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A Narrative Review – Role of Autophagy in Drug Resistance Mechanism and its Potential in Methanolic Extract of Arabidopsis thaliana By HPLC Method

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ABSTRACT

Autophagy, a cellular self-degradation process, plays a complex role in various biological processes, including drug resistance. Autophagy can both promote and inhibit cell death, depending on the cellular context and the type of stress. Autophagy can Promote Drug Resistance, Inhibit Drug Resistance, Combining autophagy inhibitors with conventional chemotherapy or targeted therapies may enhance their efficacy by preventing autophagy-mediated drug resistance. Arabidopsis thaliana, a small flowering plant, has emerged as a powerful model organism in plant biology. Its rapid life cycle, small genome size, and ease of cultivation have made it an invaluable tool for understanding fundamental biological processes. Arabidopsis completes its life cycle in just six to eight weeks, allowing for rapid generation times and efficient genetic studies. Chloroquine is known to inhibit autophagy, a cellular process involved in the degradation and recycling of cellular components. Methanolic extraction is a widely used technique in natural product research to extract bioactive compounds from plant and animal sources. Its versatility, efficiency, and relative safety have contributed to its widespread use in various industries. High-Performance Liquid Chromatography (HPLC) is a versatile analytical technique widely used to separate, identify, and quantify components in complex mixtures. Arabidopsis thaliana might absorb chloroquine from the methanolic extract. In Arabidopsis, chloroquine treatment has been shown to disrupt autophagy, leading to the accumulation of cellular debris. HPLC analysis could then be used to quantify the amount of chloroquine present in the plant tissue.

Key Words: Autophagy, Drug Resistance, Methanolic Extract, HPLC, Arabidopsis thaliana

INTRODUCTIONS

Autophagy is a highly controlled cellular disintegration and recycling process seen in all eukaryotes, including yeast. Autophagy is an evolutionarily conserved intracellular mechanism that degrades and recycles cytoplasmic components via vascular/lysosomal pathways. It enables the cell to withstand food deprivation and other biotic and abiotic stressors. Hormone and amino acid concentrations control autophagy. Autophagy-related genes were initially discovered in yeast and play a variety of activities, including enzyme, signaling, transporter, scaffold, and ubiquity-like protein. Currently, more than 30 autophagy- related genes have been found in yeast, animals, and plants. Autophagosome formation is mediated by autophagy-related proteins (AGS) that form the autophagy activating kinase (ULK) complex and regulatory proteins such as AMP-activated protein kinase (AMP), mammalian target of rapamycin complex, vacuolar protein sorting Beclin 1, B cell lymphoma 2 (BCL-2), and other proteins. Autophagy is a self-degradative process that plays a crucial role in balancing energy sources throughout critical developmental stages and in response to nutritional stress. Autophagy also performs housekeeping functions by removing misfolded or aggregated proteins, cleaning damaged organelles including mitochondria, endoplasmic reticulum, and peroxisomes, and eliminating intracellular infections. Thus, autophagy is often regarded as a survival process, despite the fact that dysregulation has been associated to non-apoptotic cell death. Autophagy can be non-selective or selective in its removal of certain organelles, ribosomes, and protein aggregates, while the processes governing selective autophagy are not fully understood. In addition to removing intracellular aggregates and damaged organelles, Autophagy promotes cellular senescence and antigen presentation, protects against genomic instability, and inhibits necrosis, making it an important preventative mechanism for cancer, dementia, cardiomyopathy, diabetes, liver disease, autoimmune disorders, and infections. Autophagy is executed and regulated at the molecular level, and its disturbance can cause illness. Christian de Duve coined the term 'autophagy', which comes from the Greek for 'eating of oneself,' over 40 years ago. It was largely based on the observed

degradation of mitochondria and other intracellular structures within lysosomes of rat liver perfused with the pancreatic hormone glucagon.

The molecular mechanism of glucagon-induced autophagy in the liver is yet unknown; nevertheless, it is tissue-specific and requires cyclic AMP-induced activation of protein kinase-A. In recent years, the scientific community has 'rediscovered' autophagy, with multiple laboratories making significant advances to our molecular knowledge and awareness of the physiological importance of this process. Although the relevance of autophagy is widely understood in mammalian systems, yeast (Saccharomyces cerevisiae) has made major fundamental discoveries in understanding how autophagy is controlled and performed at the molecular level. Currently, 32 different autophagy-related genes (Atg) have been identified in yeast through genetic screening, and many of these genes are conserved in slime mould, plants, worms, flies, and mammals, highlighting the importance of the autophagic process in starvation responses across phylogeny. Autophagy begins with an isolating membrane, also known as a phagophore, which is most likely produced from a lipid bilayer provided by the endoplasmic reticulum (ER), trans-Golgi, and endosomes, however the exact genesis of the phagophore in mammalian cells is debated. This phagophore extends to engulf intracellular cargo such as protein aggregates, organelles, and ribosomes, which are then sequestered in a double-membraned autophagosome. The filled autophagosome develops by fusion with the lysosome, which promotes the breakdown of autophagosomal contents by lysosomal acid proteases. Lysosomal permeases and transporters return amino acids and other degradation byproducts to the cytoplasm, where they can be repurposed for macromolecule assembly and metabolism. Thus, autophagy may be viewed as a cellular 'recycling factory' that enhances energy efficiency via ATP synthesis and conducts damage management by eliminating non-functional proteins and organelles.

CLASSES OF AUTOPHAGY

1. Macroautophagy

A double membrane bound autophagosome forms and unites with a vacuole, dissolving the cell content and recycling the products back into the cell's cytoplasm for reuse.

2. Microautophagy

Cytoplasmic components are immediately ingested by invagination of the tonoplast membrane, resulting in autophagic bodies in the vacuole. It is commonly employed to degrade store proteins during seed germination and development senescence.

3. Mega-autophagy/ Chaperone mediated autophagy

The Tonoplast Ruptures allow vacuolar hydrolases to enter the cytoplasm and destroy cytoplasmic substances. It frequently reflect the ultimate stage of planned cell death.

IT CAN BE DIVIDED INTO FOUR STEPS

1. During nucleation, the autophagosome membrane is formed from its source.
2. The expansion phase lasts until the autophagosome is fully formed.
3. Initiation: Proteins required to commence membrane formation are recruited.The ATG5-ATG16L1 complex lipidates the microtubule-associated protein 1A/1B-light chain 3 (LC3), recruiting autophagy targets.
4. LAMP-2 and Rab7 have a role in autophagosome-lysosome fusion during degradation.

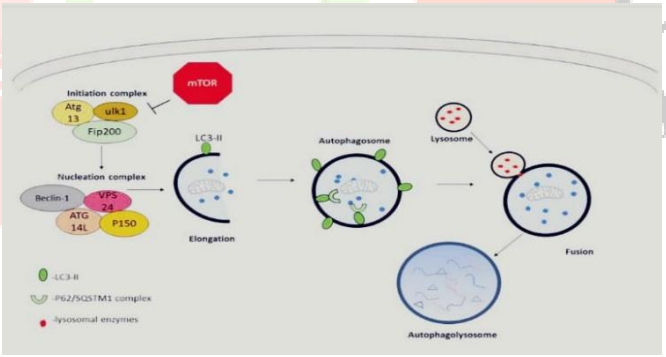

Fig:1 Showing division of Autophagy in four steps

THE MOST IMPORTANT AUTOPHAGY INHIBITORS ARE:

1. Chloroquine (CQ) is a final stage autophagy inhibitor that disrupts the autophagosome-lysosome fusion phase. CQ and its derivative, hydroxychloroquine (HCQ), are the only FDA-approved medications now being used in clinical studies, and they are frequently coupled with normal therapy.
2. Bafilomycin A1 (BafA1) inhibits V-ATPase, preventing autophagosome-lysosome fusion and lysosomal acidification.
3. 3-Methyladenine (3-MA) inhibits class III phosphatidylinositol 3-kinase (PI3K), preventing autophagy early on. It is not considered a selective autophagy inhibitor because it also inhibits class I PI3K. Indeed, under some circumstances, it can stimulate autophagy.
4. Spautin-1, an inhibitor of USP10 and USP13, inhibits autophagy by degrading the PIK3C3/VSP34-Beclin 1 complex.

Lys 05, a CQ derivative, suppresses autophagy by accumulating in the lysosomes. Autophagy, on the other hand, is a general response that is triggered by a range of cellular stresses in a short period of time (minutes to hours). Furthermore, it does not need significant genetic or epigenetic changes or selective pressure. Indeed, medicines that target autophagy are being explored in clinical trials alongside traditional chemotherapy or targeted treatment. However, there are limitations and issues that must be solved. Autophagy serves a dual purpose that is very depending on the circumstances. Knockdown of autophagy-related genes raises the prevalence of cancer in various tissues and, in certain cases, contributes to cell death through a process known as autopsis. Furthermore, autophagy is required for physiological activities in many cells, including immune system control,

metabolism, and senescence. Indeed, autophagy inhibitors such as CQ and HCQ, which have mostly been used to treat malaria and autoimmune illness, have a variety of adverse effects ranging from skin rash to muscular weakness to gastrointestinal and neurological issues, as well as permanent retinopathy. The degree of adverse effects is particularly critical with long-term therapies. In addition, CQ may worsen chemotherapy-related damage in organs such as the kidney, brain, heart, and hematopoietic cells. Another key issue is pharmacological specificity, since both CQ and HCQ do not specifically inhibit autophagy. They instead collect in acidic cellular compartments and interfere with lysosomal activity, disrupting autophagy and other cellular processes. For these reasons It would be nice to have a precise marker to identify those individuals in whom autophagy plays a significant role and enhance therapy efficacy. Unfortunately, this marker is not currently accessible since measuring autophagy in vivo, particularly in people, is extremely difficult. Autophagy can also be activated in response to chemotherapeutics, serving as a drug resistance mechanism.

EXTRACELLULAR AND INTRACELLULAR FACTORS OF AUTOPHAGY

1. Growth factors
2. Nutrient deprivation/under starvation
3. Stress oxidative salt ER stress
4. During pathogen invasion
5. Protein aggregation/organelle aggregation.
6. Drought
7. Senescence

AUTOPHAGY RELATED GENES

1. Yeast-34 Autophagy Related Genes (ATG)
2. Rice-33(Arabidopsis, Wheat, Soya)

PLANT PROFILE

Arabidopsis thaliana, also known as thale cress, mouse-ear cress, or arabidopsis, is a tiny plant in the mustard family (Brassicaceae) that is native to Eurasia and Africa. It is commonly found along road shoulders and disturbed terrain, and is classified as a weed. A. thaliana, a winter annual with a brief life cycle, is a prominent model organism in plant biology and genetics. For a sophisticated multicellular eukaryote, A. thaliana's genome is quite tiny, measuring roughly 135 megabase pairs.It was the first plant to have its genome sequenced, and it is a valuable resource for studying the molecular biology of several plant features, such as flower formation and light sensing. Arabidopsis thaliana is an annual (rarely biannual) plant that typically grows to 20-25 cm tall. The leaves form a rosette at the plant's base, with a few more leaves on the flowering stem. The base leaves are green to somewhat purple in color, 1.5-5 cm long, and 2-10 mm wide, with an entire to coarsely serrated border; the stem leaves are smaller and unstalked, with an entire margin. Trichomes are tiny unicellular hairs that coat the leaves. The blooms are 3 mm in diameter, grouped in a corymb, and have the structure of the typical Brassicaceae .The fruit is a siliqua 5-20 mm length, with 20-30 seeds. Roots are basic in structure, having a single primary root that extends vertically downward to produce smaller lateral roots. These roots interact with rhizosphere microorganisms, such as Bacillus megaterium. A. thaliana may go through its whole lifespan in six weeks. After around 3 weeks, the center stalk develops flowers, which self-pollinate. In the lab, A. thaliana can be cultivated in Petri plates, pots, or hydroponics, as well as under fluorescent lights or in greenhouses. The methanolic extract of Arabidopsis thaliana has been investigated for its bioactive components, some of which may regulate autophagy. The extract has exhibited a variety of pharmacological activities, including anti-cancer activity. Compounds produced from Arabidopsis thaliana may interact with the autophagy process, either directly altering autophagic activity or influencing the signaling pathways that control autophagy. This interaction might either improve the efficacy of existing chemotherapeutic medicines or give a new approach to drug resistance. Autophagy is an important component of cancer cells' drug resistance mechanisms, serving as both a survival strategy and a possible therapeutic target. The study of methanolic extracts from Arabidopsis thaliana provides a prospective avenue for creating novel drug resistance methods by harnessing the plant's bioactive chemicals to control autophagy and improve the efficacy of cancer therapies.

NATIVE HABITATS

Europe: Found from the Mediterranean to Scandinavia and Spain to Greece Asia:
Found in the Yangtze River basin
Africa: Found in tropical alpine ecosystems, possibly including South Africa

Arabidopsis thaliana (mouse-ear cress) can be found in the following Indian states: Himachal
Pradesh
Jammu Sikkim
Uttar Pradesh
Uttarakhand

Arabidopsis thaliana is a plant that can be found in the western Himalayan region of India. It has been found in a variety of habitats, including human-disturbed lawns and undisturbed river valleys.

SCIENTIFIC CLASSIFICATION
KINGDOM-PLANTAE
CLADE-TRACHEOPHYTES
CLADE-ANGIOSPERM CLADE-EUDICOTS CLADE-ROSIDS
ORDER-BRASSICALES FAMILY-BRASSICACEAE GENUS-ARABIDOPSIS
SPECIES-A.thaliana
BIONOMIAL NAME-Arabidopsis thaliana
SYNONYMS-Arabis thaliana

Fig:2 Arabidopsis thaliana

PHYIOLOGICAL ROLES OF AUTOPHAGY IN PLANTS

1. Autophagy in Nutrition Starvation Sucrose, Carbon and nitrogen stimulate AtATG7,AtATG8, and Yellowing of leaves expression of AtSEN1,a senescence maker gene, accelerates senescence. Involved in roots hair production and root elongation under nutrients.
2. Autophagy in the Oxidative Stress Response -Reactive oxygen species activated derivatives of highly poisonous cell death causes damage to carbohydrates, DNA, lipids and Reduced degradation efficiency.
3. Autophagy in programmed cell

ROLE OF AUTOPHAGY

1. Leaf senescence
2. Seed Development
3. Reproductive Development
4. Vascular Development
5. Nutrient Starvation
6. Drought Stress
7. Heat Stress
8. Plant microbe interaction

1. LEAF SENESCENCE

Leaf senescence is regarded as an essential developmental phase due to its crucial function in remobilizing resources from adult leaves to support growing organs (such as developing seeds). Because autophagy is a breakdown and recycling mechanism, it is reasonable to assume that it is active during leaf senescence. However, early senescence has been seen in numerous Arabidopsis atg mutants, a rice atg7 mutant, and a maize atg12 mutant, which contradicts this concept.One potential explanation for this trait is that the downregulation of flavonoid production in atg mutants induces oxidative stress, which then promotes the manufacture and storage of excess SA, resulting in leaf yellowing. The discovery that several ATG transcripts are increased in older leaves lends evidence to autophagy's role in senescence.For example, in Arabidopsis, 15 ATG genes are activated during senescence. Apple, barley, and soybean all show enhanced expression of ATG genes during senescence. Similarly, 30 and 27 ATG genes were found to be elevated in maize's older leaves and leaf tip.

2. SEED DEVELOPMENT

Autophagy has an influence on seed production not only due to its importance during senescence and nutrient remobilization, but also because it functions during seed development. A recent research in Arabidopsis found that practically all ATG genes were elevated in siliques during seed formation. In maize, numerous ATG genes showed increased transcript quantity in the endosperm but not in the embryo. Furthermore, an ATG8 lipidation test revealed that the buildup of ATG8-PE adducts in maize endosperm began at 18 days after pollination (DAP) and continued until the final time point employed in the experiment, 30 DAP, demonstrating that autophagy was engaged during endosperm development. Autophagy may play a role in transporting precursors to PSVs, which are the sites of precursor processing to the mature form.

3. REPRODUCTIVE DEVELOPMENT

Despite their lower fertility, Arabidopsis atg mutants may complete normal life cycles and generate viable seeds. One exception is the Arabidopsis atg6 mutant, which has a pollen germination defect. Autophagy abnormalities, however, may not contribute to this phenotype because ATG6 and the PI3K complex are engaged in a variety of biological activities. The first direct evidence linking autophagy to reproductive development was discovered in wheat. During wheat floret development, many floret primordia are aborted before reaching the fruitful floret stage, a process exacerbated by long-day circumstances. Autophagy was discovered in ovary cells that were going through programmed cell death (PCD) in aborting florets.Transmission electron microscopy (TEM) revealed the creation of double-membrane vesicles, which eventually merged with the vacuole and released single-membrane structures into it. Additionally, ATG4 and ATG8 were elevated throughout this process.

4. VASCULAR DEVELOPMENT

Vascular tissues are crucial to plants because they play critical roles in mechanical support and long-distance transport. The two primary conductive tissues are xylem and phloem, which are made up of cells that are highly specialized for this role. The primary conducting cells in xylem are tracheary elements (TEs), which go through PCD and totally remove their cellular

contents as they differentiate. The importance of autophagy in xylem formation was originally revealed in poplar. Several ATG genes were upregulated during poplar xylem fibre cell PCD, and autophagy was seen in fibrous and pioneer roots in the field.Autophagy also plays a role in TE development in Arabidopsis, as indicated by a lower xylem cell number in an atg5 mutant compared to WT, activation of many ATG genes during TE differentiation, and the presence of autophagosome-like structures in developing cells. Heterologous Arabidopsis RabG3b overexpression in poplar increased xylem development and growth.

5. NUTRIENT STARVATION

Autophagy has been extensively researched as an abiotic stress response, and one common stressor that triggers autophagy is nutritional deprivation. Aside from Arabidopsis, autophagy has been demonstrated to operate in responses to nutritional restriction (carbon or nitrogen) in apple, barley, foxtail millet, grapevine, maize, pepper, rice, and wheat, as evidenced by overexpression of ATG genes or hypersensitivity of autophagy-defective mutants to starvation. A recent study found that overexpressing ATG18a in apples enhanced tolerance to nitrogen deprivation and autophagy under these circumstances. Compared to WT plants under N depletion circumstances, multiple pathways and their related genes were further activated in response to N depletion in ATG18a overexpressing plants

6. DROUGHT STRESS

Drought is another major environmental stress that plants may experience. Autophagy was originally shown to have a role in the response to drought stress in Arabidopsis, as evidenced by ATG18a upregulation and osmotic stress-induced autophagosome formation. Several studies in crops have revealed that ATG genes are upregulated in response to drought stress, including apple, barley, foxtail millet, pepper, rice, tomato, and wheat. Arabidopsis atg5, atg7, and RNAi-ATG18a mutants, which are unable to activate autophagy during drought, are extremely vulnerable to drought stress, indicating that autophagy is critical for plant survival in drought circumstances. Autophagy-defective tomato and wheat plants exhibited similar characteristics. Furthermore, overexpressing ATG18a in apples boosted autophagy activity and drought tolerance, indicating that autophagy plays an important role in drought responses.

7. HEAT STRESS

Arabidopsis atg5 and atg7 mutants are very susceptible to heat stress, suggesting that autophagy is also active during heat reactions. Under heat stress, pepper and tomato showed an increase in autophagosome production and activation of ATG genes. Silencing ATG5 or ATG7 in tomato plants resulted in lower autophagy induction during heat stress, compromising heat tolerance. Furthermore, a natural thermotolerant pepper line demonstrated stronger autophagy activity than a thermosensitive pepper line. These findings imply that autophagy may provide heat tolerance to plants. Heat or heat-induced ROS can have hazardous consequences, such as protein aggregation. It has been demonstrated that autophagy-defective plants in Arabidopsis and tomato collect more insoluble proteins during heat stress, implying that autophagy may be used to eliminate protein aggregates. Arabidopsis requires the transcription factor WRKY33 for heat tolerance. Tomato contains two homologues of Arabidopsis WRKY33: WRKY33a and WRKY33b. Silencing either WRKY33a or WRKY33b reduced heat tolerance, indicating that they operate during heat stress. Heat inhibited the activation of the autophagy-related genes ATG5, ATG7, NBR1a, and NBR1b in WRKY33a- or WRKY33b-silenced plants, indicating that the WRKY33s may be positive autophagy regulators in tomato.

8. PLANT MICROBE INTERACTIONS

Autophagy has a role in crop species' responses to biotic stress, and manipulating autophagy affects disease resistance in various species. For example, in bananas, the autophagy inhibitor 3-methyladenine (3-MA) reduced resistance to Fusarium oxysporum f.sp. Cubense.New research reveals that autophagy can play both antimicrobial and promicrobial functions in plant-pathogen interactions. Autophagy enhances resistance to necrotrophic infections, whereas autophagy-defective Arabidopsis mutants are more vulnerable to these pathogens.

DRUG PROFILE

Chloroquine is an antimalarial drug that was once widely used to prevent and treat malaria. However, due to the emergence of chloroquine-resistant strains of malaria, its use is now limited.

Chemical and Physical Properties:

* Chemical Name: 7-Chloro-4-(4-diethylamino-1-methylbutylamino) quinoline
* Molecular Formula: C18H26ClN3
* Molar Mass: 319.88 g/mol
* Appearance: White to yellowish-white crystalline powder
* Solubility: Soluble in water and ethanol
* Melting Point: 57-60°C

PHYSIOLOGICAL ACTIVITY

- Chloroquine is easily absorbed through the gastrointestinal tract.
- It has a wide distribution throughout the body, including the liver, spleen, kidneys, and lungs.
- The liver metabolizes chloroquine.
- It is eliminated through the urine and stool.

ACTIVITY OF CHLOROQUINE WITH ARABIDOPSIS THALIANA

It's worth noting that this experiment appears to entail exposing a living organism (Arabidopsis thaliana) to a potentially toxic drug (chloroquine). It is critical to ensure that the experiment is carried out responsibly and ethically, with adequate safety precautions and respect to any legislation. Here's a description of the experiment's possible results, as well as some key points to consider.

POTENTIAL OUTCOMES:

- Arabidopsis thaliana may absorb chloroquine from methanolic extracts. HPLC analysis may then be used to determine the concentration of chloroquine in plant tissue.
- Chloroquine may affect the plant's metabolic pathways, potentially altering the production of specific metabolites. HPLC could be used to detect and measure these changes.
- Chloroquine can have harmful effects on plants, affecting growth, development, and overall health. These effects could be seen visually and even quantified using a variety of methods.
- To get accurate results, carefully construct the experimental design to account for aspects such plant age, growth circumstances, and chloroquine concentration.
- Develop an accurate HPLC method for measuring chloroquine and its metabolites.
- To derive meaningful conclusions concerning chloroquine's effects on Arabidopsis thaliana, detailed study of HPLC data is necessary.

KEY OBSERVATIONS AND POTENTIAL MECHANISMS:

- Studies indicate that chloroquine can cause stress responses in Arabidopsis. This includes the activation of genes related to oxidative stress, heavy metal stress, and pathogen defense.
- Chloroquine inhibits autophagy, which degrades and recycles cellular components. Chloroquine administration in Arabidopsis has been demonstrated to inhibit autophagy, resulting in the accumulation of cellular waste.
- Chloroquine can impact plant growth and development. Depending on the dose and length of exposure, it can limit root growth, change leaf morphology, and affect flowering timing.
- Chloroquine may interfere with a variety of metabolic processes in Arabidopsis.
  This can include changes to photosynthesis, respiration, and the formation of secondary metabolites.
- Understanding Plant Stress Responses: Arabidopsis is a useful model organism for investigating plant stress responses. Chloroquine can be employed to examine the molecular mechanisms behind these reactions.
- Arabidopsis is an effective model for investigating autophagy. Chloroquine can be used to study autophagy's role in a variety of plant processes, including development, stress response, and pathogen defence.
- Drug Discovery: Research into chloroquine's effects on Arabidopsis can reveal its mechanism of action and lead to the development of other medications with similar or improved properties.
- Ethical considerations: Conducting plant research responsibly and ethically is crucial to avoid harm to organisms.

CONCLUSION

Autophagy, a fundamental cellular process that degrades and recycles cellular components, has received substantial research for its function in a variety of physiological and pathological situations, including cancer. Autophagy plays a dual role in cancer, acting as a tumor suppressor and a promoter of drug resistance. Plants, especially Arabidopsis thaliana, are rich in bioactive chemicals with potential medicinal use. Methanolic extracts of A. thaliana have been found to contain a variety of chemicals, including flavonoids, terpenoids, and phenolic acids, some of which are thought to regulate autophagy. High-performance liquid chromatography (HPLC) can be utilized to identify and quantify particular chemicals in A. thaliana's methanolic extract. This knowledge can aid in understanding the processes by which these chemicals influence autophagy and may lead to medication resistance. More study is needed to completely understand the role of autophagy in drug resistance and to design viable therapeutic options targeting autophagy pathways.

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