Scopoletin: a review of its source, biosynthesis, methods of extraction, and pharmacological activities

Abstract: Scopoletin, also known as 6-methoxy-7 hydroxycoumarin, is one of the naturally occurring coumarin commonly found in many edible plants and plays an important role in human health. Despite the various potential pharmacological properties, the biosynthesis process, method of extraction, and mechanism of action on this compound have not been documented well. In this current review, the biosynthesis pathway, distribution of scopoletin in the plant kingdom, and extraction techniques are elaborated. The in vitro, in vivo, and in silico pharmacological studies are also discussed on antioxidant, antimicrobial, anticancer, anti-inflammation, and neuroprotective aspects of scopoletin. This study may help to understand the benefit of scopoletin containing plants and would be beneficial for the prevention and treatment of diseases.

Keywords: biosynthesis; extraction method; scopoletin; source; therapeutic potential.

1 Introduction

Plants have been widely used in folk medicine system. Many plants were recorded to have medicinal properties and provide a substantial therapeutic effect for the treatment of several ailments including infectious diseases. Natural product is also considered as a potential source for discovery and development of new drug. It was recently found an increase in the use of natural-based products in healthcare systems in many countries, either developed or developing countries. Natural-based product has also attracted attention by researcher due to its advantages. Moreover, the use of natural products in health system promotes a significant role in pharmaceutical industry, because chemical compounds are more expensive compared to herbal [1]. It has been reported that by the end of 2013, 547 natural products have been approved by FDA, in which more than 40% of FDA-approved natural products are derived from plants [2].

Coumarin is a secondary metabolite that is characterized as 1,2 benzopyrones. This compound is distributed in plants and fungi [3]. Scopoletin, also known as 6-methoxy-7-hydroxycoumarin, is one of the naturally occurring coumarin commonly found in many edible plants and plays an essential role in human health [4]. Structurally, scopoletin is identified to have two aromatics rings supplemented with a hydroxyl group substitution together with a methoxy group and one oxo group (Figure 1).

In nature, the presence of scopoletin is often related to the defence mechanism of plant towards infection by parasite and microbial [5]. This compound has been isolated from a wide range of medicinal plants [6]. Scopoletin can be easily detected since it gives off fluorescent characteristics under UV light. Various biological properties of this compound have also been elucidated [4]. The present study was undertaken to review scopoletin, including sources, extraction, biosynthesis, as well as its pharmacological properties.

2 Biosynthesis, source distribution, and extraction of scopoletin

A number of studies have been published in elucidating the biosynthetic pathways of scopoletin at a molecular level [7–10]. However, despite the importance of coumarin and its derivatives, including scopoletin, in plant and human life, the biosynthesis of these active compounds remains poorly documented and may differ between species. Scopoletin has modifications in its benzene ring, and genetic studies in Arabidopsis thaliana support that scopoletin is
biosynthesized from the phenylpropanoid pathway via ortho-hydroxylation of cinnamate, \( p \)-coumarate, caffeate, and ferulate [7, 8]. Phenylpropanoid pathway serves as a starting point for the production of many important compounds, including lignan, flavonoids, and coumarins [11].

In the very first step of phenylpropanoid pathway, phenylalanine ammonia-lyase (PAL; EC 4.3.1.5) catalyses the deamination of phenylalanine to yield \( \text{trans-cinnamic acid} \). Later, cinnamate-4-hydrocylase (C4′H; EC 1.14.13.11) catalyses the hydroxylation of cinnamate to yield \( \text{\( p \)-coumarate} \) (also known as \( 4\)-coumarate). C4′H is a cytochrome P450-dependent monooxygenase that also involved in lignin biosynthesis [12]. In a work carried out by Vanholme et al. [13], C4′H mutant line displayed significantly reduced growth rate and lignin accumulation, suggesting the importance of this enzyme in the phenylpropanoid metabolism. CoA ligase (\( 4\)-CL; EC 6.2.1.3) transforms \( \text{\( p \)-coumarate} \) into \( \text{\( p \)-coumaroyl-CoA} \), a precursor of downstream metabolites, which is esterified with shikimic or quinic and then enters the biosynthesis pathway (Figure 2). Besides the \( \text{\( p \)-coumaroyl-CoA} \), caffeoyl-CoA and feruloyl-CoA may also be used as substrates for scopoletin biosynthesis. Mutation in \( \text{CCoAOMT1} \) gene which encodes caffeoyl CoA O-methyltransferase 1 (EC 2.1.1.104), revealed to decrease the level of scopoletin in \( \text{ccoaomt1} \)-repressed \( \text{A. thaliana} \) roots compared to wild-type roots [8]. This mutation affects the production of feruloyl-CoA, which is catalysed from caffeoyl-CoA by caffeoyl CoA O-methyltransferase, moreover, indicating the importance of \( \text{CCoAOMT1} \) gene for scopoletin biosynthesis. In \( \text{A. thaliana} \), feruloyl-CoA is subjected to ortho-hydroxylation catalysed by feruloyl CoA-6′-hydroxylase (F6′H; EC 1.14.11.61), which then the 6′-hydroxyferuloyl-CoA undergoes trans-cis spontaneous isomerization and non-enzymatic lactonization to form scopoletin [9]. Some studies revealed that mutation in \( \text{f6′h1} \) gene which is responsible for F6′H extremely reduced fluorescence signal of scopoletin in \( \text{A. thaliana} \) roots, indicating that F6′H is 2-oxoglutarate dependent dioxygenase essential and specific for scopoletin synthesis in \( \text{A. thaliana} \) [14].

Scopoletin has been identified at different levels of concentrations across plant parts, in many different species and plant families. Table 1 summarized the presence of scopoletin in various botanical families, such as \( \text{Asteraceae} \), \( \text{Convolvulaceae} \), \( \text{Rubiaceae} \), \( \text{Solanaceae} \), and \( \text{Moraceae} \).

Several extraction methods have been developed to extract scopoletin from different plants and natural products. The most commonly used method is traditional maceration with methanol [14, 30], aqueous methanol.
### Table 1: Scopoletin in various species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Family</th>
<th>Organ</th>
<th>Scopoletin content</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acer saccharum Marsh.</td>
<td>Sapindaceae</td>
<td>Leaf and stcker wood</td>
<td>161.9 nmol/g FW</td>
<td>[15]</td>
</tr>
<tr>
<td>Aegle marmelos</td>
<td>Rutaceae</td>
<td>Fruit</td>
<td>0.014%</td>
<td>[16]</td>
</tr>
<tr>
<td>Arabidopsis thaliana</td>
<td>Brassicaceae</td>
<td>Shoot</td>
<td>0.0720 nmol/g FW (shoot)</td>
<td>[7, 10, 17]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Root</td>
<td>14.7 nmol/g FW (root)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Callus</td>
<td>3.02 nmol/g FW (callus)</td>
<td></td>
</tr>
<tr>
<td>Argyreia speciosa</td>
<td>Convulvulaceae</td>
<td>Root</td>
<td>2.49% (w/w) DW</td>
<td>[18]</td>
</tr>
<tr>
<td>Artemisia annua</td>
<td>Asteraceae</td>
<td>Leaf</td>
<td>0.04477% DW (leaf)</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Flowering aerial part</td>
<td>0.83% FW/2.78% DW</td>
<td>[20]</td>
</tr>
<tr>
<td>Artemisia iwayomogi</td>
<td>Asteraceae</td>
<td>Leaf</td>
<td>204 mg/g</td>
<td>[21]</td>
</tr>
<tr>
<td>Atractylodes macrocephala</td>
<td>Asteraceae</td>
<td>Root</td>
<td>5.478 μM</td>
<td>[22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole herb</td>
<td>4.5 mg/g extract</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aerial part</td>
<td>0.062 μg/mL</td>
<td>[24]</td>
</tr>
<tr>
<td>Clitoria ternatea</td>
<td>Fabaceae</td>
<td>Aerial part</td>
<td>0.190 μg/mL</td>
<td>[24]</td>
</tr>
<tr>
<td>Convolvulus pluricaulis</td>
<td>Convolvulaceae</td>
<td>Whole plant</td>
<td>0.1738%</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aerial part</td>
<td>0.252 μg/mL</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf</td>
<td>0.324 μg/mL</td>
<td>[24]</td>
</tr>
<tr>
<td>Evolvulus alsinoides</td>
<td>Convolvulaceae</td>
<td>Aerial part</td>
<td>0.01–0.55% (aerial parts)</td>
<td>[26]</td>
</tr>
<tr>
<td>Fabiana imbricata</td>
<td>Solanaceae</td>
<td>Leaf, petioles, stem,</td>
<td>≤5 nmol/g FW (leaf, petioles,</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>underground stem, tuber tissue</td>
<td>stem, tuber tissue</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Leaf and stem bark</td>
<td>±25 nmol/g FW (underground stem)</td>
<td></td>
</tr>
<tr>
<td>Houttuynia cordata</td>
<td>Saururaceae</td>
<td>Whole plant</td>
<td>0.001–0.016%</td>
<td>[29]</td>
</tr>
<tr>
<td>Ipomoea batatas</td>
<td>Convolvulaceae</td>
<td>Leaf, petioles, stem,</td>
<td>&lt;5 nmol/g FW (leaf, petioles,</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>underground stem, tuber tissue</td>
<td>stem, tuber tissue</td>
<td></td>
</tr>
<tr>
<td>Lasianthus lucidus</td>
<td>Rubiaceae</td>
<td>Leaf and stem bark</td>
<td>1.4 μmol/g DW</td>
<td>[30]</td>
</tr>
<tr>
<td>Lycium barbarum</td>
<td>Solanaceae</td>
<td>Fruit</td>
<td>8 mg/kg DW</td>
<td>[31]</td>
</tr>
<tr>
<td>Manihot esculenta</td>
<td>Euphorbiaceae</td>
<td>Roots</td>
<td>3.5–52.9 nmol/g FW</td>
<td>[32]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9.8–137.9 nmol/g FW</td>
<td></td>
</tr>
<tr>
<td>Melia azedarach</td>
<td>Meliaceae</td>
<td>Kernel of ripe fruit</td>
<td>0.018 g/kg of seed kernel</td>
<td>[33]</td>
</tr>
<tr>
<td>Morinda citrifolia</td>
<td>Rubiaceae</td>
<td>Fruit</td>
<td>24.26–65 ppm (μg/g FW)</td>
<td>[34]</td>
</tr>
<tr>
<td>Morinda tinctoria</td>
<td>Rubiaceae</td>
<td>Leaf</td>
<td>1.58% (w/w) DW</td>
<td>[35]</td>
</tr>
<tr>
<td>Morus alba</td>
<td>Moraceae</td>
<td>Fruit</td>
<td>53.1–220.5 μg/g</td>
<td>[36]</td>
</tr>
<tr>
<td>Morus atropurpurea</td>
<td>Moraceae</td>
<td>Fruit</td>
<td>33.5–998.3 μg/g</td>
<td>[36]</td>
</tr>
<tr>
<td>Morus laevigata</td>
<td>Moraceae</td>
<td>Fruit</td>
<td>57.6 μg/g</td>
<td>[36]</td>
</tr>
<tr>
<td>Pelargonium sidoides</td>
<td>Geraniaceae</td>
<td>Root</td>
<td>34–48 mg/kg</td>
<td>[37]</td>
</tr>
<tr>
<td>Scopolia carnolica</td>
<td>Solanaceae</td>
<td>Leaf underground parts</td>
<td>0.04–0.07% (w/w) DW</td>
<td>[38, 39]</td>
</tr>
<tr>
<td>Solanum xanthocarpum</td>
<td>Solanaceae</td>
<td>Callus</td>
<td>1.66–2.28 mg/g DW</td>
<td>[40]</td>
</tr>
<tr>
<td>Synephrus-excelsus</td>
<td>Asteraceae</td>
<td>Flower</td>
<td>3.41 mg/g extract</td>
<td>[41]</td>
</tr>
<tr>
<td>Weigela sp.</td>
<td>Caprifoliaceae</td>
<td>Leaf</td>
<td>40–280 mg/kg of plant</td>
<td>[42]</td>
</tr>
</tbody>
</table>

[23, 31, 41], ethanol [43, 44], and aqueous ethanol [45–47]. Other organic solvents such as dichloromethane and ethyl acetate were also utilized as extracting agents [34, 48].

Soxhlet extraction method with various organic solvents such as ethanol [23, 24], methanol [18, 49], hexane [50], and dichloromethane [26] was also frequently used to extract scopoletin, as well as reflux extraction with methanol [19, 51] and ethanol [52]. On the other hand, extraction using ultrasonication technique generally requires less extraction time compared with maceration or soxhlet extraction that typically lasts for several hours or days. Wang et al. [53] performed the extraction of 12 coumarins including scopoletin from Bamboo leaves with ethanol in 30 min by ultrasonic extraction. Several publications also reported the extraction of scopoletin with...
ultrasonic method using ethanol or aqueous ethanol [54–56], as well as methanol [37, 40, 57].

One particularly interesting research is the comparison of several scopoletin extraction methods from *Convolvulus pluricaulis* reported by Tatke and Rajan [58]. In this study, the performance of conventional extraction methods like soxhlet and reflux extraction were evaluated along with novel extraction techniques such as ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), and supercritical fluid extraction (SFE). According to the study, the highest yield of scopoletin was obtained by MAE (45.1% yield), followed by UAE (yielded 23.55 and 31.87% for probe and bath ultrasonicator, respectively), reflux (10.95% yield), soxhlet (9.58% yield), and SFE (7.99% yield). Overall, novel extraction methods were found to be better than conventional ones, since they require less organic solvent and give higher yields of scopoletin in a shorter time. While SFE yielded lower scopoletin than other extraction methods, but it has major advantage as an environmentally friendly technique. It is considered as green technology due to its ability to produce extract with little or no organic solvent. The most frequently used extraction agent in supercritical fluid extraction is carbon dioxide (CO2), since it is non-flammable, non-toxic, and has low critical temperature which is critical feature in extracting heat sensitive and reactive compounds. It is also easily separated from the extract without leaving solvent residue in the product. Jokić et al. [27] employed SFE with CO2 for the extraction of scopoletin from *Helichrysum italicum* flowers with yield 6.31%.

Shifflett et al. [59] performed the extraction of scopoletin and five other polyphenols in tobacco leaves and tobacco products by using pressurized liquid extraction (PLE) technique. Similarly, Jamaludin et al. [60] reported the application of high hydrostatic pressure (HHP) extraction with ethanol solvent to extract three bioactive compounds including scopoletin from noni fruits. The study highlights the use of ambient temperature for the extraction process to minimize the degradation of thermally labile compounds.

A new pressurized cyclic solid–liquid (PCSL) extraction technique was utilized by Zarrelli et al. [20] for the extraction of scopoletin and artemisinin from *Artemisia annua*. The study demonstrated that PCSL extraction method is 15 times more effective than conventional maceration in shorter time with little or no use of organic solvents. The method employed only water or hydro-alcoholic solutions as extracting agents.

Another alternative for scopoletin extraction is matrix solid phase dispersion (MSPD), a method commonly used for sample preparation. A study published by Li et al. [61] described the application of matrix solid phase dispersion extraction of scopoletin and four other compounds from *Euphorbia fischeriana*, followed by ultra-performance liquid chromatography coupled with the quadrupole time-of-flight tandem mass spectrometry (UPLC/Q-TOF-MS) analysis. The result of this study shows that MSPD extraction gives higher recovery and improves the detection capability than those of conventional extraction methods, due to its ability of separation and enrichment of the target analytes.

Aside from all the advantages, modern extraction technologies such as supercritical fluid and high-pressure technology may not be feasible for low-budget laboratories, due to the requirement of special equipment associated with high pressure and the need for careful adherence to the specific temperature in the extraction process. For this reason, the conventional extraction method such as traditional maceration is still actively being used.

A number of studies also reported the isolation method for scopoletin. Sethiya et al. [23] used column chromatography with 80–120 mesh silica gel as the adsorbent and chloroform, methanol, and toluene (8:1:1) as the eluent to isolate scopoletin from *Canscora decussata*, followed by further purification by preparative chromatography. Meanwhile, Tripathi et al. [62] employed extraction with aqueous acetonitrile, followed by partition with chloroform, redissolved in methanol, and crystallization to obtain pure scopoletin from *A. annua*. They also utilized column chromatography with 60–120 mesh silica gel as the stationary phase and 4% methanol in chloroform as the eluent to isolate the remaining scopoletin in the filtrate. This method yielded 0.3% scopoletin from stems of *A. annua* with 85% purity. Another isolation method was reported by Shaw et al. [63] who isolate scopoletin from *Sinomonomium acutum*. The concentrated acetone extract of *S. acutum* was partitioned into chloroform soluble fraction, followed by two times column chromatography using 70–230 mesh and 230–400 mesh silica gel with eluent n-hexane/EtOAc mixtures. Further purification by HPLC was applied with a yield around 100 mg from 5 kg of dried stem *S. acutum*. The isolated scopoletin compound was confirmed using the comparison of IR, MS, 1H and 13C NMR spectra data with those in the published literature and reference standard of scopoletin.

The content of scopoletin in the plant extracts was determined mainly using chromatographic methods, such as thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography mass spectrometry (LC-MS), and gas chromatography mass spectrometry (GC-MS). TLC becomes the method of choice for screening of fractions from separation procedures, since it provides a more straightforward and low cost
analysis compared to other analysis methods [64, 65]. Meanwhile, HPLC is widely used for the identification and quantification of scopoletin from plant extracts. HPLC techniques allow accurate determination of a wide range of analytes from small anions to complex biomolecules [66–69], in heterogeneous samples without being isolated in pure form. Both diode array detector [70–73] and fluorescence detector [74] were used as the detector of HPLC to identify and quantify scopoletin in plant extracts. LC-MS [65, 75] and GC-MS [10, 76] provide more accurate and sensitive analysis of scopoletin, and both are usually employed to complement HPLC method to confirm scopoletin in the sample. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was also employed to determine scopoletin in plasma sample [77].

Of all chromatographic approaches for scopoletin analysis, HPLC method is the most commonly used, since it offers a more selective, accurate, and reproducible analysis than TLC. Moreover, it also allows simple sample handling and relatively cheaper analysis cost over GC-MS or LC-MS. Hence, HPLC is more suitable for routine analysis procedures [78].

A non-chromatographic method was also utilized for scopoletin measurement. Thomaz et al. [79] developed an electroanalytical method using modified carbon paste electrode based on solid state differential pulse voltammetry (DPV) to assess the authenticity of noni products. Meanwhile, a screen-printed electrode modified with composite of silver nanoparticles and hexagonal boron nitride nanosheet (NS/AgNP) was prepared by Yue et al. [22] for the determination of scopoletin in *Atractylodes macrocephala*. The results of these studies indicate that electrochemical method was comparable with TLC and HPLC. Therefore, it provides an alternative strategy for a simple, rapid, and low-cost assessment of scopoletin in plant extracts or products for routine quality control procedures.

3 Pharmacological properties of scopoletin and its mechanism of action

In this section, an overview of the pharmacological activities of scopoletin such as antioxidant, antimicrobial, antiinflammation, immunomodulator, anticariogenic, anti-metabolic disorder, neuroprotective activity and their mechanism of action are presented.

3.1 Antioxidant activity

A number of literature studies revealed that scopoletin possesses antioxidant activity and potentially used as a therapeutic compound for reactive oxygen species (ROS) mediated diseases [45, 80–82]. Scopoletin exhibits potent antioxidant effect when tested against hydroxyl radical and lipid peroxidation by scavenging free radicals, including 2,2-diphenyl-1-picrylhydrazym (DPPH), and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS). DPPH radical is converted into a stable DPPH by taking up hydrogen donated by the carboxyl group [83]. Mogana et al. [81] revealed that the EC$_{50}$ of scopoletin for scavenging DPPH and ABTS free radicals were 647.89 ± 0.07 µM and 191.51 ± 0.01 µM, respectively. The ability of scopoletin in stabilizing the radicals possibly indicates its antioxidant activity through ROS scavenging activity via hydrogen atom transfer (HAT) mechanism. Furthermore, osteopenic diseases including osteoporosis are always related to excessive osteoclastic bone resorption. It has been reported that ROS also play crucial role in osteoclast differentiation from murine macrophase RAW 264.7 cells. Lee et al. [84] studied the scavenging effect of scopoletin in differentiation of macrophase RAW 264.7. The results showed that scopoletin at 10 µM decrease the intracellular level of ROS as well as inhibiting superoxide anion production during osteoclast differentiation.

An *in vivo* study revealed that scopoletin was found to have potential as an antioxidant by affecting antioxidant enzymes in hypertensive rats. Hypertensive rats were orally administrated with 10 mg/kg scopoletin, and further, the serum concentration of nitrite oxide (NO) was greater on scopoletin-treated hypertensive rats [85]. NO is known as a potent vasodilator – the widening of blood vessels [86]. Moreover, it has been reported that scopoletin (10 mg/kg bw) possessed an antioxidant activity by inhibiting NO production. This compound also demonstrated significant protective effect and reveals a promising candidate of quenching free radical [49]. The inhibition of NO synthesis plays a pivotal role in the progress of hypertension by managing blood pressure regulation. It leads to the activation of renin–angiotensin vasoconstrictor system in severe hypertension conditions [87, 88].

3.2 Antimicrobial activity

Phenolic coumarins are known to play a protective role against microbial invasion in plants [89, 90]. This present
review may give useful insight for developing efficient antimicrobial agents from natural resources. Some studies have documented that scopoletin and its derivatives exhibit antibacterial and antifungal activities [30, 91–93]. Buathong et al. [91] reported that scopoletin shows high antibacterial activity and is able to inhibit both Gram-positive bacteria, such as *Staphylococcus aureus* ATCC 43300 and *Enterococcus faecium* UCLA 192 with minimum inhibitory concentration (MIC) value of 128 μg/mL, and Gram-negative bacteria *Stenotrophomonas maltophilia* DMST 19079 with MIC value of 256 μg/mL. In term of its structure activity relationship, Yang et al. [94] indicated that O–CH₃ group and OH group at the positions C-6 and C-7 have been shown to significantly affect antibacterial activity of scopoletin.

Antibacterial resistant has become serious health problem and it needs to find alternative approach to overcome this problem. One of appealing strategy is the use of natural product including active compounds. Scopoletin isolated from *Lasianthus lucidus* Blume was reported to have an inhibition activity against multidrug-resistant *Pseudomonas aeruginosa* strain DMSC 37166 and ATCC 27853 with MIC value of 0.66 μg/mL [30]. Further, field emission scanning electron microscopy (FE-SEM) analysis also displayed remarkable morphological changes in *P. aeruginosa* after exposure of scopoletin. The methanolic extracts of *L. lucidus* and scopoletin, generated cellular lysis, inflated swollen cell wall, and prolonged cells compared to untreated *P. aeruginosa* [30]. This condition is quite similar to the mechanism of antibiotics in β-lactam group, which inhibit penicillin-binding protein leading to deformed cell wall, including cell elongation, dissolved cell wall, and cell lysis [95, 96]. Clinical research on gingivitis patients disclosed that *Morinda citrifolia* L. extract that contains scopoletin significantly decreased the gingival index in patients [97]. Gingival index was developed for the assessment of the gingival condition. The above-mentioned extract was found to inhibit the growth of oral streptococci i.e. *Streptococcus mutans* and *Streptococcus mitis*, both are known to be associated with dental caries [98].

On the other hand, many studies were also conducted to know the effect scopoletin, against the fungal strains [99–101]. Scopoletin isolated from the skin of cassava root exhibited inhibitory activity on the tested *Candida* strains. The mechanism of action may be related to the ability of scopoletin in increasing oxidative stress and accumulation of ROS [101], which further regulate the apoptosis in yeast [102]. Treatment with scopoletin significantly decreased in the total biomass of *Candida* yeast, which might be due to the action of the scopoletin on the quorum sensing chemical pathways of yeast resulting in biofilm dispersion [103, 104].

### 3.3 Immunomodulator and anti-inflammation

Inflammation is a physiological response of the immune system to tissue damage resulting from various factors such as pathogens, toxic compounds, or tissue injury. In response to this condition, the cells release inflammatory chemical signals to initiate the healing process and produce cytokine pro-inflammatory responses [105]. Many studies have demonstrated anti-inflammatory action of scopoletin and its derivatives not only in vitro but also in vivo studies [49, 70, 106]. The anti-inflammatory properties of scopoletin may be mediated by several mechanisms of action.

A preliminary study showed that scopoletin was able to inhibit the production of cytokines including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) in Raw 264.7 macrophages in vitro [107]. Consistently, an in vivo study using animal model was conducted to provide information about acute oral toxicity and inflammation activity of scopoletin isolated from the root of *Hypochaeris radicata* [49]. Oral administration of scopoletin isolated from methanolic root extract of *H. radicata* and *Crossostepheum clinensis* restored the induction of cytokines expression, including TNF-α, IL-1β, IL-6, inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2) as a result of inflammation in the paw oedema [49, 106]. Pre-administration of scopoletin demonstrated an appreciable reduction of oedema by 92.88%, which is comparable to the standard drug indomethacin with 95.25% of reduction [49]. The anti-inflammatory mechanism may be correlated with the increased level of antioxidant enzymes, including superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) [108]. Another study demonstrated that scopoletin-rich *Morinda elliptica* leaf extract mitigated osteoarthritis progression by reducing inflammatory cytokines under osteoarthritis induction. Down-regulation of inflammatory cytokines further suppressing oxidative stress and inflammation at the joint, suggesting that scopoletin may exert anti-inflammatory activity [47].

In the case of immunomodulator, the use of natural products as immunomodulators has become the centre of studies due to the role of immune system for the protection and treatment of several diseases. A number of studies have showed that some coumarins are able to enhance immune response by activating macrophages to degrade extracellular pathogens [3, 109]. A study from Alkorashy et al. [110] reported that scopoletin enabled to regulate phagocytosis signaling pathway by increasing activity of macrophages and regulating genes expression which is
associated with phagocytosis. One of the affected genes is CD64, which is known to contribute on phagocytic process and autoimmune inflammatory responses [111]. Scopoletin was found to down-regulate CD64 expression, it indicates that scopoletin may possess a balanced immunomodulatory activity [110]. Additionally, scopoletin also appears to favour phagocytosis by decreasing overexpression of Rho family GTPases during inflammation conditions.

### 3.4 Anticarcinogenic

Cancer is one of the universally known fatal diseases, and many studies have been carried out using natural compounds in treating the disease. Cancer creates curiosity within the scientific community. According to 2018 worldwide cancer data, lung and breast cancers are the most common type around the world, with 12.3% of the total number are new cases. Meanwhile, colorectal cancer is the third most common, with 1.8 million new cases diagnosed [112]. Various cancer treatments which were designed and employed to cure cancer are associated with several side effects. Natural products have been intensively explored as alternative sources of new anticancer drugs.

Scopoletin has been reported to possess anticancer activity against several cancer cell lines. Tian et al. [113] reported that scopoletin inhibited the growth of human cervical cancer cell lines with IC$_{50}$ values in the range of 7.5–25 µM. This compound can induce apoptosis against HeLa cervical cancer cell lines. Scopoletin at 15 µM enhanced the expression of Bax, Caspase 3, 8 and 9. In contrast, it also inhibited the expression of anti-apoptosis Bcl-2. Furthermore, in order to confirm the effect of this compound on the cell cycle, flow cytometry analysis was then performed. The result showed that scopoletin also inhibited the HeLa cells at G2/M. Scopoletin also exhibited strong activity in inhibiting the growth of breast cancer cell lines and lead to the morphological change of tested cell lines. The compound at 500 µg/mL significantly inhibited the growth of MDA-MB-435 cell lines compared to the control. At this concentration, scopoletin revealed the anticancer activity by promoting the apoptosis using flow cytometry analysis [114]. In addition, scopoletin isolated from *Eupatorium laevigatum* possessed cytotoxic effect against various human cancer cell lines such as, HT-29, NCI-H660, MCF-7, and RXF-393 cell lines with IC$_{50}$ values in the range of value of 19.1 and 23.3 µg/mL [115]. It has been known that ultraviolet B may induce irradiation of keratinocytes. This compound can be utilized for the prevention and treatment of photoaging skin. A study by Kim and team [116] resulted that scopoletin at 300 µM downregulate the expression matrix metallopeptidase (MMP)-1 protein expression.

It has been known that nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) plays an in important role to mediates resistance towards cancer therapeutic agents by inhibiting cell apoptosis and intracellular signaling pathways that involved in chemoresistance. Therefore, modulation of signalling pathway is beneficial to overcome this problem. According to previous study, scopoletin exhibited potential antitumor activity against several cancer cell lines in which leukaemia cell lines are more sensitive to this compound. In order to investigate the molecular interaction of scopoletin in regulating intracellular signalling pathway, docking study was further performed. In this study, the interaction of compound with four protein targets such as o NF-κB, I-κB kinase β, I-κB kinase β-NEMO complex, and NF-κB-DNA complex was analysed using in silico model. The result showed that scopoletin has strong interaction with, I-κB kinase and NF-κB-DNA complex with energy binding were less than –7 kcal/mol. Moreover, this compound interacted with some key protein residues e.g. Arg144, Lys147, adenine18, and guanine19 [117].

In the case of in vivo study of the anticancer properties of scopoletin, several studies have also been performed using scopoletin-rich extract prepared from the leaf of *M. citrifolia*. The extract contained scopoletin in high concentration was then investigated its mechanism of anticancer activity in vivo using leukaemia induced BALB/c mice. The animal was treated with the extract (100 or 200 mg/kg BW). The result showed that scopoletin-rich extract significantly reduces bone marrow myelo-blasts levels of leukaemia-induced mice. The extract also downregulated the expression of anti-apoptotic genes and significantly upregulated cancer suppressor genes CSF3, SOCS1, PTEN, and TRP53 [118]. Moreover, using xenograft animal model, the potency of scopoletin as anticancer has also been investigated. Scopoletin at 100 and 200 mg/kg BW significantly inhibited vascularization in matrigel plugs implanted in nude mice with percentage inhibition of 59.72 and 89.4%, respectively. In the xenograft animal model, the compound at doses of 100 and 200 mg/kg BW exhibited a strong inhibition effect on tumour growth with percentage inhibition of 34.2 and 94.7%, respectively. This result indicated the potency of scopoletin as the chemotherapeutic agents [50].

### 3.5 Anti-metabolic disorder

Diabetes mellitus (DM) type 2 is the most common metabolic disorder in the world. DM type 2 is characterized by
insulin resistance. Plant-derived compound, including scopoletin, has been extensively studied for their potency in DM treatment. According to the previous study, scopoletin significantly stimulated reactivation of insulin-mediated Akt/PKB phosphorylation in high-glucose-induced HepG2 cell line. This compound also significantly increased the expression of peroxisome proliferator-activated receptor γ 2 (PPARγ2). The result indicated that scopoletin can improve insulin resistance in vitro model and might be useful to regulate metabolic disorder [119]. Jang and team also evaluated the effect of scopoletin as antidiabetic. In this study, scopoletin inhibited the activity of α-glucosidase and α-amylase with IC₅₀ values of 85.12 and 37.36 μM, respectively. The results indicated that scopoletin can be used as a natural antihyperglycaemic. The study was then proceeded by using animal model. The administration of scopoletin in streptozotocin (STZ)-induced diabetes in mice showed that postprandial blood glucose levels were significantly suppressed in the scopoletin group compared to the control [120].

Furthermore, the potency of scopoletin as an antidiabetic type 1 has been investigated by Choi and co-workers (2017). In this study, scopoletin at the dose of 0.01% w/w was able to reduce the blood glucose streptozotocin-induced diabetic mice. Scopoletin, along with metformin as positive control, downregulated the expression of the hepatic gene, which regulates triglyceride and cholesterol. Scopoletin also significantly suppressed the activities of hepatic fatty acid synthase and phosphatidate phosphohydrolase [121]. In diabetic conditions, glucose uptake is crucial to reduce hyperglycaemic. According to the previous study, scopoletin possessed antidiabetic effect by stimulating glucose transporter type 4 (GLUT4) translocation via upregulating the activation of phosphatidylinositol-3-kinase (PI3K) and AMP-activated protein kinase (AMPK) pathway. This compound enables the promotion of phosphorylation AMPK and enhances the expression of PM-GLUT4 [122].

The effect of scopoletin in glucose uptake was also investigated by Kalpana and team [123]. The study focused on investigating the mechanism of scopoletin in improving insulin sensitivity in high fructose diet (HFFD)-fed rat model. Scopoletin was administrated to the animal model for 45 days. The glucose level and lipid profile, as well as AMPK protein expressions were observed. The results showed that scopoletin significantly lowered glucose level and lipid profile. The compound also exhibited the activation of AMPK pathway. This finding indicated that scopoletin is able to improve insulin signaling activation of AMPK.

Another study also reported that scopoletin at a dose of 10 mg/kg BW prevents acute pancreatitis. The administration of the compounds attenuated the severity of pancreatitis induce cerulain [124]. Beside its effect toward diabetic conditions, scopoletin also exhibited potency in regulating the production of triglycerides. Yang et al. have investigated the effect of scopoletin on lipoprotein lipase activity in 3T3-L1 adipocytes. It was found that scopoletin significantly increases lipoprotein lipase and the LPL mRNA level in adipocytes. The result indicated that scopoletin might facilitate the clearance of plasma triglycerides [125]. Furthermore, the prevention effect of scopoletin in diet-induced obese mice was also performed. It was found that scopoletin reduces fatty acid, plasma acetaldehyde, and triglycerides compared to control. Scopoletin also significantly activated hepatic AMPK compared to control [126].

### 3.6 Neuroprotective activity

Scopoletin possessed strong neuroprotective activity. Molecular docking was performed in order to investigate the interaction of compound on enzymatic target including acetylcholinesterase (AchE) via computational study. In silico study results revealed that this compound significantly interacted with AchE in which its binding energy was low and thus high stable protein-ligand complex. According to the docking results, the scopoletin compound was found to have close interaction with key amino acid residues such as Tyr337, Glu202, and Tyr133. In addition, the energy binding of this compound against butyrylcholinesterase (BuChE) was somewhat similar with tacrine and rasagilline [127]. The docking results obtained further support the strong inhibition activity of AchE demonstrated by scopoletin.

A study by Luo and team [128] reported that scopoletin showed a good affinity for gamma-aminobutyric acid (GABA) transaminase and GABAₐ receptors, suggesting its potential in modulating neural transmission balance. GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (CNS) and plays a critical role in normal brain function [129]. GABA transaminase is a mitochondrial enzyme that controls the level of GABA in brain and glutamate. The imbalance of GABA and glutamate is known to link with stress and anxiety disorders [130, 131]. Recent studies suggest that the release of inflammatory cytokines may be linked with enhanced activation of the threat- and anxiety-related brain circuitry and amygdala, which is related to the CNS [132, 133] and contribute to behavioral changes [134].

An in vivo study using lipopolysaccharide (LPS)-induced male C57BL/6 mice displayed that scopoletin...
treatment regulated inflammatory responses by inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signalling pathways in which GABA transaminase is involved [128]. In addition, molecular docking analysis demonstrated the potential interaction of scopoletin and the active site of GABA transaminase [128], which was similar binding mode with vigabatrin, a medication used to treat epilepsy and anxiety problem [135]. These findings suggest that scopoletin may exert protection activity against inflammatory-mediated neurodegeneration.

Other studies mentioned that scopoletin isolated from *Tilia amurensis* and *Argyreia speciosa* roots displayed significant neuroprotective properties against neurotoxicity in glutamate-induced HT22 and amyloid beta (Aβ42)- and H2O2-induced PC12 cells, respectively [127, 136]. *In silico* docking analysis and *in vitro* studies demonstrated interaction between scopoletin and various proteins involved in Alzheimer’s disease, including Aβ42, AChE, and BuChE, suggesting scopoletin potential in multitarget-directed ligand approach. Scopoletin also has been found to suppress NF-κB signaling resulting in reduced CNS inflammation in experimental autoimmune encephalomyelitis animal model [137]. By modulating CNS inflammation and dendritic cell activation, scopoletin demonstrates its potential as a treatment candidate for the modulation of inflammatory conditions in neurogenerative disorders.

### 4 Conclusions

Scopoletin is a coumarin derivative widely distributed in the plant kingdom, particularly in edible plants. Various extraction methods have been developed to extract scopoletin from different plants and natural products. However, conventional method such as traditional maceration still remains commonly used over the novel extraction method, due to the simplicity and feasibility for low-budget laboratories and scaling-up. Numerous *in vitro* and *in vivo* animal studies support the role of scopoletin in the prevention and treatment of various diseases, including inflammation, infectious diseases, non-communicable and metabolic related-diseases, as well as neurogenerative disease. Even though a large number of studies have been demonstrated pharmacological activity of scopoletin, further animal studies and clinical trials are necessary for better understanding and investigation of its potential as a plant-derived drug candidate.

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