_2O_2 induced cell death and reduce levels of apoptotic cells and ROS [53]. The baseline dose for Eastern mice (<200 μg/ml) showed anti-dopaminergic and anti-adrenergic activity in mice [54]. Scopoletin has a diabetes-stimulating effect. It can stimulate GLUT4 transport and regulate glucose uptake via plasma membrane activation of PI3K and AMPK pathways in 3T3-L1 adipocytes [55]. After continuous oral administration, Scopoletin exhibits a weak urate reduction effect. Sphingolipids have an inhibitory effect on XOD activity in serum and liver [56].

The separation process begins with an extraction process designed to attract the scopoletin compounds present in the plant. According to several studies, the most widely used solvent is methanol. This is because these compounds are relatively easy to dissolve in these compounds. The first extraction process in traditional separation is usually carried out by immersion method for ± 72 hours. The solvent used enters the cell through the simple cell wall containing scopoletin. The cell content is dissolved due to differences. The concentration between the solution inside and outside the cell. The high concentration solution will be added and replaced with a low concentration solvent (diffusion process). Other extraction methods (such as the Soxhlet extraction method) have been carried out at temperatures of 40 ° C to 60 ° C, and the reflux method has been used. The extraction method, with the help of heating, will speed up the dissolving process. After heating, the particles at high temperatures will move faster than at low temperatures. The contact between the solute and the solvent is more effective. Other extraction methods include using supercritical CO2 at 80 ° C to 85 ° C for 1-2 hours. The Soxhlet extraction method was used 0.93% to obtain the extraction results [62] from the process of extracting scopoletin compounds. As observed from the scopoletin separation process results in Table 2, the number of isolates obtained was significantly different. Separate 0.3% *Artemisia annua* stems by diafiltration for 6-8 hours to obtain isolates, then using column chromatography and dissolving scopoletin in a methanol-chloroform mixture to obtain extract [1].

Analytical methods that can assist the identification process of scopoletin compounds include thin layer chromatography as initial identification. Ultraviolet-visible spectrophotometer and HPLC can be used for further identification. High-performance liquid chromatography can identify target compounds based on the following principle: when a sample moves through the stationary phase (which can be either solid or liquid), the sample will be taken up by the mobile phase (it can be a liquid or a gas). Analytical methods that contribute to the process The various components in the sample are separated according to their different affinities to the stationary phase. Components that can interact strongly with the stationary phase move more slowly, so they can be separated from other components that interact weakly. In addition, several complementary identifications must be carried out to identify the structure of the compounds obtained from the separation process, for example, using infrared-FT, nuclear magnetic resonance, and mass spectrometry.

TLC (Thin Layer Chromatography) is a qualitative test to determine the purity of a compound. Based on a number of studies (Table 3), good thin layer chromatography results were obtained, namely the ratio of $C_4H_8O_2$: CH_3OH: H_2O (100: 6: 4), and an Rf value of 0.91 [65]. For several compounds in multiple eluents, the Rf value is very typical. The Rf value is the ratio of the eluent distance to the mobile phase on the TLC plate. Compounds with a greater Rf show lower polarity, and vice versa. This is because the stationary phase is polar. The more polar compounds are held firmly in the stationary phase, resulting in a lower Rf value. A good TLC Rf is 0.2 to 0.8. If Rf is too high, all you have to do is reduce the eluent's polarity and vice versa. The value of Rf can be used as proof of multiple identifications. If the Rf value has the same value, it can be said that the compound has the same or similar properties as a comparison. The success of the separation depends on the difference in the components' solubility to be separated in the solvent [45]. Most of the coumarin compounds are active against ultraviolet rays. This is because coumarin has a conjugated double bond. It is known that ultraviolet absorbent rays can either absorb conjugated bonds or have a chromophore. Scopoletin is a coumarin compound, showing blue fluorescence when exposed to 365 nm of ultraviolet light [75]. Staining under 365 nm ultraviolet light indicates that the compound has at least two conjugated double bonds. This appearance is due to the strength of the interaction between ultraviolet light and the chromophore which is bound by additional pigments present in the dye. Visible light fluorescence is the light emitted by these components when electrons are excited from a basic energy level to a higher energy level and then return to their original state when the energy was released. Hence, the visible stain on UV lamps looks very bright due to silica. The gel used does not fluoresce under 365 nm ultraviolet light [76].

HPLC stands for high-performance liquid and can be described as a method of separating molecules from a liquid medium that is subjected to high pressure. The function of HPLC is to determine or measure or analyze the levels of active ingredients in a sample (drugs, food, or herbs). Based on the results of several studies (Table 4), aroma pole analysis (2013) Vipul et al. (2013) showed that the retention time of scopoletin was 19,583, which indicated that the compounds of scopoletin and standard scopoletin were the same, namely the retention time of 19,579 minutes [46].

Table 5. The data from the FTIR spectrum of standard Scopoletin.

Peaks (cm^{-1})	Functional group	Reference
3337.44	O-H Alcohol group present	
2850.97	C-H group present	
1702.90	Carbonyl C=O group present	

Peaks (cm^{-1})	Functional group	Reference
1628.09	CH=CH group present	[21]
1565.06	Benzene ring present	
1510.53	Benzene ring present	
861.46	Due to disubstitution of benzene	

Fourier transformed infrared (FTIR) spectroscopy can quickly measure functional groups without damage and is able to analyze several components simultaneously. Basically, FTIR spectroscopy is the same as dispersion IR spectroscopy. What distinguishes it is the development in the optical system before the infra-red beam passes through the sample. Table 5 shows the data obtained from standard FTIR spectroscopy and possible scopoletin functional groups. In infrared spectrum analysis [21], the peak was at 3341.44 cm^{-1}, which is the result of the OH alcohol group present, the peak of 2875.05 is the result of the CH group present, the peak of 1703.42 is the result of the carbonyl C = O group present, at the peak of 1606.75 is the result of the CH = CH group present, and at the peak of 1568.83; 1511.16; 861.50 is the result of the benzene ring present. In the study [30] infrared spectrum analysis, the peak at 3318 was the result of the OH alcohol group present, the peak 2926-290 was the result of the CH group present, the peak 1698 was the result of the carbonyl C = O group present, the peak 1602 was the result of the CH = CH group present, as well as the peaks 1567.68 and 1517.01 are the results of the benzene ring present. In a study [32] infrared spectrum analysis, peaks at 3325 (υO-H), 3055 (υC-H of C = CH), 2920 (υC-H of –CH3, CH2), 2850 (υC-H of –OCH3), 1705 (υC = O) of δ lactone, 1604 (υC = C of aromatic ring). In a study [33] infrared spectrum analysis, peaks at 3325 (υOH of the alcoholic group), 3001 (υ= CH stretching), 2848 (υC-H stretching of CH3), 1705 (υC = O stretching), 1612; 1566; 1504 (υC = C stretching of cumarone and benzene), 1427 (υC-H of CH3 group), 1288; 1134 (υC-O stretching of OH), and 864 (Disubstitution of benzene).

Table 6. The data from the NMR spectrum of standard Scopoletin.

Standard Scopoletin			
No. of H atom	**δ value, ppm**	**Integration, Multiplicity (J, HZ)**	**Reference**
3	6.23	1H, d(9.2)	[21]
4	7.88	1H, d(9.6)	
5	7.14	1H, S	
8	6.79	1H, S	
C-6-OMe	3.93	3H, S	

Table 6 shows the data obtained from the scopoletin NMR spectrum standard. In the study [21] the ^1H NMR spectrum showed two double peaks, and the coupling constant at δ 6.22 and 7.88 ppm was 9.2 Hz, defined as H-3 and H-4, respectively. At δ 7.13 and 6.79 ppm showed two single aromatic peaks defined as H-5 and H-8, and at δ 3.93 ppm showed methoxy groups. In the study [32] the ^1H NMR spectrum showed two double peaks, and the coupling constant at δ 6.2 and 7.6 ppm was 9 Hz, defined as H-3 and H-4, respectively. At δ 6,8 and 6,9 ppm showed two single aromatic peaks defined as H-5 and H-8 and at δ 3,7 ppm showed methoxy group. The study [77] ^1H NMR spectrum showed two double peaks, and the coupling constant at δ 6.22 and 7.88 ppm was 9.5 Hz, defined as H-3 and H-4, respectively. At δ 7.12 and 6.79 ppm showed two single aromatic peaks defined as H-5 and H-8, and at δ 3.97 ppm showed methoxy groups. The study [78] ^1H NMR spectrum showed two double peaks, and the coupling constant at δ 6.09 and 7.83 ppm was 9.5 Hz, defined as H-3 and H-4, respectively. At δ 7.10 and 6.65 ppm showed two single aromatic peaks defined as H-5 and H-8, and at δ 3.77 ppm

indicated a methoxy group. In the study [79] the ^{1}H NMR spectrum showed two double peaks, and the coupling constant at δ 6.01 and 7.69 ppm was 12 Hz, defined as H-3 and H-4, respectively. At δ 6.86 and 6.61 ppm showed two single aromatic peaks defined as H-5 and H-8 and at δ 3.81 ppm indicated a methoxy group. In the study [80] the ^{1}H NMR spectrum showed two double peaks, and the coupling constant at δ 6.18 and 7.50 ppm was 9.2 Hz, defined as H-3 and H-4, respectively. At δ 6.77 and 6.84 ppm showed two single aromatic peaks defined as H-5 and H-8, and at δ 3.88 ppm showed methoxy groups.

Table 7. The molecular weight data from the mass spectrum of standard Scopoletin and isolated compound V.

No. Sample	M-1 Peak	Molecular weight	Reference
Standard Scopoletin	191.10	192.10	[21]
Isolated Compound V	191.10	192.10	[21]

Table 7 shows the molecular weight data of the mass spectra of the standard Scopoletin and isolated compounds V. The mass spectrum of the standard compounds separated from Scopoletin and V shows the M-1 peak at 191.10, which indicates the same molecular weight of 192.10. Therefore, it can be determined that the isolated compound V was Scopoletin [21].

8. Conclusions

Based on the literature review results, scopoletin can be extracted from *Morinda citrifolia* L, *Helichrysum italicum*, *Convolvulus pluricaulis*, *Artemisia annua*, Lasianthus lucidus, and *Morus alba* L. The highest yield of scopoletin extract was found in noni (*Morinda citrifolia* L) namely 0.93% using the Soxhlet extraction method. The isolation process of scopoletin from *Artemisia annua* by column chromatography followed by recrystallization gave the highest isolate yield that was 0.3%. Scopoletin identification can be made using Thin Layer Chromatography (TLC), High-Performance Liquid Chromatography (HPLC) where the HPLC system of 0,01M acetic acid: acetonitrile: methanol (60:20:20) mobile phase, UV detector at 254 nm, C18 (250 × 4.6 mm, 5 μm) column, 1 ml/min flow rate has the fastest analytical time among other HPLC system. Fourier Transform Infrared Spectrophotometer (FTIR), Nuclear Magnetic Resonance, and Mass Spectrometry can be used to identify. Based on in vitro studies, scopoletin has pharmacological activities, including as an antihepatotoxicity, antibacterial, antifungal, antitubercular, and antioxidant. Pharmacological activities proved *in vivo* antithyroid, antihypertensive, anti-proliferative, anti-inflammatory, neurological, anti-dopaminergic and anti-adrenergic, antidiabetic, and antihyperuricemic activities.

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Conflicts of Interest

The authors declare no conflict of interest.

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