REVIEW ARTICLE

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Potential Anti-Senescence Effect of Extract from *Andrographis paniculata* Herbal Plant and Its Bioactive Compounds: A Systematic Review

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The rapid aging of the global population is a major worldwide issue because of the close relationship between age and the development of several diseases. Aging or senescence is among the most widely studied topics at the moment. However, no pharmaceuticals have been developed that claim to possess anti-senescence properties. *Andrographis paniculata*, is a medicinal plant found widely throughout tropical and subtropical Asia. This review aims to identify the potential anti-senescence effect of *A. paniculata* extract and its bioactive compounds. By following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, five databases were used and *in vivo* and *in vitro* studies were included in this review. *A. paniculata* extracts and their bioactive compounds exert anti-senescence properties through their anti-inflammatory and antioxidant properties. This herb and its compounds enhanced memory, cognitive function and behaviour in Alzheimer's disease. The extract also promoted cell cycle progression and proliferation in the skin. In addition, andrographolide exhibited anti-senescence effects in endothelial cells through the activation of PI3K/Akt/Nrf and PI3K/Akt/AP-1 pathways. *A. paniculata* along with its bioactive compounds including andrographolide and 14-deoxyandrographolide, may have the potential to be used as anti-senescence through anti-inflammatory and antioxidant properties. However, the specific markers to evaluate the senescence are necessary to be conducted. Any clinical trials should be done to establish these findings. Since in clinical settings this potential herbal may be used for long-life time, the safety profile and toxicity of *A. paniculata* should be considered.

Keywords*: herbal plants, Andrographis paniculata, andrographolide, bioactive compounds, senescence*

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Introduction

Aging or senescence is a progressive reduction in physiological function that involves changes in anatomy, endocrine systems, neural circuitry, and behavior.[1] The accelerated senescence of the world population is a significant global concern due to the strong correlation between age and the onset of many illnesses. Senescence acts as a central role to the progression of age-related diseases including arthritis, cancer, and neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease. Currently, one of the most popular areas of study is senescence due to the unprecedented increase in worldwide life expectancy. It is projected that the number of humans over 65 years old will reach 1.6 billion by the year 2050. Multiple non-communicable diseases commonly co-occur with senescence, resulting in increased disability and vulnerability to frailty.[2-4]

Senolytic medications such as dasatinib and quercetin, also known as anti-senescence drugs, possess the ability to eliminate senescent cells. These drugs are being considered as possible alternatives for the prevention and treatment of chronic diseases. However, the chronic usage of dasatinib can result in serious and lethal outcomes, such as pleural effusion (PE) and pulmonary arterial hypertension (PAH). On the other hand, the prolonged use of quercetin is still uncertain.[5-8] Herbal medicine is recognized for its potential as an anti-senescence treatment, and numerous experimental investigations have investigated herbal remedies like ginseng or *Panax ginseng* and *Zingiber officinale* or ginger on senescence. Elderly people tend to suffer from cardiovascular diseases which requires them to consume anti-coagulant medication for a whole lifetime. However, ginseng and ginger should not be consumed together with anti-coagulant medication such as aspirin, clopidogrel, or warfarin as it may increase the risk of bleeding.[9-14] Hence, it is essential to investigate alternative herbal plants that have the potential to exhibit anti-senescence effect.

Andrographis paniculata, also known as the "King of Bitters", is a medicinal plant found widely in Southeast Asia, including Indonesia, and has been traditionally used in the community. Previous studies have been conducted to evaluate the anti-inflammatory and antioxidant effects of *A. paniculata* extract or its bioactive compounds.[15–17] However, to the best of our knowledge, those studies have not explored in detail the *A. paniculata* effect in senescence process. Here, we summarized the results of the research regarding *A. paniculata* extract and its bioactive compounds available for anti-senescence *in vivo* and *in vitro* studies limited to the last 10 years.

Material and methods

This systematic review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The inclusion and exclusion criteria were chosen in order to investigate the molecular mechanism using both *in vivo* and *in vitro* approaches. Literature discussing *A. paniculata* extract along with its main bioactive compounds such as andrographolide, neoandrographolide, and isoandrographolide were included in this study.[18]

Eligibility Criteria

This review encompassed senescence *in vivo* and *in vitro* models as demographic groups. Elevated oxidative stress, elevated glucose levels, and inflammation triggered accelerated senescence. Any illness unrelated to age or animal research that did not specifically investigate senescence pathways were not considered. Both *in vivo* and *in vitro* models were utilized in this study due to the limited availability of clinical data on this subject. The intervention groups consisted of *A. paniculata* extract and its bioactive components. All the studies included used one of these strategies for the indicated population categories. This review specifically examined the individual effects and molecular mechanisms of *A. paniculata* and its bioactive components, excluding studies that employed *A. paniculata* in combination with other substances. Any articles demonstrating the impact of *A. paniculata* or its bioactive components on senescence molecular pathways were considered in the analysis. Only research published in English and without time restrictions was considered. Excluded were in-silico research, clinical trials, review papers, meta-analyses, studies on foods not used for therapeutic purposes in the clinic, and other irrelevant study designs.

Search Strategy

A search strategy using five electronic databases; PubMed, Scopus, Embase, Web of Science, and Wiley Online Library, was conducted. The search was revised on February 15, 2024, utilizing the specified databases. The search query employed was "*Andrographis paniculata*" and "aging or

senescence" as outlined in the supplementary table (Table 1). The search results and search terms were discussed to confirm the search terms and study identification. References were managed via Mendeley.

Table 1. The search term used for this review.

Database	Search Term	Filter
Pubmed	(*Andrographis paniculata*) AND (aging OR senescence)	10 years; English
Scopus	(*Andrographis paniculata*) AND (aging OR senescence)	2014-2024; English
Embase	(*Andrographis paniculata*) AND (aging OR senescence)	2014-2024
Web of Science	(*Andrographis paniculata*) AND (aging OR senescence)	2014-2024
Wiley Online Library	(*Andrographis paniculata*) AND (aging OR senescence)	2014-2024

Selection Process

The titles of the initial 147 articles were assessed and 31 duplicate articles were eliminated, obtaining final 116 articles which abstract were evaluated. Discrepancies, such as inconsistencies with the study selection criteria, were addressed through comprehensive discussions until an agreement was established. Articles that did not match the inclusion criteria of this study were excluded (n=93). The 23 papers were separately reviewed in full text and any disagreements were settled through discussions. Eleven papers were included when the selection process concluded. Figure 1 presents a summary of the selection process.

Data Collection Process

Data were gathered using Microsoft Excel and included the following details: herbal medicine or bioactive compound of herbal medicine, type and age of animal model or *in vitro* model, induced senescence/age-related diseases, sex of animal model, outcome, and mechanisms. The data were retrieved separately from relevant studies using the provided data. The data retrieved by the three authors was compared and any differences were resolved through discussions. The data extracted was summarized in Table 2 (*in vivo* studies) and Table 3 (*in vitro* studies). This study primarily focused on investigating the effects and molecular mechanisms of *A. paniculata* in senescence.

Risk of Bias Assessment

The chosen publications were evaluated using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias (RoB) tools, which were adapted from the Cochrane RoB. This tool includes ten domains: sequence generation, baseline characteristics, allocation concealment, random housing, blinding (Caregiver/Investigator blinding), random outcome assessment, blinding (outcome assessor blinding), incomplete outcome data, selective outcome reporting, and other sources of bias. This tool generates a single question for each domain, enabling reviewers to assess bias risk by indicating yes, no, or unclear. The conclusions reached by each author will be collected via open discussion. Figure 2 presents a summary of the risk of bias created with SYRCLE's tool to be displayed in an illustration to provide readers with an easy understanding of the risk of bias in the studies included. They determined that baseline similarity was required to clearly define the characteristics of both the control and the experimental populations. The criteria were as follows: All animals in both groups must be of the same species, within the same age group, and have similar weights. Random housing, random outcome assessment, blinding of common assessors, and incomplete outcome data were evaluated based on being mentioned in the study. If not specified, they were categorized as "unclear." After carefully examining the publications, the review authors independently identified certain selective outcomes and other biases. Bias in each domain was classified as low, unclear, or high. There were no tools for assessing the risk of bias on *in vitro* studies. Therefore, the selected *in vivo* studies were not being assessed for the risk of bias.

Effective Measures, Synthesis Methods, Reporting Bias Assessments, Certainty of Evidence

The data from the population, interventions, and results were organized in Microsoft Excel. This app was selected for its user-friendly interface. The effective measures weren't employed because of the experiment's outcome variations. Determining if each study included was free from selective outcome reporting involved reviewing the methodology and outcomes sections. The certainty of the evidence was not established due to the lack of follow-up with meta-analysis in this investigation.

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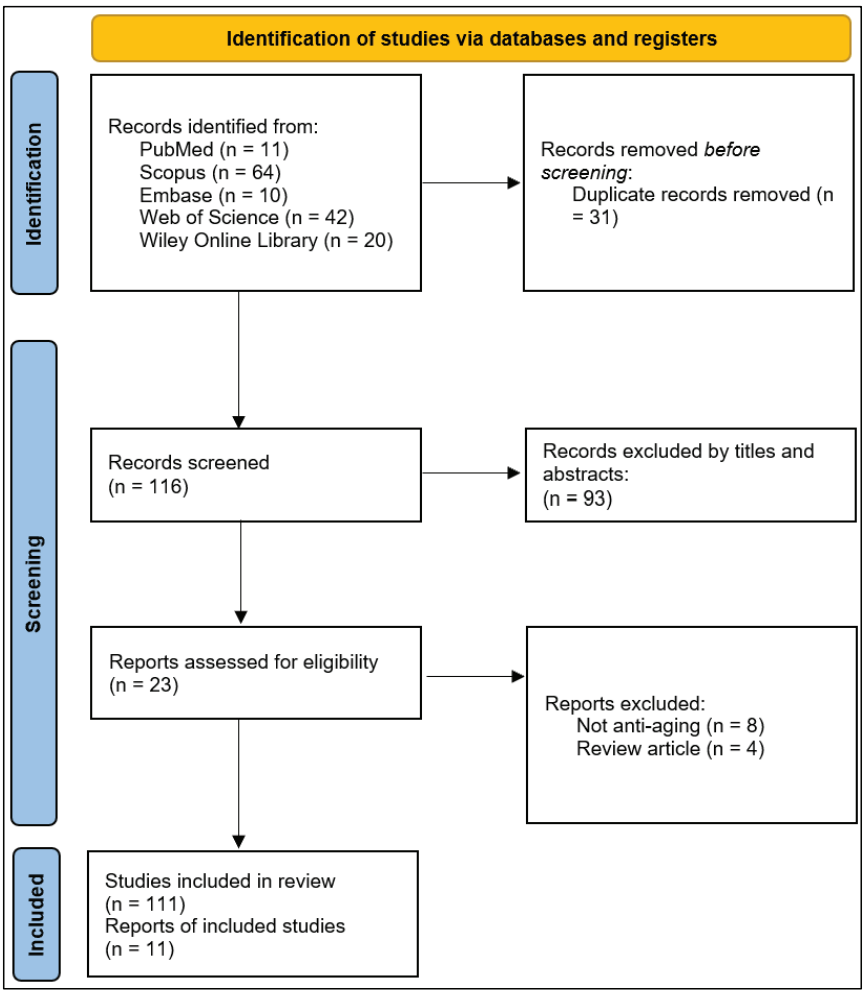

Figure 1. Illustration of the study selection process.

Results

Study Selection and Characteristics of Included Studies

Searching 5 databases, including PubMed, Scopus, Embase, Web of Science, and Wiley Online Library, led to a finding of 147 articles. Thirty-one duplicates were identified and eliminated, and 93 irrelevant studies were discarded from the review after screening their abstracts. Twenty-three research received a thorough assessment for suitability, and 12 studies were found unsuitable thus being excluded. Only 11 papers were obtained during the study selection process, as depicted in Figure 1. Eleven articles were reviewed to summarize the effect of *A. paniculata* extract or its bioactive constituents on senescence, based on *in vivo* and *in vitro* studies.

Table 2 and Table 3 displays every study that was included. Most investigations used bioactive compounds, particularly andrographolide, instead of plant extracts. One investigation involved the use of plant extract and two studies involved the use of neoadrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydro-andographolide.

Risk of Bias

The baseline characteristics of the control and experimental groups remained similar in all trials. Most studies have an unclear risk of bias in the random outcome assessment, blinding of outcome assessors, and incomplete outcome data domains. None of the studies analyzed exhibited selective outcome reporting or other forms of bias. Although certain biases were present in the animal research, they were included in the review because they met the established inclusion criteria, and most studies did not have conflicting interests. Furthermore, some studies were published in reputable journals, hence their conclusions may be considered credible. A quality assessment was done to assist

Table 2. Summary of extracted data from *in vivo* studies.

Herbal Medicine (Plant Part/ Compound	Type and Age of Animal Model (Mice or Rats)	Induced Senescence/Age-Related Disease	Sex	Outcome	Mechanism	Citation Number
Andrographolide	Balb/c mice model, 4-5 weeks old	Arthritic, CFA-induced (injected with complete freund's adjuvant)	M & F	Anti-arthritis by supression of inflammatory and pro-inflammatory cytokines	Reduce paw edema, reduce p-p38 expression, suppress CD40, reduce TGF-β1, IL-6, IL-1β	19
Andrographolide	Octodon degus, 7-72 months old - female (adult 56 months old, young 12 months old)	Based on age - brain senescence	M & F	Improve memory in brain senescence	Activate Wnt signaling pathway through direct inactivation of the enzyme GSK3β (recovered β-catenin level, increased in the Ser9 phosphorylation levels, recovered Cyclin D1 and CAMK-IV protein level)	25
Andrographolide	Octodon degus, female (aged 56 months old, young 12 months old)	Based on age - brain senescence	F	Reduce neuroinflammation and anti-oxidant	Reduce Aβ load and aggregation in brain, decrease IL-6 expression in the hippocampus and cortex, decrease phosphorylated JNK expression	33
Andrographolide	Octodon degus, female (aged 72 months old, young 36 months old)	Based on age - brain senescence	F	Improve cognitive impairment and synaptic dysfunction impairment	Increase synaptic plasticity, increase the efficiency of basal synaptic transmission	3
Andrographolide	Octodon degus, female (aged 56 months old, young 12 months old)	Based on age - brain senescence	F	Recover the hippocampus-dependent cognitive performance, improve synaptic strength, recover synaptic functions	Improve memory by novel object recognition (NOR) test, recovered GluN2A subunit of the NMDA receptor, decrease Tau phosphorylation, decrease Aβ40 and Aβ42 levels	34
Andrographolide	AβPPswe/PS-1 mice, 7 and 12 months old	Based on age - brain senescence	M	Reduce cognitive impairment, prevent LTD (long term depression)	Decrease Aβ depositions, decrease Tau phosphorylation, recover synaptic function, recover spatial memory, inhibit GSK-3β	23
Andrographolide	WT C57BL/6 mice (2 months old and 10 months old), APPswe/PSEN1ΔE9 mouse (7 months old)	Based on age and type of animal - brain senescence	N/R	Increase proliferation of neural progenitor cells, increase neurogenesis	Activate Wnt/β-catenin signaling pathway, increase NeuroD1 level	24

readers evaluate the data sources included in this review, promoting a thorough grasp of their integrity and reliability.

Anti-senescence Effect of A. paniculata

The individual study results are presented in Table 2 (*in vivo* studies) and Table 3 (*in vitro* studies). There were 11 articles in this review including 7 articles using *in vivo* models and 4 articles using *in vitro* models. Most of the selected studies used *Octodon degus* as a model of brain senescence. Other studies used Balb/c mice for arthritis models, AβPPswe/PS-1 mice for AD models, and wild type (WT) C57BL/6 mice. Andrographolide is a primary bioactive component of *A. paniculata*, which were used in most of the selected studies. Previous study on Balb/c mice induced with complete Freund's adjuvant (CFA) showed that andrographolide showed its efficacy in treating arthritis by decreasing inflammatory mediators such as nuclear factor kappa B (NF-κB), cyclooxygenase-2 (COX-2), and phospho-p38 which play crucial roles in inflammation and contribute significantly to the development of arthritis by triggering a signaling pathway that results in the creation of tumor necrosis factor (TNF)-α, interleukin (IL)-1β,

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Table 3. Summary of extracted data from *in vitro* studies.

Herbal Medicine (Plant Part/Compound)	Type of *i n vitro* Model	Induced Aging/Age-Related Disease	Sex	Outcome	Mechanism	Citation Number
Andrographolide 7.5 mcM	EA.hy926 endothelial-like cells	TNF-alpha-induced	N/A	Anti-oxidant, anti-inflammation, atherosclerosis prevention	Inhibit ROS generation, inhibit HL-60 adhesion, increase cellular GSH content and GCLM gene expression, induce HO-1 gene expression, PI3K/Akt pathway activation (induce HO-1, GCLM gene expressions, and GSH synthesis)	41
Andrographis paniculata	Epidermal stem cells	N/R	N/A	Anti-senescence	Induce cell cycle progression, increase integrin beta1 (CD29), upregulate VEGF secretion	35
Andrographis paniculata methanolic extract, andrographolide, neoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandographolide	Human dermal fibroblasts, adult (HDFa)	H_2O_2-induced for 1 h, LPS-induced	N/A	Anti-inflammation anti-oxidant	ME and andrographolide showed antioxidant anti-inflammatory activities via reduced IL-6 and TNFα production, 14DAP showed antioxidant activity and pro-collagen type I production	39
Andrographis paniculata methanolic extract, andrographolide, neoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandographolide	Human, adult, low calcium, high temperature (HaCaT) keratinocytes	H_2O_2-induced for 1 h, LPS-induced	N/A	Anti-inflammation anti-oxidant	ME and andrographolide showed antioxidant anti-inflammatory activities via reduced IL-6 and TNFα production, 14DAP showed antioxidant activity	38

IL-6, IL-17, matrix metalloproteinases (MMPs), and other substances.[19–22] In this study, andrographolide also showed better effect than dexamethasone in reducing these inflammatory mediators.[19]

AβPPswe/PS-1 mice are transgenic models for AD with AβPP and PS-1 mutant transgenes. The study used young (7-months-old) and adult (12-months-old) AβPPswe/PS-1 mice and showed that andrographolide improved cognitive abilities and reduced various neuropathological indicators of AD, such as protecting postsynaptic proteins, decreasing Aβ aggregate maturation, recovering synaptic functions (LTP), and enhancing spatial memory performance.[23]

The prior research evaluated neurogenesis on young and aged wild type (WT) C57BL/6 mice, as well as in APPswe/PSEN1ΔE9 mice, a model for AD and showed that andrographolide increased cell proliferation and the production of new neurons in the dentate gyrus of the adult hippocampus, called neurogenesis, in both wild-type mice and a mouse model of AD by reduction of glycogen synthase kinase (GSK)-3β. However, this compound did not affect Ak strain transforming (Akt), extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK), casein kinase (CK), and S6 kinase.[24]

Most *in vivo* studies utilized young and aged *O. degus* as a model for studying brain senescence. Wnt ligands play a role in regulating the cell proliferation, fibrosis, and cellular morphogenesis in the central nervous system.[25-28] Prior research has shown a significant connection between changes in Wnt signaling and the development of neurodegenerative conditions, like AD.[2,25,29,30] Andrographolide can penetrate the blood-brain barrier and restore Wnt signaling in the aged *O. degus* brain by directly deactivating the enzyme GSK-3β, increasing β-catenin levels, enhancing Ser9 phosphorylation levels, and restoring Cyclin D1 and

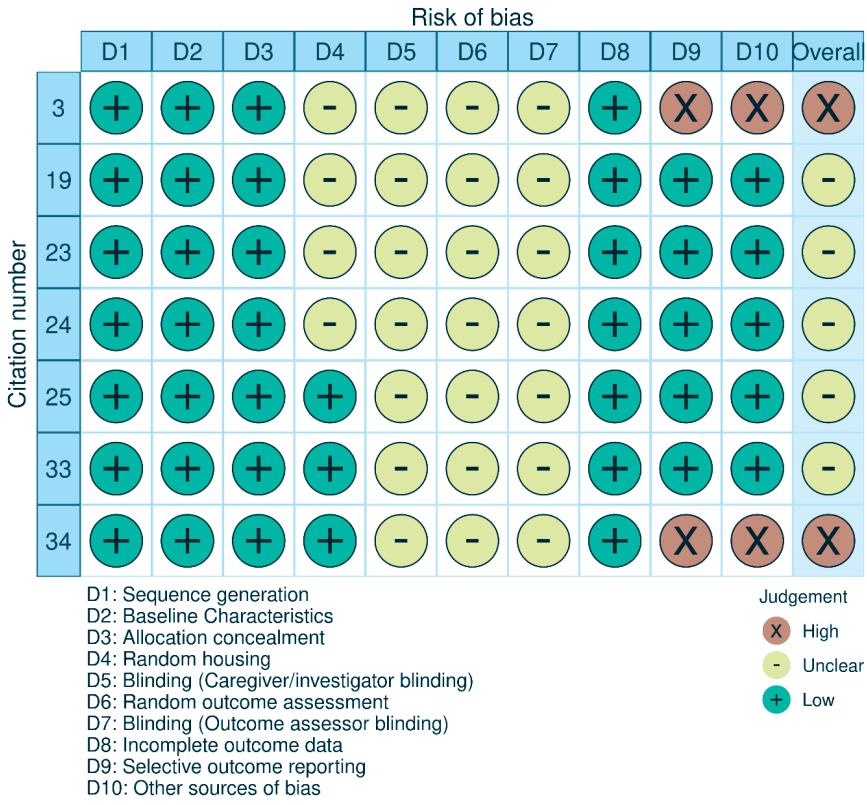

Figure 2. Summary of risk of bias.

CAMK-IV protein levels. These findings strongly indicate a potential improvement in memory processes.[25,31]

Andrographolide decreased Aβ load and aggregation, reduced neuroinflammatory markers in *O. degus* brain, and lowered levels of GFAP, IL-6, COX-2, CaMKII, and JNK proteins. Andrographolide significantly decreases 4-HNE and N-Tyr levels and protects against oxidative stress-induced damage. COX-2 is naturally present in the central nervous system and plays a role in essential brain functions such as synaptic activation, memory consolidation, and functional hyperemia. Andrographolide has been shown to reduce COX-2 levels in normal endothelial brain cells. However, after much research over the past decade, the data on the involvement of COX-2 in neurodegenerative diseases like AD remains unclear.[32,33]

Andrographolide enhanced spatial learning and memory in aged *O. degus*. Andrographolide altered electrophysiological parameters in *O. degus* by reducing basal synaptic transmission efficiency, enhancing long-term synaptic plasticity, restoring postsynaptic response stability, and increasing basal synaptic transmission efficiency. The study analyzed the protein levels of N-methyl D-aspartate receptor subtype 2B (NR2B), GluR1, and gamma-aminobutyric acid (GABA) receptors in the postsynaptic region. Andrographolide specifically enhanced the expression of NR2B in the hippocampus. Andrographolide therapy increased the expression of postsynaptic density protein 95 (PSD95) protein in postsynaptic scaffold proteins.[3]

Similar findings of enhanced synaptic plasticity were reported that andrographolide was found to restore the N-methyl-D-aspartate (NMDA) receptor through analysis of postsynaptic proteins including NMDA and PSD95. Andrographolide lowered Tau phosphorylation and decreased Aβ aggregates in the brains of old animals, which contributed to the improvement of AD. Andrographolide also enhanced memory through the new object recognition (NOR) test.[34]

The *in vitro* studies analyzed in this review utilized different cell types. Previous study utilized human epidermal stem cells (EpSCs) to study the impact of *A. paniculata* extract (APE). The study indicated that APE seemed to promote cell cycle progression and triggered the proliferation of EpSCs.[35] Integrin β1, which supports the survival of human adult epithelial progenitor cells (ePCs), was upregulated by APE treatment. Integrin β1 is essential

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for angiogenesis in the epidermis during wound healing. APE increased the release of VEGF, which activates integrin β1 to facilitate endothelial cell proliferation or migration via the ERK1/2 pathway, a MAPK signaling pathway. APE also enhanced the formation of type 1 collagen through VEGF mediation.[35–37]

Previous study examined the anti-senescence properties of *A. paniculata* methanolic extract (ME), andrographolide, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide on human dermal fibroblasts (HDFa) and human, adult, low calcium, high temperature (HaCaT) keratinocytes. ME containing 0.87% andrographolide exhibited antioxidant activity at 5 µg/mL and reduced IL-6 production and TNFα mRNA levels in both cells, indicating anti-inflammatory effects. At concentration of 0.1 µg/mL, 14DAP exhibited antioxidant properties but did not reduce TNFα mRNA levels or IL-6 production in either cell type. On the other hand, 14DAP reduced the synthesis of pro-collagen type I. The pure andrographolide compound demonstrated anti-inflammatory properties by reducing IL-6 secretion and TNFα mRNA levels at a concentration of 5 µg/mL in HDFa cells. Andrographolide sodium bisulfate reduced the protein expression of p65 and lowered the production of TNFα in HaCaT cells. Furthermore, neoandrographolide and 14DAP11-12 demonstrated no antioxidant or anti-inflammatory effects.[38-40]

Andrographolide on EA.hy926 endothelial-like cells exhibited anti-inflammatory and antioxidant properties by suppressing TNFα-induced reactive oxygen species (ROS) generation, intercellular adhesion molecule (ICAM)-1 expression, and human leukimia (HL)-60 cell adhesion. This inhibition was likely linked to the suppression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activation, upregulation of heme oxygenase (HO)-1 and glutamate-cysteine ligase modifier (GCLM) expression, and increased glutathione (GSH) content through the phosphoinositide 3-kinases (PI3K)/Akt/nuclear factor erythroid 2-related factor (Nrf)2 and PI3K/Akt/activator protein (AP)-1 pathways.[41]

Discussion

We reviewed *in vivo* and *in vitro* article in the last ten years on using *A. paniculata* extract and its bioactive compounds for anti-senescence. Eleven articles were analyzed according to the described research selection criteria. As a result, it was confirmed that *A. paniculata* extract and its bioactive compounds have anti-senescence effects through various mechanisms summarized in Figure 3.

Safety and Toxicity of A. paniculata Extract and Its Bioactive Compound

Based on previous study using leaf ethanolic extract of *A. paniculata* to assess the acute toxicity effect, the mice were ingested to various concentrations of *A. paniculata* extract such as 0, 300, 2000, or 5000 mg/kg BW and observed for 14 days. Then, the mice were euthanized, and the internal organs and blood samples were collected. All treated mice survived, and no side effects were observed. The upper fixed dose of 5000 mg/kg BW of *A. paniculata* extract showed no significant acute toxicological effects.[42] Another previous study conducted on mice using andrographolide to study the acute toxicity effect, found that no significant changes of the body weight, organ weight, hematological and biochemical parameters were seen compared to control groups. Additionally, observation for 14 days showed no mortality, and the dose of 2000 mg/kg BW of andrographolide showed no significant acute toxicological effect.[43] Another acute toxicity study used 5000 mg/kg BW of andrographolide on mice and found no death nor adverse effect signs observed indicating that the LD_{50} of andrographolide was more than 5000 mg/kg BW in mice.[44]

Another study has evaluated the sub-chronic toxicity effect of *A. paniculata* standardized extract (AP-Bio) in Sprague Dawley rats for 90 days. There were no side effects of *A. paniculata* extract in 300, 600, and 900 mg/kg BW concentrations. The ophthalmoscope examination, urinalysis, hematological and biochemistry parameters, absolute and relative vital organ weights, and histopathological findings found no significantly different compared to control. The median lethal dose (LD_{50}) of *A. paniculata* extract was determined to be greater than 5000 mg/kg BW, whereas the no observed adverse effect level (NOAEL) of *A. paniculata* extract was found to be 900 mg/kg BW.[45] Another study conducted on Wistar rats using 250 and 500 mg/kg BW of andrographolide to study the subacute toxicity effect for 21 days, found no significant changes in body weight gain, behavior, mortality, hematological and biochemical parameters, vital organ weight, and histopathology. In addition, the white blood corpuscle (WBC) and lymphocyte were significantly increased by 7-14% and 21-24% respectively, along with a reduction in urea by 17-24% indicating the immune-stimulant and renal protective effects of andrographolide.[44]

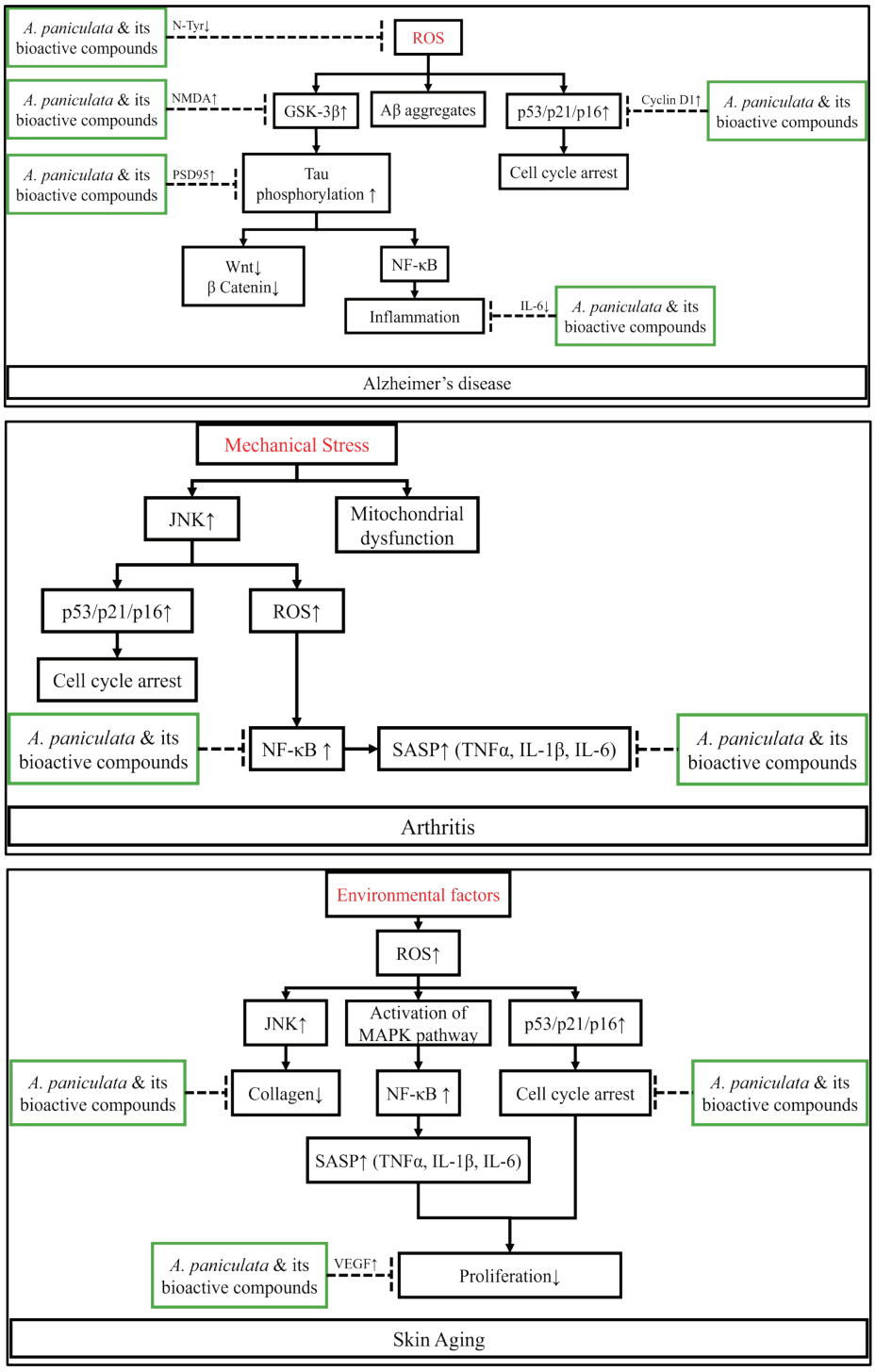

Figure 3. Proposed mechanisms of *A. paniculata* extract and its bioactive compound as an anti-senescence.

Bioavailability and Pharmacokinetics of A. paniculata Extract and Its Bioactive Compound

In a previous study, rabbits were administered *A. paniculata* orally at the dose of 7.04 mL/kg BW. Blood samples were collected at intervals of 0.0, 0.5, 1.5, 2.0, 3.0, and 5.0 hours following administration. The andrographolide levels were analyzed by high performance liquid chromatography in plasma, stomach, and liver. The results showed that the maximum andrographolide concentration reached in organ (C_{max}) values were 1.1893 µg/mL in the stomach, 0.2136 µg/mL in the plasma, and 0.0051 µg/mL in the liver. The time taken to reach the maximum concentration in the organ (T_{max}) values were 1 hour in the stomach, 1.5 hours in the plasma, and 2 hours in the liver. Meanwhile, the area under the

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curves (AUC) values were 2.4824 µg h/mL in the stomach, 0.4340 µg h/mL in the plasma, and 0.0038 µg h/mL in the liver. The absorption percentages in the stomach, plasma, and liver were 28.28%, 2.93%, and 0.05%, respectively.[46] It was necessary to develop the *A. paniculata* extract or its bioactive compounds in a specific formulation to increase its bioavailability. Thus, andrographolide-loaded pH-sensitive nanoparticles were compared with pure andrographolide in Wistar albino rats orally and showed that the nanoparticles increased the bioavailability by 121.53%, increased the AUC values by 2.2-fold, increased the C_{max} values by 3.2-fold, and decreased the T_{max} values by 4.0-fold.[47]

A. paniculata Extract Along with Its Bioactive Compounds, Andrographolide and 14-deoxyandrographolide, Improved the Progression of Senescence

Most research utilized *O. degus* of varying ages and Babl/c mice, AβPPswe/PS-1 mice, and C57BL/6 mice in different *in vivo* conditions. *O. degus*, a rat native to Central Chile, exhibits many genetic and physiological characteristics associated with neuropathological changes, such as reduced neural plasticity, cognitive decline and neuroinflammation. These characteristics are similar to AD and indicate that *O. degus* could be a more dependable model for studying the pathogenesis of AD.[2,26,33,34] Previous studies have suggested that the rodent *O. degus* is a promising model for pre-clinical research in biomedicine. *O. degus* is considered a natural model for human physiology and pathology due to its development of human-like traits. Rats and mice are nocturnal animals with behaviors that differ dramatically from humans. *O. degus* is a diurnal mammal, which is advantageous for endocrinological and circadian research.[48,49] *O. degus* are well-suited for laboratory settings and are cost-effective to breed due to their similar maintenance requirements to other laboratory rats.[49]

The most commonly utilized strain in the field of behavioral neurology and biomedical research is C57BL/6 mice.[50,51] Using C57BL/6 mice as a model for age-related cognitive decline might significantly advance research on age-related cognitive deficiencies and neurodegenerative conditions like AD. C57BL/6 mice are classified as old when they reach 18 to 24 months of age. Mortality becomes a concern after 24 months and hinders the analysis of data collected from C57BL/6 mice that are considered too advanced in age.[51]

Arthritis, a condition associated with senescence, affects elderly individuals globally, and cellular senescence is believed to have a role in its development.[52-54] Cellular senescence is a state in which cells permanently stop dividing, become resistant to programmed cell death, and continuously release a specific set of molecules known as senescence-associated secretory phenotype (SASP). SASPs are a collection of inflammatory molecules, including cytokines, chemokines, and enzymes. SASPs are a collective term for inflammatory factors associated with senescence.[52,55] Common SASPs, such IL-6 and IL-8, may stimulate the secretion of SASP factors, creating a positive feedback loop that strengthens senescence. IL-1α on the cell membrane, a component of SASP, can increase the transcription of IL-6 and IL-8 by boosting the binding capacity of NF-κB and C/EBPβ to DNA.[52,56] Andrographolide exhibited anti-inflammatory properties in arthritis which decreased the production of inflammatory molecules like IL-6.[19] It can be concluded that arthritis as a degenerative disease undergoes cellular senescence mechanisms in which SASPs are activated. As mentioned above, IL-6 is one of the SASPs in senescence perspective, and it can be concluded that andrographolide reduced SASP production in age-related disease.

Andrographolide also improved cognitive performance in an AD animal of varying ages by reducing the active state of GSK-3β, inhibiting long term depression (LTD) in the carbonic anhydrase 1 (CA1) region of the hippocampus, and decreasing Tau phosphorylation.[23] Additional studies suggest that the anti-inflammatory properties of andrographolide may contribute to reducing the late pathological changes seen in the brains of transgenic mice. These effects could help protect against the neuroinflammatory response caused by glial cells, potentially reducing cognitive impairment in an AD model.[23,57–60] Furthermore, there is evidence of the etiopathology of free radicals in people with AD.[23,61,62] Free radicals induce post-translational changes in various proteins, such as oxidation, glycation, and nitrotyrosination, leading to a decrease in protein function. All of these impacts are very important in AD, as mentioned before.[23,63] Multiple studies have shown that antioxidants have neuroprotective effects against Aβ-induced cell damage.[23,64,65] Therefore, andrographolide may have antioxidant capabilities that contribute to the neuroprotection of cognitive abilities seen in the study on AβPPswe/PS-1 transgenic mice treated with andrographolide.[23]

Adult neurogenesis or formation of new neurons in the adult is regulated by Wnt signaling pathway. This

mechanism is impaired in various illnesses that impact the central nervous system, such as AD, schizophrenia, and mood disorders, and is also influenced throughout the natural senescence process.[24,66,67] Previous experiment conducted on neurogenesis by enhancing the expression of the proneural Wnt target gene NeuroD1.[24] Hippocampal neurogenesis decreases with senescence in various species, including humans. Studies show that neurogenesis can be enhanced even in later stages of senescence. This study demonstrates that andrographolide can promote neurogenesis in elderly mice.[24,67–69] Andrographolide's effect on neurogenesis may be linked to the suppression of GSK-3β.[70] GSK-3β plays a crucial role in the Wnt/β-catenin signaling system, controlling the growth, differentiation, and development of neurons.[24,71–74] Stimulation of neurogenesis with GSK-3β inhibition has been shown both in laboratory settings and in living organisms. Cultured adult hippocampal progenitors were treated with the GSK-3β inhibitor lithium, resulting in increased cell division. *In vivo* administration of lithium stimulated cell division and the determination of neuronal destiny in the hippocampus of a mouse model of AD.[24,75] However, other mechanisms linked to the biological effects of andrographolide involve the suppression of PI3K/Akt and NF-κB pathways.[24,76,77]

AD is the leading cause of age-related dementia and the most common neurodegenerative disorder worldwide. With no effective treatment or preventive therapies currently available, AD is considered a major public health threat.[78,79] Histopathological hallmarks of AD include the deposition of amyloid-β (Aβ) plaques and the formation of neurofibrillary tangles.[80] As AD progresses, several cellular processes, including Ca^{2+} homeostasis, metabolism, oxidative stress and inflammation, are compromised and accumulation of unfolded/misfolded proteins such as tau and Aβ is observed.[81,82] Among these processes, oxidative stress and inflammation have been linked to several chronic degenerative diseases. Accordingly, recent investigations have suggested that neuroinflammation can be induced by oxidative stress, leading to neuroplastic deficits, mitochondrial dysfunction and impaired signaling pathways.[83] Along with the damage induced by Aβ per se as well as by hyperphosphorylated tau protein, the above mechanisms make the pathology of AD highly complex, necessitating that it be addressed from various angles.[84,85]

The inflammatory response can trigger the production of proinflammatory mediators such IL-6, COX2, TNFα, and inducible nitric oxide synthase (iNOS) to combat the various AD characteristics identified. Both types of biomarkers are recognized as crucial indicators of AD.[33,86,87] COX2 levels were lower in older animals compared to younger animals. Treatment with Andrographolide at a low dose of 2 mg/kg, but not at a high dose of 4 mg/kg, boosted COX2 levels in older animals. CaMKII levels were altered in accordance with research indicating that COX2 expression in macrophages is controlled by CaMKII.[33,88]

Females are often underrepresented in *in vivo* studies in scientific journals. Most scientific research predominantly involves male subjects, perhaps overlooking important variations when searching for common characteristics, unlike the included study which recently showed that female degus experience age-related synapse damage, while males do not. Young females aged 12-24 months had more efficient synaptic transmission than males, but older females between 60-84 months showed a notable decrease in synaptic efficacy compared to males. In addition, older females have a lower number of functioning axonal terminals, requiring the activation of twice the number of axons to achieve the same levels of long-term potentiation as older males.[3,89] The data show that age mostly impacts females, with no significant changes detected between male and female degus in brain asymmetry, size, and morphology of hippocampal areas.[3,33] Furthermore, the cognitive assessment of behavioral activities revealed that young females with strong short-term memory skills performed poorly on long-term memory tasks. Conversely, older females with impaired short-term memory excelled in long-term memory assignments.[90] The studies suggest that females do not equally retain systems for short- or long-term memory.

Similarly, andrographolide also boosted NR2B and PSD95 levels, improving synaptic efficacy by decreasing the number of axons needed to produce the maximum response. Thus, prolonged andrographolide treatment may enhance cognitive function in females experiencing age-related cognitive decline.[3] Tau is an early indicator of AD, particularly when it is phosphorylated. Tau contains many amino acid locations that can be modified by various kinases, such as GSK-3β, MAP/microtubule affinity-regulating kinase, and cyclin-dependent kinase 5 (CDK5).[3]

Skin aging is divided into two categories such as intrinsic aging caused by cellular senescence and extrinsic aging caused by UV exposure.[91,92] The photoaging or UV-induced skin aging reduces the collagen production which induces the wrinkle

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formation. Additionally, skin aging is associated with ROS production as well as the activation of pro-inflammatory cytokines.[91] In this review, *A. paniculata* extract including andrographolide and 14DAP inhibited the progression of senescence in the skin through its anti-inflammatory and antioxidant properties.[38,39]

Cellular senescence is characterized by cell cycle arrest while the metabolites remain functional.[93] However, senescence does not experience apoptosis. Several markers are necessary to be analyzed to determine the senescence process. Typically, markers are categorized into primary and secondary markers. Primary markers include cell cycle arrest and structural alteration. Cell cycle arrest markers include p16/pRB, p21/p53, and reduced Edu/BrdU levels. Structural change markers are senescence-associated β-galactosidase staining (SA-β-gal), DNA-SCARS. Meanwhile, secondary markers consist of additional traits including senescence-associated secretory phenotype (SASP) expression, increased ROS, DNA-damage response (DDR), apoptosis exclusion, and morphology (enlarged and flattened). SASP is a group of pro-inflammatory cytokines and chemokines which acts to induce senescence in the microenvironment through paracrine signaling. To identify cellular senescence process, the representative of each primary and secondary markers should be evaluated.[94] Exploring the interconnections between biological mechanisms of senescence and diseases, especially subclinical pathologies, is essential for developing preventive strategies, therapies, and enhancing our quality of life.[95]

Limitation of Current Study and Future Perspectives

The presence of inflammatory response and oxidative stress is closely linked to numerous diseases and serves as the main driver for the progression of age-related diseases. *A. paniculata* possesses anti-inflammatory and antioxidant properties, making it suitable for various medical applications. Moreover, cellular senescence becomes the therapeutic approach in senescence and age-related diseases.[96-98] Furthermore, it is possible that *A. paniculata* can be used to treat disorders that were not specifically identified in this study, by targeting the same pathological mechanism. However, this hypothesis requires a thorough investigation to confirm its validity.

Although in this study *A. paniculata* has demonstrated its protective effects against age-related diseases, it is important to focus on the amelioration of cellular senescence by analyzing markers of senescence such as cell cycle arrest markers, SA-β-gal activity, and other traits including SASP, ROS, and DDR markers.[94] This could be used as an opportunity for further research aiming to assess the mechanism of cellular senescence.

The most frequently identified compounds in *A. paniculata* are andrographolide, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide.[38,39] The included study mostly utilized andrographolide which is a major compound in the plant. Nevertheless, this offers an opportunity to assess the effect of alternative compounds on cellular senescence and age-related diseases. Furthermore, it is essential to thoroughly investigate the pharmacological effects of *A. paniculata*, as well as its bioactive compounds, in order to obtain deeper data that can support future clinical applications. Further study is also needed to explore the pharmacological effect of *A. paniculata* in humans. However, the specific primary and secondary of senescence markers are necessary to be conducted.

Conclusion

A. paniculata extract and its bioactive compounds such as andrographolide and 14-deoxyandrographolide exhibited beneficial effects on anti-senescence in various age-related diseases or parts of the body including arthritis, AD, skin aging, and endothelial cell senescence. The common main mechanisms of action of *A. paniculata* extract and its bioactive compounds are through antioxidant and anti-inflammatory activities. Additionally, the herb and its compounds improved memory, cognitive, and behaviour by restoring Wnt, β-catenin, GABA, Cyclin D1, and CAMK-VI protein levels, and decreasing GSK-3β in AD. The extract also induced cell cycle progression and proliferation through activating integrin β, VEGF, and the production of collagen type-1 in the skin. In endothelial cell senescence, andrographolide exerted anti-senescence via PI3K/Akt/Nrf and PI3K/Akt/AP-1.

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Authors Contributions

Concepting the ideas, literature searching, manuscript preparation, and manuscript editing were conducted by NGK, WA, and AJB. All authors reviewed the manuscript.

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