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# Effects of Bioactive Compounds on Autophagy: A Systematic Review

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**Abstract:** Autophagy, a crucial cellular process for maintaining homeostasis, plays a significant role in the degradation and recycling of cellular components. Dysregulation of autophagy has been implicated in numerous diseases, including neurodegenerative disorders, cancer, and metabolic conditions. Given the increasing interest in natural, plant-derived compounds for their therapeutic potential, understanding how these compounds influence autophagy is vital. This review aims to provide a comprehensive analysis of the molecular mechanisms through which natural compounds regulate different types of autophagy by targeting specific markers and regulatory signaling pathways such as AMPK, mTOR, and AKT. It also aims to highlight the current gaps in the literature and suggest future research directions to understand these relationships. Research is conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. Studies were extracted from different databases (Scopus, PubMed and Google Scholar) up to 28 February 2024. Inclusion criteria included original studies published in English that examined pure botanical compounds from plant species with direct association to autophagy pathways. A total of 3056 studies, comprised of 68 cell-based studies, 55 animal-based studies, and 39 studies that employed both models were analysed and categorized according

to their botanical families and species with a focus on their autophagy activities. This review identified a total of 103 studies investigating the effects of pure compounds on macroautophagy, 2 studies examining microautophagy, and no studies focusing solely on chaperone-mediated autophagy (CMA). However, 4 studies explored the combined effects of macroautophagy and CMA. Additionally, 9 studies focused exclusively on autophagy-related signaling pathways alone, while 40 investigated both macroautophagy and signaling pathways. It highlights the significant role that isolated bioactive compounds from botanical species play in the regulation of autophagy across a range of diseases and future studies can build upon the findings to pave the way for the development of effective plant-based therapies targeting autophagy pathways for disease treatment.

**Keywords:** autophagy, bioactive compound, mTOR pathway, AKT pathway, AMPK pathway

## 1. Introduction

Autophagy is a fundamental cellular process that facilitates the degradation and recycling of cellular components, which plays a crucial role in maintaining cellular homeostasis. It is a highly regulated mechanism that allows cells to remove damaged organelles, aggregate proteins, and pathogens, to ensure optimal function and survival under stress conditions. Autophagy complex regulation involves different signaling pathways, proteins, and cellular processes. It can generally be divided into three subtypes: macroautophagy, microautophagy and chaperone-mediated autophagy, differentiated in terms of their pathways to transport materials to the lysosome[1]. The regulation of these pathways is complex and involves several keys signaling networks. Some of the extensively studied mechanisms are the negative regulator pathway, mechanistic target of rapamycin (mTOR) signaling pathway, positive regulator AMP-activated protein kinase (AMPK) pathway, and the nutrient sensing pathway PI3K-AKT. Autophagy is also facilitated by over 40 autophagy-related genes (ATGs) involved in the different stages, from initiation to their degradation[2].

As autophagy is essential for various physiological processes, including development, aging, and immune response, disruption of this process is linked to diseases such as neurodegeneration, cancer, and metabolic disorders. In cancer, the autophagy pathway can help suppress tumour initiation but may also help in promote its survival by providing nutrients during stress[3]. In neurodegenerative diseases, disruption in autophagy contributes to the accumulation of toxic protein aggregates, a key factor in the progression of conditions such as Alzheimer's and Parkinson's[4]. In recent years, the role of plant-derived compounds in regulating autophagy has been gaining traction, particularly because many bioactive

substances within plants can influence this critical cellular process. As discussed extensively elsewhere, crude extracts, which are complex mixtures obtained directly from plants and contain a broad spectrum of bioactive compounds, hold significant potential in affecting autophagy pathways[5]. However, the complex composition of these extracts is a challenge, as it is difficult to isolate and understand the precise mechanisms by which individual components impact autophagy.

The biological activity of crude extracts is also highly variable, as it can be influenced by factors such as extraction techniques, plant source, and even environmental conditions, which can significantly influence their efficacy[6]. This complicates reproducibility and limits the findings across studies. In autophagy modulation, crude extracts lack specificity, as they often interact with multiple pathways, making it challenging to specify the precise mechanisms of action. Their complex composition also limits mechanistic insight, as the presence of multiple bioactive compounds complicates the identification of specific molecular targets or pathways involved. This restricts the ability to understand how individual components impact autophagy and exert their effects. In contrast, pure compounds, which consist of a single type of molecule, offer several advantages in autophagy research, particularly in studying their effects. The isolation of active ingredients ensures precision, as it contributes to controlled experimental conditions, reduced variability, and increased reproducibility across studies. It also enables detailed mechanistic insights by clarifying specific pathways and molecular targets involved in autophagy regulation.

This systematic review seeks to elucidate how pure compounds modulate autophagy, specifically by addressing the limitations associated with crude extracts and highlighting the mechanistic insights of pure compounds. This paper offers a clearer understanding of the role of bioactive compounds in autophagy regulation for the development of more targeted therapeutic strategies. Additionally, the review will identify existing knowledge gaps in the literature and propose future research directions to strengthen the understanding of the interactions between bioactive compounds and autophagy. Incorporating these foundational studies will provide an important framework to understand the current landscape of research and their effects on autophagy for future investigations in this area of study.

## 2. Materials and Methods

### *2.1. Search Strategy*

This review was conducted using the publication standard known as Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). To ensure a

comprehensive literature search, the research articles of interest were extracted from three database Scopus, Pubmed and Google scholar, until 28th February 2024. Google Scholar was included to ensure a broader range of sources, including grey literature and studies that may not have significant or positive results. This inclusion is vital for identification of relevant studies that report null findings, which are often overlooked in traditional databases. With the integration of multiple databases, this paper aims to reduce publication and confirmation bias and to enhance the transparency and robustness of our systematic review methodology.

### *2.2. Study Selection*

The study selection process was conducted by two independent reviewers to ensure consistency and reliability in the evaluation of potential studies for inclusion in this systematic review. A PRISMA flow diagram (Figure 1) illustrates the study selection process with details of number of papers identified, screened, assessed and included in the final review. The following criteria were established to determine eligibility:

Inclusion Criteria:

1. Original Research: Only original peer-reviewed journal articles were considered.

2. Publication Stage: Articles must be at the final publication stage, ensuring that all peer-review processes have been completed.

3. Language: Only studies published in English were included to maintain uniformity in data interpretation.

4. Focus on pure bioactive compounds of botanical species: Studies must involve the application of pure bioactive compounds of botanical species in research, specifically those that demonstrate a linkage to autophagy mechanisms.

5. Study Design: Eligible studies included *in vitro* cell models and *in vivo* animal models that investigated neuroprotection.

6. Publication Year: There were no restrictions on the year of publication, allowing for a comprehensive review of relevant literature.

Exclusion Criteria:

1. Non-Original Research: Studies classified as reviews, virtual screenings, letters, case studies, conference papers, opinions, reports, or editorial articles were excluded to focus solely on original research.

2. Non-Botanical Species: Studies that investigated species other than botanical sources were not considered eligible for inclusion.

3. Crude extracts: Research focusing on botanical crude extracts rather than pure compounds was excluded to ensure the review concentrated on bioactive compounds of plant.

4. Lack of Autophagy Examination: Studies that did not specifically investigate autophagy pathways were excluded, as this review aims to explore autophagic mechanisms.

This systematic approach facilitated a thorough and unbiased selection of studies, contributing to the robustness of the review's findings. These exclusion criteria are applied to ensure the systematic review remained focused and relevant, to ensure quality and applicability of the findings.

### *2.3. Data Extraction*

Two reviewers conducted full-text screening to extract data from the selected studies. The information gathered for the systematic review included both the common and scientific names of the botanical species, the plant part utilized, its source, types of extracts, and preparation methods, along with studies on their phytochemical constituents. Additionally, details on study settings, specific experimental models, and the autophagy mechanisms examined, as well as other neuroprotective properties of the species, were recorded. The reported plant names were also cross verified using The World Flora Online (https://www.worldfloraonline.org/) to ensure accurate species identification and taxonomy.

## 3. Results

### *3.1. Search Result*

Figure 1 indicates the search strategy flow and its result. The search resulted in a total of 3056 studies which were available from inception to 7$^{th}$ September 2024. During the screening process, a total of 101 studies were excluded from title and abstract review, and 2832 studies were further excluded after full-text screening. In the subsequent article analyses, botanical species that reported to have neuroprotective activity with autophagy

linkage were categorized based on their family and species level (Table S1), and the types of diseases investigated using these bioactive compounds were analysed (Table 1). Hence, a total of 161 studies were included in this systematic review consist of 68 cell-based studies, 54 animal-based studies, and 39 both *in vitro* and *in vivo* studies (Table 2). Further breakdown of the types of autophagy and signalling pathway involved were also reported (Table 3).

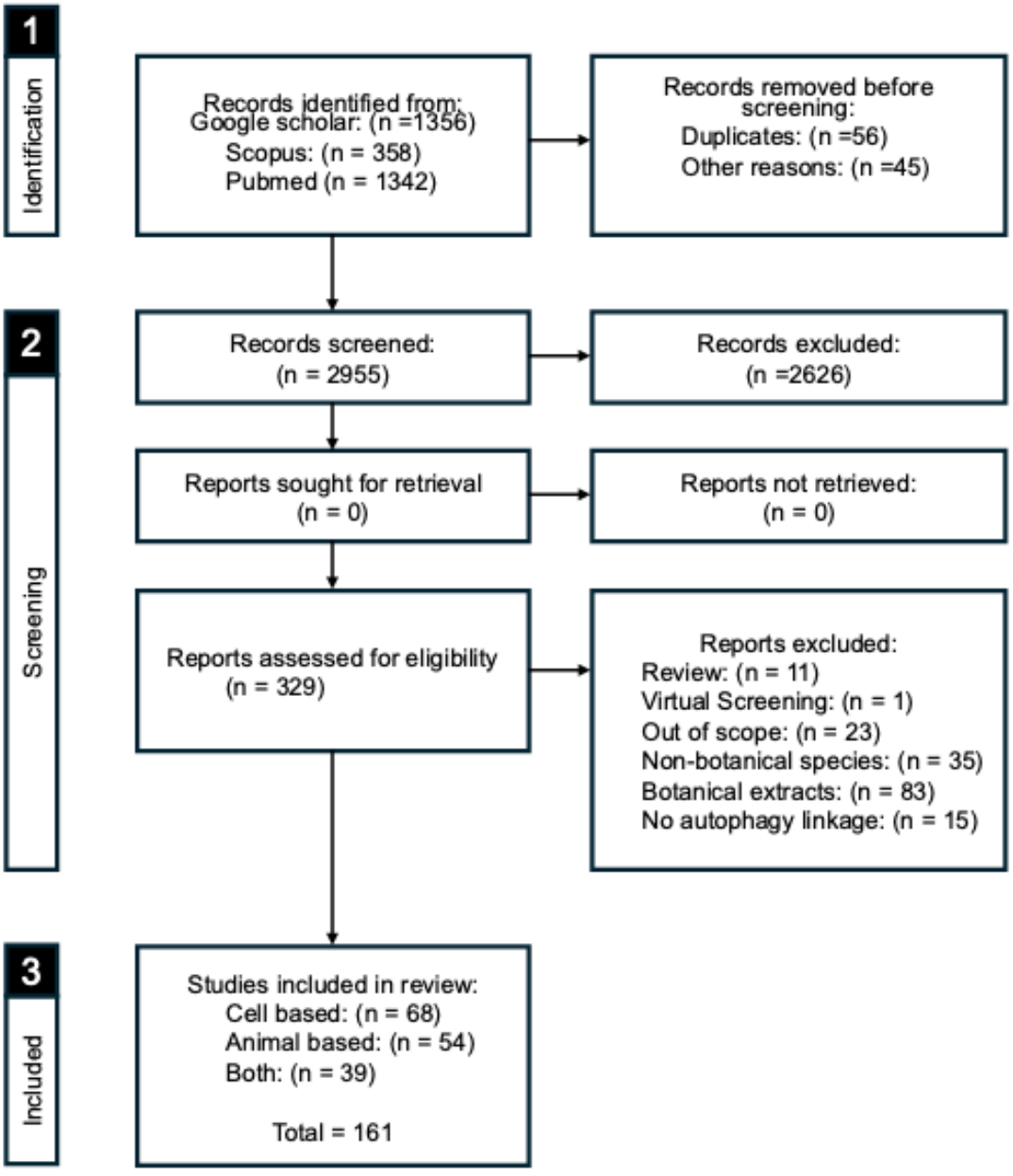


**Figure 1.** PRISMA Flow Diagram of the Systematic Review Process. The diagram outlines the systematic review methodology, from the initial identification of studies to the final inclusion of eligible studies. The diagram also specifies the number of studies identified, screened, included, and excluded at each stage of the review process.

**Table 1.** Disease Spectrum in Autophagy Research. This table categorizes diseases investigated in studies exploring the modulation of autophagy pathways by pure compounds.

| Primary Disease | Number of publications |
|---|---|
| Alzheimer's Disease | 31 |
| Parkinson's Disease | 40 |
| Cancer | 17 |
| Neurological and Neurodegenerative Disorders | 36 |
| Neuropathology and Neurological Conditions | 23 |
| Retinal and Visual Disorders | 2 |
| Others | 12 |

**Table 2.** Study Design and Experimental Models. Table summarizes the study designs employed in the review, differentiating between in vitro, in vivo, and combination of both.

| Type | Number of publications |
|---|---|
| *In vitro* | 68 |
| *In vivo* | 55 |
| Combination | 39 |

**Table 3.** Autophagy Pathways Investigated. Table provides an overview of the different types of autophagy examined across the studies, specifying the number of publications focusing on macroautophagy, microautophagy, chaperone-mediated autophagy, and related signalling pathways.

| Autophagy Type | Number of publications |
|---|---|
| Macroautophagy | 103 |
| Microautophagy | 2 |
| CMA | 0 |
| Signalling pathway | 9 |
| Macroautophagy and signalling pathway | 40 |
| Macroautophagy and CMA | 4 |

## 4. Discussions

### *4.1. Overview of The Autophagy Process*

Autophagy is a vital cellular process responsible for the degradation and recycling of cytoplasmic components, playing a crucial role in maintaining cellular homeostasis. This dynamic mechanism is particularly important under conditions of stress, nutrient deprivation, or during the removal of damaged organelles and proteins. Autophagy can be categorized into different types, including macroautophagy, which involves the formation of autophagosomes to engulf large cargo; microautophagy, where lysosomes directly engulf cytoplasmic material via membrane invagination; and chaperone-mediated autophagy (CMA), which selectively targets proteins with a KFERQ-like motif for lysosomal degradation[7]. Distinction between the categories is made by observing different markers implicated during each process, such as LC3 for macroautophagy, ESCRT machinery for microautophagy, and LAMP2A for CMA. The regulation of autophagy is complex and highly context dependent. Autophagy operates within a regulatory framework involving the Akt, mTOR, and AMPK signaling pathways, which can either promote or suppress the

autophagy process depending on cellular conditions. These are summarised in detail in Table 4.

**Table 4.** Autophagy Markers and Detection Methods. Table detailed specific types of autophagy, the key markers associated with each autophagy type, and the commonly employed detection methods used across the reviewed studies.

| ***Autophagy Distinction*** | | | |
|---|---|---|---|
| **Types** | **Description** | **Key markers** | **Detection method** |
| Macroautophagy | • Non-selective<br>• Engulfment by autophagosomes | ULK1, Beclin-1 and LC3II/I ratio | • Western Blot<br>• Immunofluorescence<br>• Electron Microscope |
| Microautophagy | • Non-selective<br>• Engulfment by lysosomes | Lysosomal membrane dynamics. ESCRT mechanics | • Electron Microscope |
| Chaperone-mediated autophagy | • Highly selective<br>• Degradation by specific protein | HSC70, LAMP2A | • Degradation route measurement |
| ***Regulatory Pathway*** | | | |
| **Pathway** | **Role** | **Mechanism** | **Detection methods** |
| AMPK | Positive regulation autophagy | Inhibit mTORC1, activate ULK1 | • Western Blot: ULK1 phosphorylation<br>• AMPK activation assays |
| mTOR | Negative regulation autophagy | Inhibit ULK1 – prevent autophagous formation | • Western Blot: ULK1, S6K |
| AKT/P13K | Inhibit autophagy | mTORC1 activation - promotes cell growth | • Western Blot: AKT phosphorylation |

### *4.1.1 Macroautophagy*

Macroautophagy is essential for cellular homeostasis and survival, particularly during stress conditions such as nutrient deprivation, hypoxia, or oxidative damage. It degrades and recycles damaged organelles, misfolded proteins, and intracellular pathogens, preventing the accumulation of toxic cellular waste[8]. This process also provides cells with energy and essential building block during metabolic stress. Impairment of macroautophagy has been linked to neurodegenerative disorders such as Alzheimer's and Parkinson's, as well as cancer and certain metabolic conditions. This process involves the formation of autophagosomes, which engulf damaged organelles and proteins and are subsequently delivered to lysosomes for degradation. The effects of pure compounds on macroautophagy are determined based on key markers, which are commonly assessed. The most common marker is the LC3-II/I ratio, which indicates the conversion of LC3-I to LC3-II and is a hallmark of double-membrane autophagosomes formation[9]. An increased LC3-II/I ratio generally indicates enhanced

autophagic activity, while a decrease may suggest impaired autophagic flux. Beclin-1 is another essential protein, typically observed during the initiation stage of macroautophagy, and elevated levels are associated with increased autophagic activity. Lastly, macroautophagy activity can also be assessed by measuring the levels of p62 (SQSTM1). As a receptor, p62 binds to ubiquitinated proteins and facilitates their degradation via autophagy[10]. High levels of p62 indicate impaired autophagic degradation, while decreased levels suggest effective clearance of substrates.

Table 3 indicates that most of the pure bioactive compounds extracted and studied primarily exert their effects through macroautophagy. Out of 161 papers, 103 are categorised under macroautophagy alone. Certain bioactive compounds can be extracted from the same plant source, with similar effects on autophagy. For instance, *oleuropein aglycone*, a polyphenol derived from olive oil, is a known enhancer of macroautophagy activity observed by the increased LC3-II/I ratio and Beclin-1 levels[11,12,13]. Similarly, hydroxytyrosol, also another polyphenolic compound sourced from olive oil, increases the LC3-II marker level[14]. A study combining the two compounds observed an upregulation of the LC3-II/I ratio and Beclin-1 levels while reducing p62 expression[15]. Green tea is another rich source of various bioactive compounds, with compounds such as epigallocatechin-3-gallate (EGCG) and catechin, which have been shown to play key roles in the regulation of macroautophagy[16,17,18]. Wogonin and baicalein are bioactive flavonoids derived from the plant *Scutellaria baicalensis*, commonly known as Chinese skullcap. Both compounds belong to the flavonoid family, with similar chemical structure and are known for their diverse biological activities. In context of autophagy, the compounds are involved in macroautophagy[19,20,21]. Goniothalamins[22] which is a bioactive compound from styryl lactone and celastrol from celastraceae[23] also increases LC3II/I ratio indicating its involvement in macroautophagy.

However, the relationship of the pure compound effects on macroautophagy are complex. For example, resveratrol which is a naturally occurring polyphenolic compound predominantly found in the skin of red grapes, berries, and certain nuts demonstrated a complex role in modulating autophagy. Resveratrol has been shown to increase autophagic activity by enhancing the LC3-II/I ratio[24,25,26,27] and promotes upregulation of Beclin-1[27,28] while reducing the levels of p62[29,30,31,32,33,34]. During low energy condition, enhanced autophagy recycles cellular components to support energy production[35]. In condition where there is high oxidative stress, autophagy plays a protective role by reducing reactive oxygen species (ROS) levels, preventing cellular damage, and activating survival pathways[36]. These

mechanisms together support autophagy as a protective response. In contrast, these studies showed resveratrol suppressing autophagy in specific disease models or under certain conditions. For example, in traumatic brain injury models and oxiapoptophagy (cell death that combines features of oxidative stress, apoptosis, and autophagy), resveratrol treatment can lead to a decrease in autophagic markers LC3-II/I and Beclin-1[37,38]. This suppression might occur because, under extreme oxidative stress, cellular processes may prioritize apoptosis over autophagy to manage cellular damage. This dual role suggests that resveratrol's effect on autophagy is context-dependent, where it promotes autophagic processes under energy-limiting conditions but potentially inhibits them in high-stress scenarios. The shift towards apoptosis may be due to mechanisms such as mitochondrial dysfunction and excessive ROS production that overwhelm autophagic pathways[39,40]. It is also possible that the observed decrease in autophagic markers is likely due to an impaired autophagic flux, and not outright inhibition, as ROS interfere with lysosomal function and steps in autophagic degradation[41]. Understanding this condition-specific response is critical for therapeutic applications, particularly in diseases marked by oxidative stress, such as neurodegenerative disorders. Future research should aim to clarify the molecular pathways through which resveratrol modulates autophagy across different metabolic states, exploring dose-response relationships and timing to maximize therapeutic efficacy while minimizing adverse effects from autophagic suppression.

Curcumin, which is also a polyphenolic compound like resveratrol exhibits a context-dependent influence on autophagy. In certain conditions, particularly those characterized by oxidative stress and low energy levels, curcumin can enhance autophagic activity. In macroautophagy, curcumin enhances autophagic activity observed from the increases in LC3II/I ratio and Beclin-1 in epilepsy[42,43] while also reducing p62[44]. However, like resveratrol, its effects can vary; under specific stress conditions, it may also induce apoptosis instead of promoting autophagy. This duality underscores the importance of the cellular environment in determining whether curcumin acts primarily as an autophagy enhancer or as an apoptotic agent. Thus, both curcumin and resveratrol demonstrate the potential to induce autophagy, but their efficacy and role can shift based on the presence of oxidative stress and energy availability.

Some pure compounds are also known to suppress macroautophagy. For example, breviscapine which is classified as a flavonoid, belongs to the broader family of polyphenols and have neuroprotective effects against focal cerebral ischemia via suppression of macroautophagy[45]. Compound such as n-butylidenephthalide[46], icariin[47] and Icariside II[48] also help to suppress macroautophagy. Another example is *Lycium*

*barbarum* polysaccharide, which suppressess macroautophagy indirectly via its signalling pathway[49]. A comprehensive list detailing the effects of each pure compound on macroautophagy is presented in Table S1.

### *4.1.2. Microautophagy*

Microautophagy is a selective degradation process in which lysosomes directly engulf specific cytoplasmic components, such as damaged organelles or misfolded proteins, through the invagination of their membranes[50]. This selectivity is mediated by molecular tags (e.g., ubiquitin) and receptors (e.g., p62/SQSTM1), which recognize and target dysfunctional or unnecessary material for degradation, ensuring cellular homeostasis. This process involves the protrusion or invagination of the lysosomal membrane, allowing for the uptake of cytoplasmic components. Additionally, endosomal invagination can create multivesicular bodies that transport these components into the lysosomal lumen. This helps in providing a rapid turnover of proteins and organelles and is important for cellular quality control.

Monitoring microautophagy typically involves detecting the transport of lysosomal transmembrane proteins into the organelle lumen. In yeast, this can be achieved through immunoblot analysis of tagged proteins[51]. In mammalian cells, exosomes serve as indicators of intraluminal vesicle biogenesis, reflecting similarities between exosomal and microautophagic pathways[52]. However, extra caution is needed when interpreting data from assays that do not distinguish between autophagic and microautophagic activities. This is due to the markers for microautophagy are less well-defined than those for macroautophagy as the process is usually observed indirectly via techniques like electron microscopy (EM). However, recent studies have identified specific proteins, such as LC3 and LAMP2 that may be involved in microautophagy[53]. The use of these markers is still an emerging area of research. Some reports have also adopted the markers during membrane fusion systems in autophagy, such as Rab and ESCRT I/III which also contribute to microautophagy[54].

Frm the paper reviewed, aloe emodin compound from aloe vera indicates autophagic enhancement due to the increased number of vesicles[55]. In contrast, in a cerebral ischemia *in vivo* model, Silymarin, derived from *Silybum marianum* (milk thistle), was found to reduce MDC (Monodansylcadaverine) staining, indicating fewer autophagic vacuoles and suggesting decreased autophagic activity[56]. Since MDC staining marks autophagosomes, this reduction may reflect inhibition of autophagosome formation or impaired autophagic flux. However, further studies using markers like LC3-II and p62/SQSTM1 are needed to confirm whether Silymarin inhibits autophagy via microautophagy or a different pathway.

### *4.1.3. Chaperone-mediated autophagy (CMA)*

Chaperone-mediated autophagy (CMA) is a selective form of autophagy that degrades specific cytosolic proteins with pentapeptide motif (KFERQ-like sequence) within the lysosomes[57]. It is a process that involves recognition of substrates by molecular chaperones, Heat Shock Cognate 70 (Hsc70) that transport targeted proteins to the lysosomal membrane[58]. In the lysosome, the substrates are then translocated across the membrane in the presence of LAMP2A, making it a key lysosomal membrane protein that forms a multimeric complex facilitating protein import[59] (Losmanová et al., 2020). CMA is usually observed from the levels of Hsc70 and LAMP2A.

Resveratrol and curcumin, two well-known polyphenolic compounds, are involved not only in macroautophagy but also in CMA. Resveratrol upregulates LAMP2 expression, promoting CMA and this is vital in the clearance of damaged or misfolded proteins as therapeutic potential for neurodegenerative diseases such as Alzheimer's disease[33,34]. Similarly, curcumin also demonstrated the ability to upregulate LAMP2 in *in vitro* models of Alzheimer's disease[43], further suggesting that it could enhance CMA activity. By modulating LAMP2 expression, both compounds show promise in improving the degradation of toxic protein aggregates, highlighting their potential as therapeutic agents in neurodegenerative conditions. Paeoniflorin is a monoterpene glycoside from the *Paeoniaceae* family which has shown neuroprotective effects as it is involved in both macroautophagy and CMA[60]. Quercetin is another type of polyphenol which has been shown to be involved in both macroautophagy and CMA[61].

### *4.1.4. The Role of AMPK, mTOR, and AKT pathways in autophagy regulation*

The regulation of autophagy is complex and involves several key signalling pathways such as mechanistic target of rapamycin (mTOR), protein kinase B (AKT), and AMP-activated protein kinase (AMPK). Understanding these pathways is crucial to understand how different compounds influence autophagic processes in health and disease. The mTOR pathway is the central regulator of cell growth, proliferation, and metabolism[62]. As a nutrient-sensing pathway, mTOR, particularly through the mTORC1 protein complex, inhibits autophagy under conditions of nutrient abundance, making it a key negative regulator of autophagy. Activation of mTORC1 phosphorylates key substrates that in turn suppress autophagic initiation[63]. During nutrient deprivation or stress, mTOR activity is inhibited, leading to activation of autophagy and is crucial for cellular adaptation especially during metabolic stress and prevents accumulation of damaged organelles and proteins. Another negative autophagy regulator is AKT or protein kinase B, important in cell survival and

metabolism and is activated by growth factors and insulin signalling. Activation of AKT promotes cell growth and survival while inhibiting autophagy via phosphorylating components involved in the autophagic process, AKT can suppress the initiation of autophagy[64]. Therefore, targeting the AKT pathway may provide a therapeutic strategy for enhancing autophagic activity in various diseases. In contrast, AMPK promotes autophagy in response to low-energy states making it a positive regulator. When cellular energy levels are low, an increased AMP/ATP ratio, activates AMPK and inhibits mTOR signalling[65]. This activation leads to the upregulation of autophagy-related genes and promotes clearance of the damaged cellular components. The interplay between mTOR, AKT, and AMPK pathways is crucial for the regulation of autophagy and understanding pure compounds affecting these signalling pathways provides valuable information into potential therapeutic strategies aimed at modulating autophagic processes in various diseases which are illustrated in Figure 2.

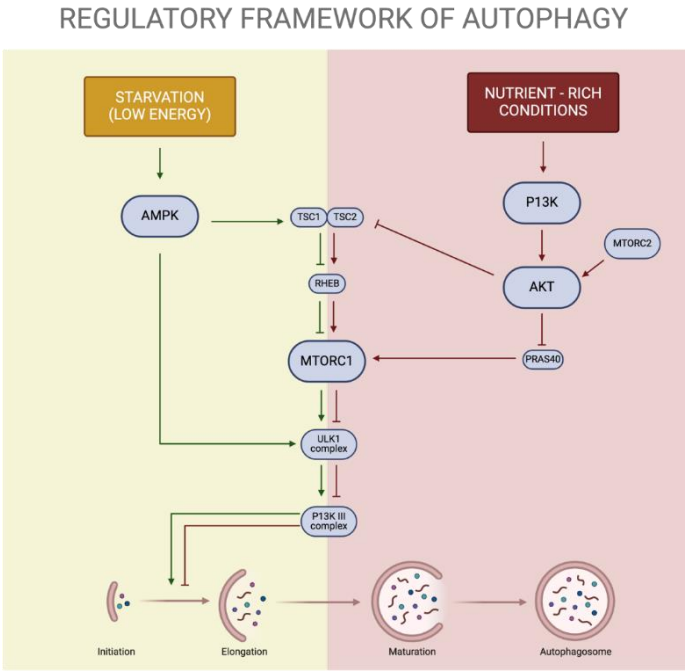


**Figure 2.** Regulatory Framework of Autophagy. This figure illustrates the regulatory framework of autophagy, highlighting the roles of key signaling pathways and complexes under different cellular conditions. Autophagy is regulated by the interplay of AMPK, mTORC1, Akt/PI3K, and their downstream effectors, which either promote or inhibit autophagy depending on nutrient availability and energy status. AMPK, AMP-activated protein kinase; mTORC1/2, mechanistic target of rapamycin complex 1/2; PI3K, phosphoinositide 3-kinase; Akt, protein kinase B; TSC1/2, tuberous sclerosis complex 1/2; RHEB, Ras homolog enriched in brain; ULK1, Unc-51-like kinase 1; PRAS40, proline-rich Akt substrate of 40 kDa. Created in BioRender. Ming, L. (2025) https://BioRender.com/c49f502.

Several bioactive compounds have shown the ability to modulate autophagy via these pathways, providing potential therapeutic benefits in various disease contexts, Resveratrol which is known to influence autophagy via macroautophagy and CMA also indicates the

ability to regulate autophagy via suppression of mTOR pathway and activation of the AMPK pathway[24,30,32,66]. Resveratrol inhibits mTOR by competing with ATP at its binding site [36] and activates AMPK through phosphorylation of ULK1, which becomes crucial under low energy or high oxidative stress conditions. Arctigenin, a lignan from burdock, suppresses the mTOR pathway in both *in vitro* and *in vivo* models of AD[67] while in a diabetic model, it not only inhibit mTOR but activates AMPK[68]. This adaptability highlights arctigenin's versatility in modulation of the autophagy pathways based on underlying metabolic conditions.

From Table S1, other bioactive compounds further illustrate this context-dependent modulation of signalling pathways. Berberine and curcumin are known to decrease mTOR levels[69,70], while curcumin also reduces AKT activity[44], aligning with its known autophagy-enhancing properties. Similarly, catechin from green tea decreases both AKT and mTOR levels[18], and wogonin from *Scutellaria baicalensis* is recognized for its ability to selectively downregulates mTOR[19]. Additionally, carnosic acid from rosemary promotes autophagy via activation of AMPK[71], while diosgenin from *Dioscorea nipponica* and piperine have been shown to reduce PI3K, AKT, and mTOR signaling pathways[72,73].

While many compounds are known to enhance autophagy, certain compounds such as alkaloid oxymatrine, suppresses autophagy by regulating the P13K/Akt/mTOR pathway[74]. This is important as suppression of autophagy in this context, offers neuroprotective effects after hypoxic-ischemic brain damage by reducing cellular stress. Hence, regulation of autophagy is highly context-dependent; therefore, understanding the specific circumstances under which activation or suppression occurs is important for developing effective therapeutic strategies. Recognizing these varying effects helps in therapeutical interventions that focuses on the beneficial aspects of autophagy modulation based on the specific pathological context.

### *4.2. Disease-Specific Context and Autophagy Regulation*

#### *4.2.1. Alzheimer disease*

Alzheimer's Disease (AD) is characterized by the accumulation of amyloid-beta (Aβ) plaques and tau tangles, leading to synaptic dysfunction and neuronal death[75,76]. In the context of autophagy, dysregulation leads to accumulation of these toxic aggregates by impairing their clearance. Within the autophagic signalling pathways, hyperactivation of the mTOR pathway is commonly observed in AD patients, effectively inhibiting autophagy and increasing protein aggregation[77]. Targeting mTOR with compounds that can enhance

autophagic activity, facilitating the clearance of Aβ plaques and tau tangles can be used as a potential therapeutic approach. Additionally, activation of AMPK supports autophagy by inhibiting mTOR, which is important for promoting the removal of toxic aggregates and may offer neuroprotective benefits in AD[78]. Furthermore, overactivation of the AKT pathway can suppress autophagy, contributing to the accumulation of Aβ.

In Alzheimer disease, several bioactive compounds have demonstrated neuroprotective effects, although their mechanism does not involve autophagy. For instance, epigallocatechin-3-gallate exerts neuroprotective properties in rat primary cortical neurons without affecting autophagic activity[79]. Similarly, limonene (+) also known as D-limonene commonly found in citrus fruit does not influence autophagy-related factors, and the use of an autophagy inhibitor did not diminish limonene's protective effect on the viability of Drosophila models of Alzheimer's disease[80]. Additionally, urolithin A exhibited only minimal neuroprotective effects and did not activate autophagy[81]. This indicates that further research should be done to understand the exact mechanism of the neuroprotective effects exerted by these compounds.

*4.2.2. Parkinson disease*

Parkinson's Disease (PD) is marked by the accumulation of misfolded alpha-synuclein protein aggregates. Similar to AD, impaired autophagy is linked to the pathogenesis of PD, leading to neuronal damage and degeneration[82]. Hyperactivation of mTOR in PD inhibits autophagy, promoting the accumulation of alpha-synuclein aggregates. Inhibition of mTOR has been shown to enhance autophagic degradation of these aggregates, potentially alleviating PD symptoms. AMPK activation can improve mitochondrial function and promote the clearance of damaged proteins in PD. Enhancing AMPK activity may therefore provide neuroprotective benefits. Similar to AD, AKT overactivation can inhibit autophagy in PD. Targeting AKT signalling may help restore autophagic processes crucial for neuronal health.

Compounds such as celastrol[23], polydatin[83], and baicalein[20] have been identified as autophagy inducers, leading to reduced α-synuclein levels in SH-SY5Y cells. Similarly, in *C. elegans*, a nematode worm animal model, β-amyrin has been shown to exert anti-PD effects by reducing α-synuclein aggregation through the LGG-1-mediated autophagy pathway[84]. In contrast, compound such as harmine extracted from *peganum harmala* helps α-synuclein clearance. Interestingly, this process remains unaffected by disruption of autophagy inhibition, lysosomal inhibition, and silencing of autophagic genes[85]. This confirms that its effect is independent of autophagy.

### *4.2.3. Cancer*

Autophagy has a dual role in cancer; it can suppress tumour initiation by maintaining cellular homeostasis and preventing genomic instability, but it can also support tumour growth under certain conditions, such as nutrient deprivation or hypoxia, by enabling cancer cells to survive and adapt to stress. In early stages, autophagy acts as a protective mechanism by eliminating damaged components and pre-cancerous cells. In established tumours, autophagy promotes cancer cell survival, progression, and resistance to therapies[86]. The balance between promoting and inhibiting autophagy is vital for cancer therapy outcomes because autophagy itself exhibits a dual role in cancer, acting to either suppress tumour initiation or promote survival and resistance in advanced stages, thus influencing whether targeting it will prevent tumour initiation or enhance treatment effectiveness, depending on the context. Many cancers exhibit hyperactivated mTOR, which inhibits autophagy and allows tumour cells to survive under stress. Targeting mTOR with inhibitors can induce autophagy and promote cancer cell death, while AMPK activation has been shown to inhibit cancer cell proliferation by promoting autophagic degradation of oncogenic proteins[87]. In some cases, AKT signalling often promotes cancer cell survival by inhibiting autophagy and inhibiting AKT may promote apoptosis via restoration of autophagic processes.

Some anticancer plants highlighted in the Moroccan toxicological review exhibit pro-apoptotic and anti-proliferative effects by inducing ROS, mitochondrial dysfunction, and caspase activation. Although autophagy was not directly assessed, these pathways overlap with autophagic regulation, suggesting a possible role for these compounds in autophagy-mediated cancer cell death[88]. Compounds like magnolol have been shown to induce cytotoxicity in HELA cells[89]. Similarly, 4,6' anhydrooxysporidinone and diosgenin exhibit cytotoxic effects on MCF-7 breast cancer cells and DU145 prostate cancer cells, respectively[72,90]. In contrast, Platycodin D induces cell death in brain tumour cells by suppressing autophagy, leading to a decline in autophagosome-lysosome formation and lysosomal proteolytic activity[91]. The different mechanisms of action among these compounds indicate the complexity of targeting autophagy in cancer treatment. For instance, while magnolol and other compounds activate pathways that promote cell death through direct cytotoxic effects, Platycodin D takes a different approach by inhibiting autophagy. This suppression is effective in cancer cells that rely on autophagy for survival under stress conditions. By reducing the formation of autophagosomes and impairing lysosomal function, Platycodin D effectively decreases the cancer cells' ability to manage stressors, leading to increased cell death. Other compounds may induce cell death through apoptosis or direct cellular damage. Hence, inhibiting autophagy can exploit the dependence of certain cancer

cells on this process for their survival. Understanding these mechanisms is crucial for developing targeted therapies that can effectively reduce tumour viability while minimizing harm to normal cells.

In neurodegenerative diseases such as AD and PD, the therapeutic goal is generally the activation autophagy via AMPK activation or mTOR inhibition to enhance the clearance of toxic protein aggregates. In cancer, the approach varies; while autophagy activation can induce cell death in certain cancers, in others, tumour cells exploit autophagy for survival, necessitating a strategy that balances autophagy activation and suppression. A comprehensive understanding of AMPK, mTOR, and AKT in autophagy regulation is therefore essential for developing targeted therapies. By selecting specific compounds and pathway targets based on the disease context, therapeutic strategies can be tailored to optimize the benefits of autophagy modulation.

### *4.3. Limitation and Future Directions*

From the analysis, the effects of pure plant compounds on autophagy are highly promising, however some limitations must be considered. One of the most prominent issues is the variability in experimental models used. Many studies in this review, despite focusing on the same disease, employed different cell lines and animal models, leading to inconsistencies in results that limits the generalization of findings. For example, varying responses across model types can lead to misinterpretations regarding the efficacy and toxicity of bioactive compounds. Research has shown that results obtained from one model may not be replicated in another due to inherent biological differences, which complicates the translation of preclinical findings into clinical applications[92]. Inconsistent dosages, treatment durations, and bioactive compound introduction methods further complicate direct cross-study comparisons as changes in methodological parameters can yield significantly different outcomes and variations in how autophagy is induced or measured. Discrepancies in study designs can hinder the establishment of clear therapeutic guidelines, making it difficult to predict patient responses based on preclinical data[93]. Establishing clear guidelines will facilitate better understanding and application of findings related to autophagy.

To overcome these issues, advanced statistical techniques such as meta-analyses, random-effects models, and sensitivity analyses are recommended for future studies. This will help to account for the differences in study designs, dosage levels, and outcomes, enabling the data synthesization to be more accurate and to make reliable conclusions on the compounds' efficacy. These methods help to ensure that any observed discrepancies in

outcomes are due to methodological nuances rather than intrinsic variations in the compounds' effects. Additionally, meta-regression approaches allow researchers to systematically adjust for specific variables, like model type or dosage, and assess their influence on outcomes, providing insight into the conditions under which a compound may be effective[94]. This approach is vital for translating preclinical findings into clinical applications, where consistency and reliability are required.

The lack of standardization in measuring autophagy markers poses another challenge. Different techniques and criteria for assessing autophagic activity result in variable outcomes, making it difficult to synthesize data and draw consistent, generalizable conclusions. While numerous studies have reported the effects of bioactive compounds on autophagy markers, there is often a significant gap in understanding the detailed mechanistic insights behind these effects. For instance, while compounds such as aloe emodin and goniothalamins have been shown to enhance autophagic activity and induce apoptosis in cancer cell lines, the specific pathways through which they exert these effects remain poorly defined[22,55]. This lack of clarity can hinder the development of targeted therapies that effectively harness the therapeutic potential of these compounds. Understanding the underlying mechanisms is crucial, as it not only informs the selection of appropriate compounds for further research but also helps in optimisation of its formulations and delivery methods for clinical applications.

Hence, it is important to enhance the standardization and reproducibility of autophagy research, and this can be achieved via specific set of recommendations for autophagy assays, bioavailability assessments, and delivery methods. These measures will address methodological variability and provide a roadmap for future research efforts, ultimately making findings more clinically applicable. Collaborative research efforts are essential to enhance the reliability of autophagy studies, with shared experimental standards and best practices in assay development improving comparability across studies. Adopting detailed reporting guidelines requiring disclosure of model types, dosages, administration routes, and endpoints can further support transparency, reproducibility, and more precise cross-study comparisons. A set of guideline[9] recently updated serves as a foundational step toward standardizing autophagy assays. Developed by expert researchers in the field, the guidelines outline essential protocols and interpretations for autophagy monitoring, setting a benchmark for consistency and accuracy across studies[9]. By following these guidelines, research groups can produce more reliable and comparable data, ultimately supporting reproducibility and advancing the field of autophagy research.

To expand our understanding and improve the therapeutic potential of these compounds, several future research avenues are recommended. The combination of multiple plant-derived compounds may offer enhanced efficacy in regulating autophagic processes. While some studies have already begun to explore these synergistic effects, further research is necessary to fully understand the potential benefits of such combinations. It is also important to study the interactions between compounds at molecular and cellular levels as it could potentially reveal more potent therapeutic strategies, especially in the context of complex diseases where single-agent treatments have shown limited success. An exploration of these synergistic or additive effects could not only improve our understanding of autophagy modulation but also pave the way for novel, multi-targeted interventions that capitalize on the complementary actions of different bioactive compounds.

Another key area for future research is in optimisation of the extraction and formulation techniques for pure compounds to ensure maximum efficacy in autophagy modulation. For studies focusing on pure compounds, ensuring consistency in purity and efficacy is critical. While some compounds are commercially sourced and come with verified purity, others are extracted in-house, where variability in extraction methods can affect the final product's quality. The standardization of extraction techniques to maintain uniformity in the purity and potency of these bioactive compounds is important as it will enhance the reproducibility of results across studies. Additionally, optimizing formulation techniques, such as improving the bioavailability and stability of these compounds will be essential. Advances in delivery methods, such as targeted formulations, can further improve the pharmacokinetic profiles of these pure compounds, thereby maximizing their therapeutic potential in modulating autophagy.

Autophagy’s therapeutic potential is not limited to neurodegeneration and cancer. It has also been investigated in the context of infectious diseases, particularly COVID-19, where its modulation may influence viral replication, immune responses, and inflammation. Recent studies suggest that targeting autophagy-related pathways could aid in the development of supportive therapies to manage viral load and disease severity[95,96]. These findings highlight autophagy as a versatile target across a wide spectrum of diseases, warranting further research beyond traditional therapeutic categories. While numerous studies have demonstrated the potential of autophagy regulation in ameliorating disease pathology, these findings required validation via human studies. For example, the positive outcomes observed in animal models of AD suggest that similar approaches could be explored in human trials targeting the underlying mechanisms of neurodegeneration. However, translating these findings to human trials requires rigorous testing to confirm

efficacy and assess the potential side effects. By focusing on disease conditions with strong preclinical support and establishing clear translational pathways, researchers can enhance the likelihood of successful therapeutic applications of autophagy-modulating strategies.

## 5. Conclusions

In conclusion, this systematic review provides a comprehensive analysis of the effects of pure plant-derived compounds on autophagy across various disease models. By focusing on isolated compounds, the review offers targeted insights into their specific roles in autophagic regulation, both *in vitro* and *in vivo*. These compounds have demonstrated the potential to modulate key autophagy pathways, which is crucial for identifying therapeutic benefits in diseases characterized by autophagy dysregulation. The findings emphasize the need for validation through human clinical trials, as current research primarily relies on preclinical data. Establishing these links is crucial to translate preclinical successes into viable treatment options. Furthermore, isolating pure compounds allows for a clearer understanding of their bioactivity compared to crude plant extracts, which may contain complex mixtures of bioactive substances that can interact synergistically or antagonistically. By addressing these gaps, this review highlights the therapeutic potential of bioactive compounds in treating diseases associated with autophagy dysregulation.

**Supplementary Materials:** Table S1: Categorization of Studies by Autophagy Type and Compound.

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