Review Paper:

Decoding Scopoletin: A Comprehensive Review

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Abstract

A naturally occurring coumarin chemical, scopoletin is present in many plants such as passion flower, stinging nettle and chicory. It has a wide range of biological activities including antibacterial, anti-inflammatory, antioxidant and anti-cancer properties. In silico studies have shown that scopoletin can interact with a number of different proteins including enzymes, receptors and nucleic acids. This suggests that scopoletin may have a number of different mechanisms of action. In vitro studies have shown that scopoletin can inhibit the growth of a variety of bacteria, fungi and viruses. It can also reduce inflammation, it can protect cells from oxidative damage and it can kill cancer cells. In vivo studies have shown that scopoletin can reduce blood pressure, it can improve blood sugar control and it can protect against liver damage. It has also been shown to be effective in the treatment of a number of other conditions, including anxiety, depression and pain.

Overall, the results of in silico, in vitro and in vivo studies suggest that scopoletin has a number of potential therapeutic applications. Nevertheless, further investigation is required to comprehensively comprehend the processes by which scopoletin operates.

Keywords: Scopoletin, Biological Activities, Therapeutic, Coumarin.

Introduction

Nature has given to mankind a comprehensive supply of cure for all the diseases. The primary healthcare requirements of almost 80% of the world's population are partially or entirely met by conventional medicines[14]. Herbal medicine has been used in medical practice as primary treatment for thousands of years and has made a significant contribution in maintaining human health. The therapeutically significant secondary metabolites are abundant in medicinal plants[44]. The benefits of using medicinal plants in treating a variety of illnesses include their safety, affordability, efficiency and readily availability.

The scientific community is becoming more interested in identifying and characterizing bioactive components from diverse plant extracts for application in the pharmaceutical sector or the formulation of functional meals. Nowadays, it is well acknowledged that diet plays a significant part in reducing risk factors and preventing many chronic illnesses[4]. Flavonoids are among the diverse group of bioactive substances generated from plants that have been proven to have several health advantages. As a result, they are regarded as a class of dietary compounds with significant medical value. These plant-based secondary metabolites are believed to encompass a variety of structurally different polyphenol classes having significant pharmacological activity such as anticancer, anti-inflammatory, antioxidant, antimicrobial, neuroprotective etc.

The secondary metabolite known as coumarin is classified as 1,2-benzopyrones. Plants and fungi both contain this chemical[45]. One of the naturally occurring coumarins frequently found in many edible plants, is scopoletin (6-methoxy-7 hydroxycoumarin) and it has a significant impact on human health. Scopoletin is structurally known to have two aromatic rings, one of which is substituted with one hydroxyl group and one methoxy group and another ring contains one oxo group. This substance has been extracted from a variety of therapeutic plants like *Angelica archangelica, Angelica dahurica, Arabidopsis thaliana, Artemisia annua, Canscora decussate, Chenopodium murale, Citrullus lanatus, Decaschistia crotonifolia, Fagraea ceilanica, Hypochaeris radicata, Ipomoea digitata, Lasianthus lucidus, Mitracarpus frigidus, Morinda citrifolia, Morus alba, Paederia foetida,* etc. (Fig. 1).

In nature, scopoletin is frequently linked to a plant's defense system against parasite and microbial invasion[25]. To find its untapped health advantages, further scientific work is required. This study emphasizes the several therapeutic applications of scopoletin in treating a range of diseases and addresses scopoletin *in silico* investigations. For the present review, different electronic databases like Pubmed, ScienceDirect, Wiley, Researchgate, Scopus and Google Scholar between the years 1997 and 2024 were searched. The search includes the keywords words like 'scopoletin', 'sources scopoletin' 'pharmacological activity', 'scopoletin+insilico studies' and 'scopoletin+therapeutic activity' etc.

Therapeutic Potential of Scopoletin: According to various research and scientific studies, scopoletin has a wide range of therapeutic activities such as anticancer, antifungal, antiviral, anti-aging, anti-arthritic and anti-spasmodic. Scopoletin is a coumarin molecule found in a variety of medicinal plants including Scopolia species as well as Artemisia, Brunfelsia, Solanum, Mallotius and other genera (Table 1).

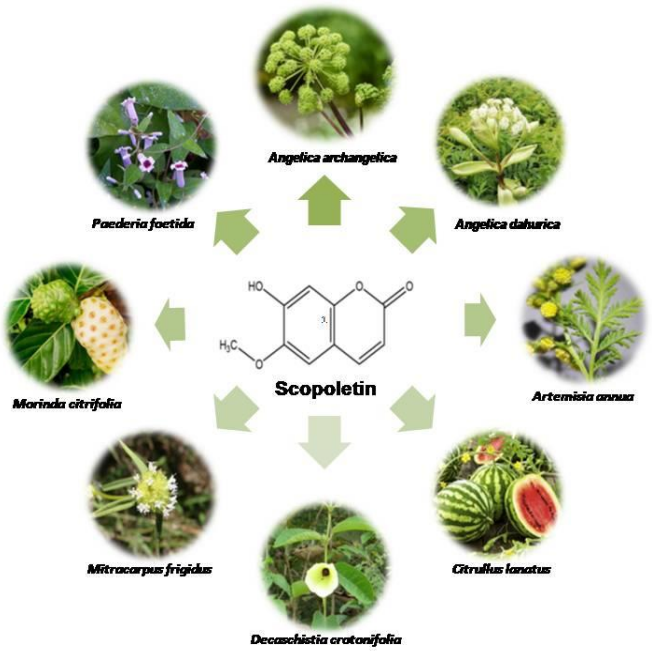

Fig. 1: Sources of Scopoletin

***In vivo* Studies**

Anti-Cancer Activity: Scopoletin is well-known for its cytotoxicity to cancer cells. It has antioxidant and anti-inflammatory properties as well as the ability to trigger apoptosis and autophagy. Seo et al[47] worked on the multidrug resistance mechanism of scopoletin on NCI cell lines by ATP-binding cassette (ABC) transporter mechanism along with the expression of EGFR oncogene and tumor suppressor gene (TP53). Zhao et al[60] investigated the anticancer potential of (*E*)-3-(4-chlorophenyl)-*N*-(7-hydroxy-6-methoxy-2-oxo-2*H*-chromen-3-yl) acrylamide (SC-III3), a newly synthesized scopoletin derivative, *in vitro* and *in vivo*.

The impact of SC-III3 on hepatocellular malignancies was investigated by conducting experiments on the human hepatocellular carcinoma cell line HepG2 cells and a xenograft of HepG2 cells in BALB/c nude mice. Cell cycle arrest and apoptosis were assessed using flow cytometry. The Western blot technique was used to detect proteins associated with cell cycle arrest, apoptosis and the ATM-Chk pathway. The viability of HepG2 cells was particularly reduced by SC-III3, perhaps due to its capacity to induce S phase arrest. SC-III3 suppresses the growth of hepatocellular carcinoma by generating reactive oxygen species (ROS) within cells, causing DNA damage and leading to a halt in the cell cycle during the S phase. However, it does not have any significant harmful effects on normal liver cells[60].

Grivicich et al[16] extracted scopoletin from the aboveground part of *E. laevigatum*. The cytotoxicity and oxidative damage in human cancer cell lines HT-29, NCI-H460, MCF-7 and RXF-393 were evaluated using the sulforodamine B test and the thiobarbituric acid reactive species assay. The cytotoxicity of NCIH460 and RXF-393 cells was influenced by the coumarin scopoletin which was extracted from the aerial parts of *E. laevigatum*. The IC_{50} values for NCIH460 and RXF-393 cells were 19.1 and 23.3 µg/mL respectively. *E. laevigatum* has the compound scopoletin, a coumarin derivative, which induces cytotoxic effects in the NCI-H460 and RXF-393 cell lines. Additionally, it is believed that oxidative damage has no relevance to scopoletin's cytotoxic effects[16].

Li et al[30] assessed the anticancer potential of scopoletin using the MMT test. Propidium iodide and annexin V-FITC were used in flow cytometry to research apoptosis and cell cycle assessments. Scopoletin's impact on cell shape and the production of apoptotic bodies in human prostate cancer (LNCaP) cells was evaluated using Hoechst 33258 staining, followed by Western blotting to examine its impact on cyclin D1 and cyclin B1 expression. In LNCaP prostate cancer cells, scopoletin produced dose-dependent growth suppression. When compared to control cells, scopoletin treatment resulted in cell shrinkage and membrane blebbing which are markers of cell death. It also produced G2/M phase growth arrest and an increase in the sub-G0/G1 cell population. After being treated to 40, 80 and 100 M of scopoletin, the cells exhibited early apoptosis in roughly 15.45, 32.6 and 21.71% of the instances respectively.

When LNCaP cells were exposed to varied scopoletin doses, cyclin D expression reduced in a concentration-dependent manner[30]. Cholangiocarcinoma cells were used by Asgar et al[2] to assess the anticancer effects of co-administering cisplatin and scopoletin. To ascertain the anticancer effects, the MTT assay, median effect principle, cell cycle arrest and apoptosis assay were used. The results showed that cisplatin and scopoletin therapy reduced the viability of cholangiocarcinoma cells in a dose-dependent manner.

The combination of these drugs greatly reduced cell growth more than any individual agent alone. Combination indices show a cytotoxic impact that is cumulative and results in a dosage reduction of more than two times for each medication. The increased cytotoxicity for the combination was caused by both the cell cycle arrest (G0/G1) and the activation of apoptosis. Additionally, cholangiocarcinoma cells were affected by a single chemical that caused cell cycle arrest and apoptosis. Non-cancer cells, however, were less impacted by the combination. Observations showed that the combination of cisplatin and scopoletin may have a good impact on the treatment of cholangiocarcinoma[2].

Worldwide, cervical cancer is a significant cause of death in women and the available treatments have serious side effects. Therefore, the development of innovative and effective treatment plans for cervical cancer is urgently needed. Cell counting and colony formation tests were used to examine Scopoletin's antiproliferative impact by Tian et al[53]. The acridine orange ethidium bromide staining method was used to identify apoptosis. Using flow cytometry, cell cycle distribution was discovered. Boyden chamber experiment was used to investigate cell invasion. Western blotting was used to evaluate protein expression. All of the cell lines' proliferation were suppressed by scopoletin.

However, with an IC_{50} of 90 M, scopoletin's cytotoxic effects on normal cells were comparatively negligible. Research on the mechanism of action of scopoletin has led to the conclusion that its anticancer effects against HeLa cervical cancer cells are a result of induction of apoptotic cell death.

Additionally, scopoletin administration increased the expression of Bax, Caspase 3, 8 and 9, while decreasing the expression of Bcl-2. At the G2/M phase of the cell cycle, *scopoletin* also prevented HeLa cells from growing. Additionally, a cell invasion study showed that *Scopoletin* reduced HeLa cell migration in a concentration-dependent manner. Here, it was discovered that *Scopoletin* might suppress the crucial PI3K/AKT pathway, which is important in the growth and development of cancer cells (Fig. 2). Scopoletin exerts anticancer effects on human cervical cancer cell lines by inducing apoptosis, cell cycle arrest, inhibiting cell invasion and PI3K/AKT signaling pathways.

Scopoletin also activates NF-kB, caspase-3 and PARP cleavage leading to apoptosis in some cell lines of blood cancer. Downregulation of cyclin-D1, proliferation of cell nuclear antigen, surviving, stat-3 and through upregulation of p53 and caspase-3, scopoletin inhibits the growth of skin cancer. Similarly, scopoletin inhibit the angiogenesis process in chick embryo chorioallantonic membranes. Scopoletin causes phosphorylation of ERK1/2 but not the p38 and JNK in urinary bladder cancer indicating its anti-angiogenic action. In human lung carcinoma, scopoletin interrupts the phosphorylation of ERK1/2, JNK and eIF-2α[53].

Anti-Fungal Activity: In 2020, Lemos et al[29] looked at scopoletin's antifungal efficacy and method of action against a strain of *Candida tropicalis* that was resistant to many drugs. In order to extract scopoletin, the plant *Mitracarpus frigidus* was first processed using HPLC. The mechanisms of action were examined using sorbitol and ergosterol bioassays, efflux pumps, nucleotide leakage, time-kill kinetics and cell density measurements in order to prevent fungal planktonic growth. The last method used to assess scopoletin's impact on Candida tropicalis biofilms was whole-slide imaging together with spectrophotometric methods.

With MIC values of 50 and 250 mg/L for scopoletin and fluconazole (positive control), respectively, it was clear that scopoletin exhibited fungistatic properties. In comparison to the growth control, scopoletin significantly reduced the growth curves and cell density of *Candida tropicalis* (91.7 % reduction). Scopoletin dramatically lowered Candida tropicalis' growth rates and cell density[29].

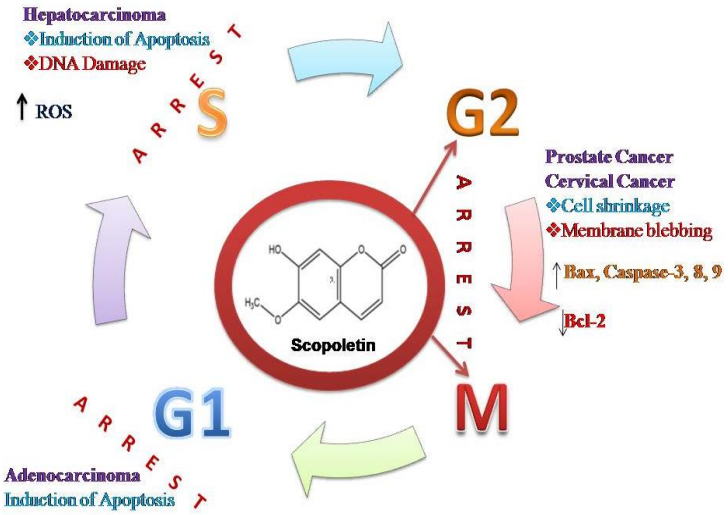

Fig. 2: Anticancer Mechanism of Scopoletin

In cell suspension cultures derived from *Ulmus pumila*, susceptible to Dutch elm disease (DED) and a resistant species, *Ulmus pumila campestris*, differently triggered aggregation of the scopoletin was observed. Scopoletin was primarily developed in the medium of the resistant challenged culture and produced fairly fast. *Ophiostoma ulmi* spore germination, which was more susceptible to this inhibitory action than mycelial development, was the target of Scopoletin's direct antifungal effectiveness *in vitro* bioassays[56].

By using virus-induced gene silencing (VIGS) to silence the gene encoding the essential enzyme for scopoletin production, it was discovered that scopoletin and its β-glycoside form were the primary sources of the blue fluorescence. According to the findings of Sun et al[52], scopoletin demonstrated potent antifungal efficacy against *A. alternata* both *in vitro* and *in vivo*.

Scopoletin, was studied by Das et al[10] for its antifungal properties. This investigation focused on seven different Candida species' planktonic forms as well as constructed biofilms. Between 67.2 and 191.4 g/mL was the typical minimum inhibitory concentration (MIC_{90}) of Scopoletin for *Candida* species using fluorescent live/dead discrimination methods. The three Candida species that responded to Scopoletin, the most were *Candida glabrata*, *Candida guilliermondii* and *Candida parapsilosis*.

By enhancing oxidative stress against the studied planktonic Candida species, Scopoletin was also discovered to enhance the buildup of intracellular reactive oxygen species (ROS). Scopoletin has antifungal properties that vary on dosage[10]. Further the anti-fungal mechanism of scopoletin includes the interference in the synthesis of fungal cell component, disruption of fungal cell walls and plasma membrane and impairment of fungal biofilm growth, formation and proliferation[58].

Anti-aging Activity: In contrast to being connected to cancer and neurodegeneration, autophagy has also been linked to aging. In the human lung fibroblast cell line IMR 90, the effects of scopoletin on autophagy and anti-aging were examined by Nam et al[37] who found that it triggers autophagy. Additionally, it has been determined that scopoletin's regulation of p53 is connected to the activation of autophagy. Additionally, scopoletin lowers the degree of SA-β-Gal staining, a sign of aging. Furthermore, the presence of scopoletin in IMR 90 cells results in a decrease in the expression levels of histone acetyltransferases while an upsurge results in the expression levels of histone deacetylases such HDAC1, SIRT1 and SIRT6.

Moreover, scopoletin raises the levels of transcription factors involved in anti-aging including Nrf-2 and p-FoxO1. Scopoletin also controls the reprogramming proteins. Therefore, results imply that scopoletin may enhance autophagy-related anti-aging with the regulation of p53 in human lung fibroblasts[37]. *Artemisia iwayomogi* has mostly been used in Korean folk medicine to treat feminine illnesses and skin whitening as well as to enhance diabetes complications and hepatic function. As a result of Kim et al[26] study, when administered to the face in an *in vivo* investigation, scopoletin displayed notable antiwrinkle results[26].

Anti-arthritic Activity: The primary component of the coumarin present in the stems of *Erycibe obtusifolia* Benth, a traditional Chinese medicine used to treat rheumatoid arthritis, is scopoletin. Pan et al[40] examined paw swelling, pathology and synovial angiogenesis to determine the anti-arthritic benefits of scopoletin in rat adjuvant-induced arthritis. It was shown that scopoletin increased the mean body weight of adjuvant-induced arthritic rats and decreased paw swelling in both inoculated and non-inoculated rats as well as articular index scores. Rats given a larger dosage of scopoletin had almost normal joint histology and reduced new blood vessel growth in the synovial tissues. The upregulation of VEGF, basic fibroblast growth factor and IL-6 in the synovial tissues of rats with adjuvant-induced arthritis was also downregulated by scopoletin.

As a result, this substance may be a viable treatment for conditions linked to angiogenesis. In conclusion, scopoletin can reduce the number of new blood vessels in the synovium and the production of significant endogenous angiogenic inducers, hence alleviating the clinical symptoms of rat adjuvant-induced arthritis and may also be used as a structural starting point for the development of more potent synthetic analogs. To combat the arthritis, scopoletin decreases the synovial angiogenesis, cell proliferation, inflammatory mediators and COX-2. It also downregulates the IL-6, MAPKs, PKC, VREB, IL-beta, TNF-alpha, IL-33, NF-kB and upregulates the apoptosis inducing factors[40].

Analgesic Activity: Chang et al[9] examined the analgesic activity of scopoletin. Scopoletin therapy in ICR mice reduced the number of writhing responses and the late-phase discomfort caused by formalin. This study showed that the treatment of scopoletin reduced the edema that was caused by the injection of carrageenan in mice. Scopoletin substantially reduced the MDA level in the edematous paw following carrageenan injection. Following the injection of carrageenan, scopoletin lowered the NO, TNF-α and PGE2 levels in the serum. Scopoletin reduced iNOS and COX-2 expressions that were brought on by carrageenan in the edematous paw. By raising the activities of SOD, CAT and GPx in the edema paw, scopoletin's anti-inflammatory processes may be connected to the drop in MDA levels. Additionally, scopoletin may modify NO, TNF-α and PGE2 synthesis which would modify its anti-inflammatory properties.

Multiple Sclerosis Activity: An animal model of multiple sclerosis (MS) called experimental autoimmune encephalomyelitis (EAE) was successfully treated with

Scopoletin, according to Zhang et al[61] in the year 2019. This was accomplished through novel regulatory mechanisms that involved the suppression of NF-β activity in dendritic cells. In EAE mice, scopoletin therapy considerably reduced the disease's severity and noticeably reduced inflammation and demyelination of the central nervous system. The reduction of MHC class II, CD80 and CD86 expression on splenic or central nervous system dendritic cells as well as the invasion and polarisation of encephalitogenic Th1/Th17 cells, were associated with the improvement of disease.

Scopoletin-treated, bone marrow-derived dendritic cells showed decreased expression of MHC class II and co-stimulatory molecules as well as decreased NF-β phosphorylation, consistent with the *in vivo* observations. According to a study, scopoletin will be useful in the development of a new MS treatment drug in the future[61].

Antihypertensive activity: The leading single factor in both the worldwide burden of illnesses affecting people and the global death rate is thought to be hypertension. The prevalence of high blood pressure is anticipated to reach 10 % globally and continues to rise across all areas. Stroke and coronary heart disease are the main contributors to this illness's high fatality rate. Scopoletin (10 mg/kg) was used to treat hypertensive rats with oxidative stress in Armenia et al[1] study. An oxidative stress inducer N-nitro-L-arginine methyl ester was utilized. Nitric oxide levels before and after the trial were also taken into account. To assess the antihypertensive action, the systolic, diastolic, mean arterial blood pressure and heart rate were employed[1,18,43].

Anti-inflammatory Activity: Traditional uses of *Angelica dahurica's* major active ingredient, scopoletin, include the treatment of headaches, rhinitis, pain and other ailments. In a mouse model of chronic inflammatory anxiety brought on by Complete Freund's adjuvant (CFA), Lou et al[32] examined the effects of scopoletin. In the elevated plus maze test and open field test, scopoletin (2.0, 10.0, 50.0 mg/kg) treatment for two weeks' dose-dependently reduced anxiety-like behaviors brought on by CFA. Additionally, scopoletin therapy reduced central and peripheral levels of IL-1, IL-6 and TNF-α in a dose-dependent manner while inhibiting microglia activation. Results show that the anxiolytic properties of scopoletin can be ascribed to the suppression of nuclear factor-kappa B and mitogen-activated protein kinase signalling pathways including anti-inflammatory activity and control of the excitatory/inhibitory balance.

***In vitro* Studies:** Synthetic drugs that are intended to treat rheumatoid arthritis (RA), a complicated inflammatory disease that mostly affects synovial joints, have severe safety risks. The acclaimed anti-rheumatic herb *Saussurea laniceps* (SL) is a great place to start when seeking natural remedies that are safe, efficient and specifically targeted for RA. Scopoletin was chosen as the anti-rheumatic alternative with the best specific affinities against the membrane proteomes of rheumatic fibroblast, like synoviocytes (FLS), the key effector cells in RA, by biomimetic ultrafiltration. Scopoletin to varied degrees suppressed RA-FLS proliferation, migration, invasion and NF-B signalling, according to *in vitro* tests. Scopoletin acts on a variety of targets, mostly tyrosine kinases, in the treatment of RA, according to network pharmacology analysis and molecular docking research combined. Overall, our current investigation revealed scopoletin as an anti-rheumatic component of SL that may bind to and inhibit tyrosine kinases, which would then inactivate NF-B in RA-FLSs[8].

***In silico* studies**

Anti-viral Activity: Scopoletin's interaction with the main protease Mpro SARS-CoV-2 was studied by Ikanovic et al[19] to ascertain its possible antiviral efficacy. The study makes use of AutoDock Vina version 1.1.2 to simulate the interaction between scopoletin and the primary proteases Mpro of the SARS-CoV-2 virus. The three-dimensional structures of the main protease Mpro of SARS-CoV-2 (PDB ID: 6Y84) and the scopoletin structure were used for analysis. Hydroxychloroquine was employed as a positive control for binding affinity and a known therapeutic effect.

The findings show that scopoletin has a similar ability to hydroxychloroquine, which is an effective antiviral in prior preclinical and clinical trials to bind to and block the major protease of the SARS-CoV-2 3Clpro. Following a successful docking investigation, it was determined that scopoletin had a -6.9 kcal/mol binding affinity for the main SARS-CoV-2 protease. This finding suggests that scopoletin and other coumarin derivatives may be useful in the battle against COVID-19, although more *in vitro* and *in vivo* research is undoubtedly required[19].

Anti-anxiety Activity: *In vivo* MAO-A inhibitory action of scopoletin isolated from the roots of *Angelica archangelica* was investigated by Kaur et al[23]. Through molecular docking experiments, the isolated scopoletin was checked for MAOA (PDB ID: 2Z5y) binding. According to molecular docking studies, scopoletin has the highest levels of affinity for MAO-A.

Anti-Alzheimer Activity: Through molecular docking, Kashyap et al[24] investigated the scopoletin effect for its antiamyloidogenic, anticholinesterase and neuroprotective potential. The findings demonstrated that practically all of the targets for Alzheimer's disease (AD) (Aβ42, AChE, BuChE, BACE1 and MAO-B) interact with scopoletin at the catalytic site residue level. Scopoletin has nearly identical binding energies to positive controls tacrine and rasagilline with BuChE and MAO-B, according to the examination of binding energies. Due to its low binding energy and excellent protein-ligand stability, scopoletin also exhibits an irreversible binding to Aβ42 and AChE.

Scopoletin is an effective MTDL that can reduce Aβ42 aggregation and important enzymes linked to AD, including AChE and BuChE, according to the results of molecular

docking. Using doneprezil as a common AChE inhibitor, Kuppusamy et al[27] investigated scopoletin's impact on the acetylcholinesterase (AChE) enzyme's crystal structure. For the docking investigations, AutoDock 4.2 was used to calculate binding energy, inhibition constant and intermolecular energy as well as conformational site analysis and docking parameters.

Studies on docking with scopoletin showed that the enzyme had tighter binding forces than donepezil. Against the target enzyme, scopoletin demonstrated several strong hydrogen bonds to many significant amino acid residues. The ability of the chemicals to inhibit AChE could be explained by several hydrophobic interactions. The future development of effective acetylcholinesterase inhibitors for the treatment of AD may result from these molecular docking analyses[27].

Antispasmodic Activity: The antispasmodic effects of scopoletin, a bioactive substance from the seeds of *Citrullus lanatus*, were examined by Wahid et al[59]. Studies conducted on the computer showed that the bioactive components (stigmasterol, quinic acid, malic acid, epicatechin, caffeic acid, rutin, p-coumaric acid, quercetin, ferulic acid, scopoletin, apigenin and kaempferol) of *Citrullus lanatus* seeds interfere with target genes related to cytosolic calcium signaling, smooth muscle contraction and inflammatory responses. Additionally, calcium/calmodulin-dependent protein kinase, myosin light chain kinase and phosphoinositide phospholipase C were discovered to have higher binding to scopoletin.

Anti-neoplastic Activity: The cells that line the bronchi and other areas of the lung, such as the bronchioles or alveoli, are where lung cancer first develops. Non-small cell lung cancer (80–85% of cases) and small cell lung cancer (10–15%) are the two primary kinds of lung cancer. By using an *in silico* technique, Lavezo et al[28] investigated the anti-neoplastic (breast and lung cancer) activity of scopoletin from the *Artemisia annua* species. The most promising structure in computer simulations that could interact with MMP-9 (metalloproteinase type 9) and EGFR, was scopoletin.

Through cell surface receptors, which are plasma membrane-based proteins, scopoletin communicates with the EGFR receptor. These receptors are triggered by environmental stimuli, resulting in intracellular signals that trigger various biochemical cascades. The transcription of genes involved in proliferation, differentiation, invasion, angiogenesis, metastasis and resistance to cell death is activated by the sequential phosphorylation of substrates[28].

Anti-hypertensive Activity: One of the main causes of stroke is hypertension. The management of hypertension can help to avoid stroke. The antihypertensive effects of noni fruit have been demonstrated. Scopoletin found in noni fruit, is used as antihypertensive. Blood pressure was formerly thought to be influenced by the brain, kidneys and endothelial issues in molecular biology research. Nitric oxide (NO), a substance with the properties of a vasoactive gas, may be produced by vascular endothelium. An additional mechanism for hypertension is a rise in peripheral resistance brought on by blood vessel vasoconstriction. Levels of NO that can produce vasodilation of blood vessels had an impact on the process of vasoconstriction and vasodilation of blood vessels.

The NOS enzyme, which is encoded by the NOS3 gene, influenced NO levels. The NOS3 protein was specifically targeted by Hijriansyah et al[17] for the anti-hypertensive effect of the active noni fruit (*Morinda citrifolia*) compound scopoletin. Blood pressure is regulated by the protein NOS3. Captopril and scopoletin were discovered to have binding energies of -5.7 and -7.6 kcal/mol respectively. The outcomes also back with the *in vivo* research[17,31].

Anti-oxidant Activity: The development of many illnesses is actively influenced by oxidative stress, which overwhelms the antioxidant defenses of biological systems. *In vitro* techniques were used to test the antioxidant properties of *A. annua* extracts and molecular docking analysis was used to identify the inhibitory potentials of 29 of the plant's bioactive chemicals against oxidative stress target proteins. SOD, CAT, GSH-Px, iNOS, eNOS, Casp-1, XOX and NOX were used as target proteins in the analysis of scopoletin by Ejembi et al[11] to determine its ability to suppress oxidative stress. The docking scores in this study were found sequentially -5.30, -7.00, -5.80, -6.70, -6.80, -5.40, -5.90 and -5.70 kcal/mol. Scopoletin can prevent several pathological disorders that might develop as a result of oxidative stress, according to the docking score results[11,36].

Anti-diabetic Activity: Diabetes is a metabolic syndrome condition that causes people to have high blood sugar levels. Because they contain anti-diabetic active ingredients, medicinal plants are used to treat diabetes. Utilizing molecular docking experiments, Adriani et al investigated the anti-diabetic impact of scopoletin *via* α-glucosidase inhibitors. Scopoletin and acarbose (standard) were found to have binding energies of -5.8 and -7.8 kcal/mol respectively. Through the inhibition of the α-glucosidase enzyme with an inhibitory value comparable to acarbose control, scopoletin has anti-diabetic properties[38]. Additionally, Tan et al[54] used the proteins α-amylase and α-glucosidase to study the anti-diabetic impact of scopoletin isolated from the *Paederia foetida* L. It was discovered that had binding energies of -6.03 and -2.92 kcal/mol respectively[54,55].

Anti-nociceptive Activity: Numerous unrelated ailments including cancer, diabetes, Alzheimer's, Parkinson's, heart diseases, stroke, arthritis, multiple sclerosis etc. are caused by the death of cells, which is a typical process that precedes inflammation. The inflammatory process is the body's reaction to harmful stimuli, which might result from an infection, irritation, or physical harm. The characteristic acute inflammatory response, which includes calor (warmth), dolor (pain), rubor (redness) and tumour

(swelling), is propagated and matured by a series of biochemical reactions. The anti-nociceptive effect of scopoletin was studied by Potluri et al[42] through molecular docking studies. For the *in silico* studies, the COX-2 protein was utilized and diclofenac was taken as standard. The binding affinities of scopoletin and diclofenac were found to be -6.91 and -7.03 kcal/mol.

Madeswaran et al[33] studied the cyclooxygenase inhibitory activity of scopoletin through *in silico* docking studies. Azelastine, a known lipoxygenase inhibitor was used as the standard. Docking results showed that the binding energies of scopoletin and azelastine were found to be -3.57 and -3.70 kcal/mol respectively. Scopoletin contributed better lipoxygenase inhibitory activity because of its structural parameters[5,6,33].

Anthelmintic Activity: In the year 2022, Peeriga et al[41] used molecular docking to investigate the anthelmintic activity of scopoletin derived from the *Decaschistia crotonifolia.* Scopoletin and albendazole were discovered to have binding energies of -7.7 and -8.7 kcal/mol respectively. These results were supported by docking study of scopoletin in *Dechaschistia crotonifolia*, which revealed good docking scores for the scopoletin when compared to conventional albendazole (Fig. 3).

Table 1
Mechanistic approach of scopoletin

Pathological condition	**Mechanism of action of Scopoletin (*in-vivo*/*in-vitro*)**
Diabetes[38,55,54]	↓Postprandial hyperglycemia ↓Serum glucose ↑Glucose uptake ↓G6PD ↑PI3K, AMPK, PKAT, GLUT4, IRS1, SOD, GSH, HDL ↓Triglyceride, Free fatty acids, LDL, VLDL, Oxidative stress, DGAT2, TLR4
Acute/chronic Liver disease[12]	↑ ALT, AST, AMPK, PI3K, SOD, CAT, GSH-Px, CPT ↓TNF-α, MPO, MDA, CYP2E1, Triglyceride, NF-kB, IL-6
Pain & inflammation[9,32]	↓ IL-1β, TNF-α, Glutamatergic transmission, IL-1, IL-6, MAPK, ↓ COX-2
Hypertension[1,18,43]	↓ IL-6, MDA, Systolic and diastolic blood pressure, TNF-α, spasmogenic activity, ↑ relaxed smooth muscles, ↓ NOS3, NO
Asthma[39]	↓ K^+ and Ca^+, IL-5, IL-10, IFN-γ
Viral infection[19]	↓ 3CLpro, Mpro
Anxiety[23]	↓ MAO-A
Alzheimer[27]	↓ Aβ42, AChE, BuChE, BACE1 and MAO-B
Spasm[59]	↓ MLCK, PIPL-C, Ca^{2+}-CAM
Cancer[28]	↓MMP-9, EGFR

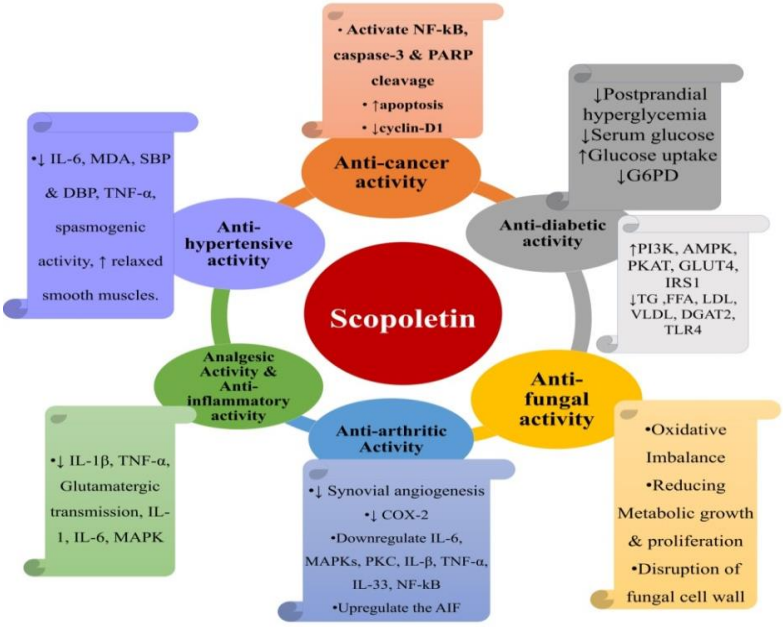

Fig. 3: Mechanism of action of scopoletin

Delivery Systems of Scopoletin: To improve the oral bioavailability, scopoletin was encapsulated into Soluplus micelles and the hypouricemic action of Soluplus-based scopoletin micelles was evaluated. Soluplus-based scopoletin micelles were prepared using a thin-film hydration method. The encapsulation efficiency of scopoletin was 87.3%±1.5% with a loading capacity of 5.5%±0.1%. After oral administration in rats, Soluplus-based scopoletin micelles exhibited significantly improved absorption in each intestinal segment compared to free scopoletin, with the duodenum and jejunum being the main absorption regions. In rats administered Soluplus-based scopoletin micelles, the $AUC_{0-\infty}$ and C_{max} were found to be 4.38- and 8.43-fold respectively.

After oral administration in rats, Soluplus-based scopoletin micelles did not alter the tissue distributions of scopoletin but significantly increased the scopoletin levels in the liver. Oral administration of Soluplus-based scopoletin micelles reduced the serum uric acid concentration to the normal level. The results suggest that the Soluplus-based micelle system greatly improves the bioavailability of poorly water-soluble drugs such as scopoletin and represents a promising strategy for their oral delivery[62].

Scopoletin was loaded *in situ* into the Zeolitic Imidazolate Framework-8 (ZIF-8), a slow-releasing material with pharmaceutical application. The expectorant activity was also evaluated. The scopoletin-ZIF-8 drug delivery system showed an encapsulation efficiency of 22.5%. A pH-dependent release profile of Scopoletin present in the drug delivery system was observed when compared to pure scopoletin. Scopoletin also presented expectorant activity at 30 and 100 mg/kg doses, but the system did not show expectorant activity at 10 mg/kg dose[46].

Biosynthetic Pathways: The biosynthetic pathway of scopoletin is a complex process that involves multiple enzymes (Fig. 4). The pathway begins with the conversion of glucose to tyrosine or phenylalanine through the shikimate acid pathway[20-22]. Tyrosine is then converted to p-coumaric acid by tyrosine ammonia lyase (TAL) or phenylalanine ammonia lyase (PAL). P-coumaric acid is then converted to feruloyl-CoA by 4-coumarate CoA ligase (4CL). Feruloyl-CoA is then hydroxylated at the 6' position by feruloyl-CoA 6'-hydroxylase (F6'H). The resulting 6'-hydroxyferuloyl-CoA is then converted to scopoletin by coumarin synthase (COSY)[52]. In addition to the enzymes listed above, the biosynthetic pathway of scopoletin also requires the participation of several other enzymes including:

- cinnamate-4-hydroxylase (C4H)
- 4-hydroxyphenylacetic acid 3-hydroxylase A (HHA)
- coumarate-3-hydroxylase (C3H)
- scopoletin 8-hydroxylase (S8H)

The expression of the genes encoding these enzymes is regulated by a variety of factors including environmental conditions, plant development and the presence of other plant secondary metabolites[51]. The biosynthetic pathway of scopoletin is found in a wide variety of plants including tomatoes, potatoes, grapes and onions. It is also found in some fungi and bacteria. The pathway is thought to have evolved from the shikimate acid pathway, which is a common pathway for the biosynthesis of aromatic amino acids.

Scopoletin is a versatile compound with a variety of biological activities. It has been shown to have anti-inflammatory, antioxidant and anti-cancer properties. It is also used in traditional medicine to treat a variety of conditions including insomnia, anxiety and high blood pressure[3]. The biosynthetic pathway of scopoletin is a complex process that is still not fully understood. However, the identification of the enzymes involved in the pathway has opened up new possibilities for the production of scopoletin and other coumarins. In the future, it may be possible to engineer plants to produce higher levels of scopoletin or to produce scopoletin in other organisms such as bacteria or yeast. This could lead to new applications for scopoletin in medicine and industry[34,49,50,57].

Toxicity, Adverse Effects and Dose of Scopoletin: Although coumarins have many pharmacological benefits, there are few reports of negative effects from exposure to high doses of the coumarin group, such as scopoletin. Preclinical studies using animal models were used to evaluate the toxicity of coumarins; chronic and subchronic studies on B6C3F1 and CD-1 mice treated with 19-300 mg/kg BW and 300-3 000 ppm coumarins respectively, showed no signs of toxicity. However, both chronic and subchronic studies found signs of liver toxicity.

Sprague-Dawley rats developed liver toxicity after receiving oral 50–500 mg/kg BW coumarins for 13 weeks. Additionally, the same rats exhibited anemia after receiving 444-5 000 ppm coumarins for 2 years. The tested dose in SD rats was found to be 50–100 mg/kg in the ether-induced arthritis model, 50–200 mg/kg in ICR mice in the Murine air pouch model and 1.5–10 mg/kg in SD rats in the drug-induced liver injury model. In the acute oral toxicity model of SD rats, the tested dose was found to be 2000 mg/kg and in female Swiss albino mice, the dose was 10-100 mg/kg[35].

Heterogeneity, Limitations, Potential Biases and Challenges in Scopoletin Research: Because scopoletin is found in a variety of plant species, its concentration and potential synergistic effects with other plant compounds can vary significantly, which can affect the observed outcomes in different studies. Research on scopoletin includes a wide range of study designs, including *in vitro* (cell culture), *in vivo* (animal models) and *in silico* studies. Studies frequently use different dosages and administration routes (oral, intravenous, topical) of scopoletin, making it difficult to establish consistent dose-response relationships and extrapolate findings across studies.

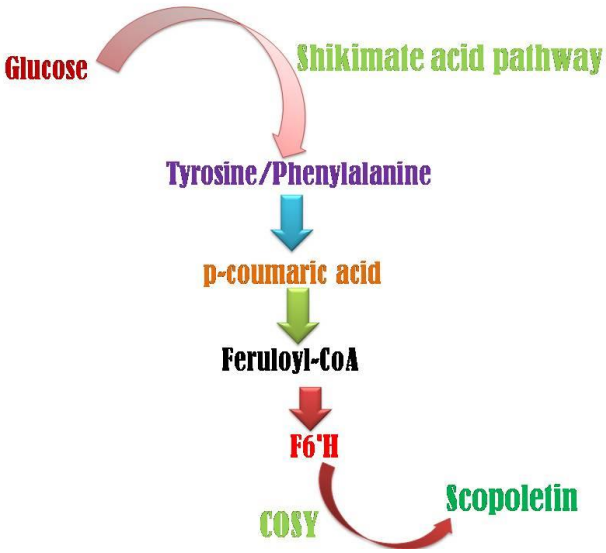

Fig. 4: Biosynthetic pathway of scopoletin

While preclinical research has showed promising outcomes for scopoletin in several disorders, clinical data in people is quite sparse. Many studies have limited sample numbers, short durations and lack strong controls, making it difficult to make solid conclusions about its efficacy and safety in people. The majority of research has concentrated on a small number of illnesses including diabetes, cancer and neurodegenerative diseases. The possible advantages of scopoletin in other circumstances require more investigation. Although some research has looked into scopoletin's modes of action, nothing is known about its exact molecular targets and pathways. A deeper knowledge of its mechanisms of action is vital for designing more effective and tailored therapy solutions[15].

The comparability and reproducibility of research findings can be enhanced by using uniform protocols for study design, dose and outcome measurements. More conclusive proof on the safety and effectiveness of scopoletin in humans can be obtained by carrying out bigger, longer-term clinical studies with strong controls. The mechanisms of action of scopoletin require more investigation in order to guide the creation of more specialized and potent treatment plans. Finding areas of consistency and inconsistency, synthesizing data and guiding future research paths may all be accomplished by doing systematic reviews and meta-analyses of previous studies. Researchers can create safer and more successful treatment plans based on scopoletin by addressing these biases and limitations and gaining a more thorough grasp of the possible advantages and disadvantages of this natural substance[7].

Future Perspectives

Because of its proven antioxidant, anti-inflammatory, anti-cancer, anti-diabetic and anti-hypertensive qualities, scopoletin, a naturally occurring coumarin compound, may find use in clinical settings to treat conditions such as cancer, diabetes, cardiovascular diseases and neurodegenerative disorders. There are few clinical trials examining the effectiveness and safety of scopoletin in people, despite the pre-clinical study results being encouraging. Scopolettin may be investigated as a preventative measure against oxidative stress-related illnesses such as cardiovascular and neurological conditions because of its strong antioxidant activity. According to studies, scopoletin may be able to prevent tumor development and angiogenesis, which would make it a viable option for treating cancer in addition to current treatments.

By increasing insulin sensitivity, scopoletin may help control blood sugar levels, which could be advantageous for diabetic patients. Its anti-inflammatory qualities may help to treat inflammatory conditions like inflammatory bowel disease and arthritis. Scopoletin may play a part in neurodegenerative illnesses like Alzheimer's since it has been demonstrated to shield neurons from oxidative stress-induced damage. According to the research, scopoletin may help to improve cardiovascular health by controlling blood pressure and lowering cardiac oxidative stress. It is still difficult to establish the right dosage and create adequate formulations for scopoletin's therapeutic use. To determine any possible adverse effects and toxicity linked to scopoletin use, more investigation is required.

Conclusion

The advantages of herbal remedies in public health are still a fertile field of study and provide scientists with complete conviction in identifying preventative methods for chronic illnesses of the human body. The absence of standardization is one key impediment to the prospective adoption of traditional medicine as the medicine of choice. Because of their wide range of biological activities and interactions, functional foods have been shown to provide health benefits. Scientific research suggests that scopoletin has beneficial benefits in a variety of deadly conditions including cancer, multiple sclerosis, analgesic, arthritic and Alzheimer's disease.

To treat these diseases, several chemotherapeutic drugs are available, but they have major side effects that might be avoided by employing natural bioactive secondary metabolites such as scopoletin. This review described scopoletin's many possible therapeutic activities which proposed a future treatment for severe disorders. Scopoletin is a coumarin compound that has been shown to have a variety of biological activities *in silico*, *in vitro* and *in vivo* studies. These activities include antioxidant, anti-inflammatory, anti-cancer and anti-diabetic properties.

In silico studies have shown that scopoletin can interact with several different proteins, including enzymes, receptors and transcription factors. These interactions are thought to be responsible for the biological activities of scopoletin. *In vitro* studies have shown that scopoletin can inhibit the activity of several enzymes involved in inflammation and cancer. It can also induce apoptosis in cancer cells. *In vivo* studies have shown that scopoletin can reduce oxidative stress, inflammation and cancer growth in animals. It can also improve blood sugar control in diabetic animals. Overall, the available evidence suggests that scopoletin has several potential therapeutic benefits.

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