A Polyherbal Antioxidant Combination Attenuates Dexamethasone Induced Sarcopenia Via Murf-1, Atrogin-1, And Autophagy Pathways

Mohd. Zubair Arshad[1]*, Abhinav Prasoon Mishra[2], Anshuman Prajapati[3], Raushan Kumar[4]

[1]*Department of Pharmacology, Advanced Institute of Biotech and Paramedical Sciences, Kanpur, UP, India.

[2]Department of Pharmaceutical Chemistry, Advanced Institute of Biotech and Paramedical Sciences, Kanpur, UP, India.

[3]Department of Pharmacology, School of Pharmaceutical Sciences, C..S.J.M. University, Kanpur, U.P., India

[4]Department of Pharmaceutics, School of Pharmaceutical Sciences, C..S.J.M. University, Kanpur, U.P., India

Corresponding Author

Mohd. Zubair Arshad

Department of Pharmacology, Advanced Institute of Biotech and Paramedical Sciences, Kanpur, UP, India.,

Email Id: zubairm49@yahoo.co.in

ABSTRACT

Background: Sarcopenia is a multifactorial syndrome characterized by loss of muscle mass and function. Dexamethasone (DEX) aggravates muscle wasting through oxidative stress, inflammation, mitochondrial dysfunction, and protein catabolism. Multi-targeted natural interventions are therefore needed. We hypothesized that a combination of *Plumeria alba* and *Asparagus racemosus* would provide superior protection against DEX-induced sarcopenia compared with individual extracts.

Objective: To evaluate the protective effects of *P. alba* and *A. racemosus*, individually and in combination, on DEX-induced sarcopenia in Wistar rats using functional, biochemical, molecular, and histopathological analyses.

Methods: Alcoholic extracts were prepared and screened for phytochemicals and antioxidant activity (DPPH assay). Male Wistar rats (n=5) received DEX (10 mg/kg, i.p., 7 days), followed by treatment with P. *alba* (100 mg/kg), A. *racemosus* (100 mg/kg), or a 1:1 combination for 28 days. Assessed parameters included body weight, muscle mass, grip strength, rotarod performance, forced swim test, serum creatine kinase (CK), antioxidant enzymes (SOD, CAT), lipid peroxidation (MDA), LC3-II/LC3-I ratio, MuRF-1, Atrogin-1, and histopathology.

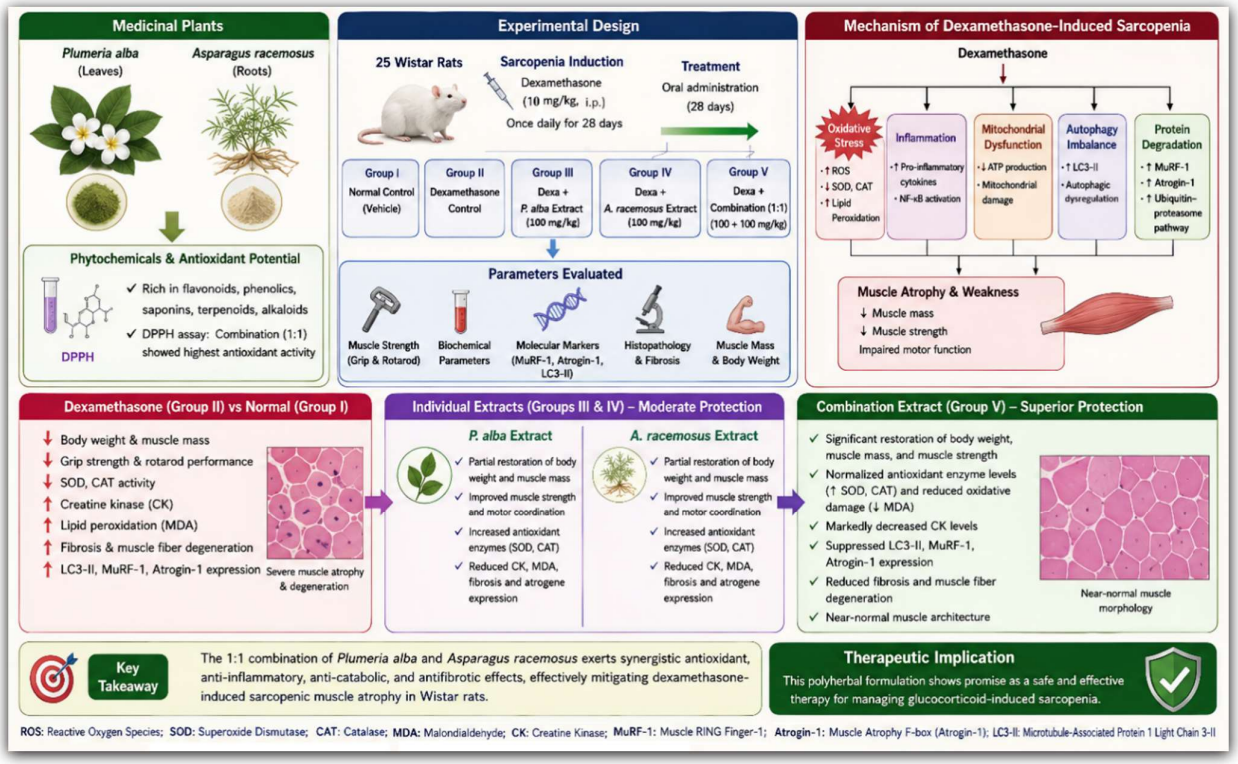
Results: The combination extract exhibited superior antioxidant activity (IC50: 31.2 ± 1.8 µg/mL; CI: 0.74). DEX induced 18.2% body weight loss and 62% reduction in muscle fiber area. Combination treatment mitigated weight loss (3.2%), restored gastrocnemius mass (86% of control), improved grip strength (93%) and rotarod latency (118.7 ± 3.8 s), normalized serum CK (76.1%), restored SOD and CAT levels (87%), and reduced MDA (60.7%). MuRF-1 and Atrogin-1 expression decreased (1.4- and 1.3-fold), LC3-II/LC3-I ratio increased (1.2 → 3.2-fold), and fiber area recovered to 91%, outperforming monotherapies ($p < 0.01$).

Conclusion: Combined extracts of *Plumeria alba* and *Asparagus racemosus* synergistically ameliorate DEX-induced sarcopenic muscle wasting via antioxidant, anti-catabolic, and autophagy-modulating mechanisms, offering a promising multi-targeted novel approach.

Keywords: Sarcopenia, dexamethasone, oxidative damage, *Plumeria alba*, *Asparagus racemosus*, novel approach.

How to cite this article: Arshad MZ, Mishra AP, Prajapati A, Kumar R. A Polyherbal Antioxidant Combination Attenuates Dexamethasone Induced Sarcopenia Via Murf-1, Atrogin-1, And Autophagy Pathways. Int J Drug Deliv Technol. 2026;16(58s): 182-201. DOI: 10.25258/ijddt.16.58s.17

Graphical Abstract



INTRODUCTION

Sarcopenia, marked by declining skeletal muscle mass, strength, and function (Nguyen et al., 2026), is a significant global health issue with low muscle power, walking inability and high chances of hospitalization (Frontera et al., 2011); affecting older adults and those with chronic metabolic and pharmacological stressors (Sayer & Cruz-Jentoft, 2022). Traditionally linked to aging, modern classifications distinguish primary sarcopenia from age-related changes and secondary sarcopenia from chronic disease, malnutrition, immobility, or prolonged glucocorticoid use (Cruz-Jentoft et al., 2019; Schakman et al., 2013). Glucocorticoid-induced sarcopenia is increasingly noted due to its widespread use in autoimmune, inflammatory, and neoplastic disorders (Sayer & Cruz-Jentoft, 2022; Schakman et al., 2013). Prolonged glucocorticoid treatment, especially dexamethasone (Williams, 2018), accelerates muscle degradation by inhibiting anabolic pathways, activating proteolytic systems, and disrupting skeletal muscle homeostasis (Bodine & Furlow, 2015; Schakman et al., 2013).

Dexamethasone triggers the ubiquitin–proteasome system (UPS), particularly the muscle-specific E3 ubiquitin ligases MuRF-1 and Atrogin-1 (Hwee et al., 2013), which are responsible for degrading contractile proteins (Bonaldo & Sandri, 2013). Concurrently, dexamethasone inhibits the IGF-1/Akt/mTOR pathway (Permpoon et al., 2025), a vital regulator of muscle protein synthesis and satellite cell activation, thereby tipping the balance toward net protein catabolism (Schakman et al., 2013). These are further aggravated by increased reactive oxygen species (ROS), mitochondrial dysfunction, and lipid peroxidation, which impair muscle regeneration (Romanello & Sandri, 2016). Elevated levels of pro-inflammatory cytokines such as TNF-α and IL-6 also contribute to muscle atrophy by activating NF-κB-mediated catabolic pathways and hindering myogenic repair (Bano et al., 2017).

Despite the clinical importance and widespread occurrence of glucocorticoid-induced sarcopenia, effective treatments remain scarce. Pharmacological options such as anabolic steroids, selective androgen receptor modulators (SARMs), myostatin inhibitors (Nguyen et al., 2026) and hormone-based therapies demonstrate varying degrees of efficacy and often present safety concerns that limit their long-term use (Bhasin et al., 2025). Although nutritional supplements including BCAAs, high-protein diets, vitamin-D (Lee et al., 2022), creatine and exercises offer some advantages, but may not adequately counter the rapid, multifactorial muscle degeneration (Cruz-Jentoft & Sayer, 2019).

However, their clinical utility is restricted by inconsistent efficacy, adverse effects, high cost, or limited long-term safety data. Therefore, the development of safe and effective therapeutic interventions capable of targeting multiple pathological pathways involved in muscle wasting remains an important unmet clinical need (Tsai, 2024).

Plant-derived bioactive compounds have garnered attention for their pharmacological properties (Oliveira et al., 2025). Phytochemicals such as flavonoids (Pandey et al., 2022), triterpenoids, and steroidal saponins demonstrate antioxidant, anti-inflammatory, anabolic, and adaptogenic activities, indicating their potential in managing muscle-wasting disorders (Rondanelli et al., 2016). Such multitarget pharmacological actions make medicinal plants attractive candidates for preventing or attenuating glucocorticoid-induced muscle degeneration (Seifi et al., 2024; Wang et al., 2025).

Unlike conventional single-target drugs, Phyto-therapeutic agents contain diverse bioactive compounds capable of modulating multiple molecular pathways simultaneously (Ankit et al., 2025). Among these botanicals, *Plumeria alba* and *Asparagus racemosus* stand out due to their phytochemical compositions. *Plumeria alba* Linn. (Apocynaceae) is a medicinal plant traditionally used for its antioxidant, anti-inflammatory, analgesic, and wound-healing properties (Chaudhuri et al., 2015; Imrana & Asif, 2020). Phytochemical investigations have identified flavonoids, phenolic compounds, iridoids, and triterpenoids that contribute to its free radical scavenging and cytoprotective activities (Zaufeen & Rahim, 2025). Similarly, *Asparagus racemosus* Willd. (Shatavari), an important medicinal plant in Ayurveda, possesses potent antioxidant, adaptogenic, immunomodulatory, and tissue-protective properties (Bopana & Saxena, 2007). Its roots are rich in steroidal saponins (shatavarins), flavonoids, and polyphenolic compounds that have demonstrated significant antioxidant and anti-inflammatory activities (Alok et al., 2013). These pharmacological properties suggest that both plants may provide protection against oxidative stress-mediated skeletal muscle damage and degradation (Wang et al., 2025).

Molecular mechanism of dexamethasone-induced sarcopenia

Sarcopenia is defined by the disruption of skeletal muscle proteostasis, resulting in a progressive reduction in muscle mass, strength, and function due to increased protein degradation and decreased synthesis (Picca & Calvani, 2021; Kim et al., 2021). The excessive activation of catabolic pathways, particularly the ubiquitin–proteasome system (UPS), plays a crucial role in muscle protein breakdown (Bonaldo & Sandri, 2013; Demontis et al., 2013). Dexamethasone, enhance the expression of muscle-specific E3 ubiquitin ligases (Hwee et al., 2013), including MuRF-1 and Atrogin-1 (Pang et al., 2023), which promote myofibrillar protein degradation (Khalil, 2018) and reduce the cross-sectional area of muscle fibers (Bodine et al., 2001). Additionally, they suppress IGF-1/Akt/mTOR signalling (Permpoon et al., 2025), thereby inhibiting protein synthesis, satellite cell proliferation, and muscle regeneration (Schakman et al., 2013; Moriya & Miyazaki, 2018).

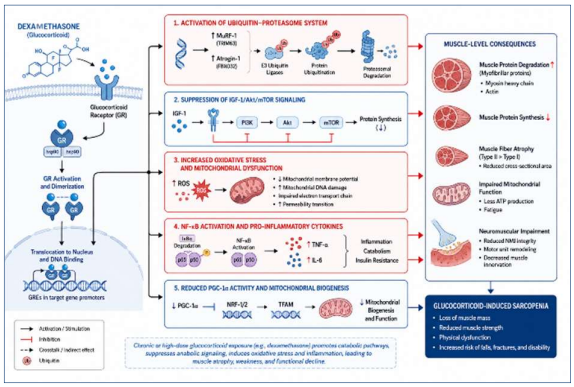


Figure 1: Overview of molecular mechanisms underlying glucocorticoid-induced sarcopenia.

Excessive ROS damages mitochondrial membranes, lipids, and DNA, leading to impaired ATP production and disrupted calcium homeostasis (Murphy, 2008; Powers et al., 2011). Prolonged oxidative stress (Agrawal et al., 2023) depletes antioxidants such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), exacerbating cellular damage and muscle wasting (Birben et al., 2012). Glucocorticoids also reduce mitochondrial biogenesis regulators like PGC-1α, contributing to mitochondrial dysfunction and impaired energy metabolism (Sandri et al., 2006). Inflammation exacerbates muscle atrophy by increasing TNF-α and IL-6 levels, which activate the NF-κB signaling pathway (Ma et al., 2018), which promotes the expression of proteolytic genes while simultaneously inhibiting myogenesis (Bano et al., 2017). These inflammatory changes destabilize neuromuscular junctions, reduce acetylcholine receptor density, and impair motor unit signalling, ultimately diminishing muscle strength and function (Iyer et al., 2021). The interplay of excessive proteolysis, suppressed anabolic signalling (Schakman et al., 2013), mitochondrial dysfunction (Chen et al., 2023; Liu et al., 2015), oxidative stress (Zhang et al., 2023), chronic inflammation (Chen et al., 2022), and neuromuscular impairment (Ma et al., 2018) drives glucocorticoid-

induced sarcopenia (Wiedmer et al., 2020). This multifaceted condition underscores the need for therapies targeting multiple pathological pathways (Hopkins, 2008).

Although many medicinal plants have been studied for antioxidant and muscle-protective effects, the synergistic anti-sarcopenic potential of *Plumeria alba* and *Asparagus racemosus* in dexamethasone-induced muscle atrophy remains unexplored. The effects of this combination on oxidative stress, atrophy markers (MuRF-1, Atrogin-1), and autophagy pathways are unknown, highlighting a critical research gap. This study aimed to evaluate the protective effects of combined ethanolic extracts of *P. alba* leaves and *A. racemosus* roots in Wistar rats, assessing body and muscle weight, muscle strength and endurance, oxidative stress biomarkers, expression of atrophy- and autophagy-related genes, and histopathological changes in skeletal muscle.

MATERIAL & METHODS

The present study was carried out at Central Laboratory of Pharmacology Department, Advance Institute of Biotech and Paramedical Sciences, Naramau, Kanpur (U.P.) India.

Study design and duration

The study followed a sequential design, including plant collection and authentication, extraction and phytochemical characterization, followed by animal experiments conducted in parallel to optimize time and resources while maintaining scientific rigor. This combined approach allows simultaneous standardization and biological validation of extracts for pharmacognostic or pharmacological studies. The research was conducted over six months, from 15th November 2025 to 15th May 2026, with all experimental phases well-structured.

Table 1: Duration of study and activities performed

Duration	Research Phase	Activities Performed
15th Nov – 10th Dec, 2025	Collection and Extraction of Plant Materials	Collection of plant materials, authentication, shade drying, powdering, degreasing and Soxhlet extraction of plant materials.
11th Dec, 2025 – 31st Jan, 2026	Extraction and Characterization of Phytoconstituent	Solvent evaporation, crystallization, phytochemical characterization and spectroscopic studies.
1st Feb – 15th March, 2026	Animal Experimental Studies	Acclimatization of rats, induction of secondary sarcopenia using dexamethasone, oral treatment with plant extracts, and periodic functional assessments.
16th March – 15th April, 2026	Biochemical and Histopathological Evaluation	Biochemical estimations, histopathological processing, and statistical analysis of experimental data.
16th April – 15th May 2026	Data Interpretation and Documentation	Compilation, interpretation, recording, and documentation of research findings.

Chemicals and Reagents

Dexamethasone sodium phosphate injection (Dexona 4 mg/mL, Zydus Healthcare, Ahmedabad, India) was used to induce experimental sarcopenia. Analytical-grade chemicals, reagents, and freshly prepared solutions were obtained from institutional laboratory facilities. Antioxidant activity was assessed using DPPH, while serum CK, SOD, CAT, and MDA were measured with commercial kits. Biochemical assays and qRT-PCR analyses were performed using validated kits and molecular reagents following manufacturer protocols at an accredited laboratory (Aakaar Biotechnologies Pvt. Ltd., Lucknow, India).

Plant collection and authentication

Fresh leaves of *Plumeria alba* Linn. (Family: Apocynaceae) and roots of *Asparagus racemosus* Willd. (Family: Asparagaceae) were collected from the natural habitat of Kalyanpur – Bithoor, Kanpur (U.P.) India. The collected plant materials were thoroughly washed with distilled water to remove adhering soil and foreign matter and subsequently shade-dried for 7-10 days at room temperature (25–30°C) for further processing.

The plant specimens were taxonomically identified and authenticated by Prof. Dr. Alka Rani, (H.O.D.) Department of Botany, Pt. Prithi Nath College, Kanpur (U.P.) India. Voucher specimens were deposited for future reference under voucher numbers PPNH/VSN/25/PA-001 (*Plumeria alba*) and PPNH/VSN/25/AR-002 (*Asparagus racemosus*). Authentication certificates were provided as supplementary material. Following authentication, the dried plant materials were pulverized separately using a mechanical grinder, and passed through a suitable mesh sieve (No. 60 & 80) to obtain uniform particle size. The powdered samples were stored in airtight containers protected from moisture and light until extraction.

Preparation of extracts

Approximately 200 g of the powdered *P. alba* leaves and *A. racemosus* roots were separately subjected to Soxhlet extraction using 95% and 70% ethanol respectively as the extraction solvent. Extraction was continued for 48 hours at 60°C until the solvent in the siphon tube became colorless, indicating complete extraction of phytoconstituents. The obtained extracts were filtered and concentrated under reduced pressure (100 – 200 mbar) using a rotary vacuum evaporator at a temperature not exceeding 50°C, rotation speed around 100-150 rpm for 45-60 mins. The concentrated extracts were further dried on a water bath to obtain semisolid masses and stored in airtight containers at 4°C until further use.

The percentage yield of each extract was calculated using the following formula:

$$\text{Percentage Yield (\%)} = \frac{\text{Weight of dried extract (g)}}{\text{Weight of crude powder (g)}} \times 100$$

Preliminary phytochemical screening

The ethanolic extracts of *Plumeria alba* leaves and *Asparagus racemosus* roots were subjected to preliminary phytochemical screening using standard qualitative methods described in pharmacognostic literature. The extracts were evaluated for the presence of major classes of secondary metabolites including alkaloids, flavonoids, saponins, terpenoids, tannins, steroids, glycosides, and phenolic compounds.

Table 2: Reagents used for preliminary phytochemical screening of *P. alba* and *A. racemosus* extracts

Phytochemical Class	Test Performed	Reagent(s) Used	Positive Indication
Alkaloids	Mayer's Test	Mayer's reagent (Potassium mercuric iodide)	Cream or white precipitate
Alkaloids	Wagner's Test	Wagner's reagent (Iodine–Potassium iodide)	Brown/reddish-brown precipitate
Alkaloids	Dragendorff's Test	Dragendorff's reagent (Potassium bismuth iodide)	Orange-red precipitate
Flavonoids	Shinoda Test	Magnesium turnings + Concentrated HCl	Pink/red coloration
Saponins	Foam Test	Distilled water	froth/foam formation
Terpenoids	Salkowski Test	Chloroform + Concentrated H_2SO_4	Reddish-brown interface
Steroids	Liebermann–Burchard Test	Acetic anhydride + Concentrated H_2SO_4	Bluish-green coloration
Tannins	Ferric Chloride Test	5% $FeCl_3$ solution	Blue-black or greenish-black colour
Phenols	Ferric Chloride Test	5% $FeCl_3$ solution	Deep blue, green, or violet colour

Fourier Transform Infrared (FTIR) Analysis

FTIR spectroscopy of the ethanolic extracts of *Plumeria alba* and *Asparagus racemosus* was carried out at Pranveer Singh Institute of Technology (PSIT), Kanpur, Uttar Pradesh, India. The spectra were recorded using standard analytical procedures in the range of 4000–400 cm^{-1} to identify the major functional groups present in the extracts.

DPPH Radical Scavenging Assay

The in vitro antioxidant activity of the ethanolic extracts of *Plumeria alba* leaves, *Asparagus racemosus* roots, and their combination (1:1) was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Various concentrations of the extracts (10, 25, 50, 100, and 200 µg/mL) were prepared in methanol. An aliquot of 1 mL of each extract solution was mixed with 1 mL of freshly prepared DPPH solution (0.1 mM in methanol) and incubated in the dark at room temperature for 30 min. Following incubation, the absorbance was measured at 517 nm using a UV–Visible spectrophotometer against a blank solution. Ascorbic acid was used as the reference standard antioxidant under identical experimental conditions.

The percentage inhibition of DPPH radicals was calculated using the following equation:

$$\text{\% Radical Scavenging Activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ represents the absorbance of the DPPH control solution and A_{sample} represents the absorbance of the test sample.

The percentage radical scavenging activity was plotted against extract concentration, and the IC_{50} value (concentration required to inhibit 50% of DPPH radicals) was determined by nonlinear regression analysis using GraphPad Prism software. Lower IC_{50} values were considered indicative of stronger antioxidant activity. All measurements were performed in triplicate, and results were expressed as mean ± SEM. Using the Chou–Talalay Combination Index (CI) method, we can estimate whether the polyherbal combination exhibits synergism, additivity, or antagonism.

For a 1:1 fixed-ratio combination, the CI at a given effect level (Fa) is:

$$CI = \frac{D_1}{(Dx)_1} + \frac{D_2}{(Dx)_2}$$

Where, D1 = dose one in the combination producing a given effect, D2 = dose of AR in the combination producing the same effect, (Dx)1 = dose one alone producing that effect, and (Dx)2 = dose two alone producing that effect.

Experimental animals and design

Healthy adult male Wistar rats (25) weighing 180–240 g were used for the present study. The animals were obtained from the animal house facility of the Advance Institute of Biotech and Paramedical Sciences, Kanpur, Uttar Pradesh, India. Prior to the commencement of the experiment, animals were acclimatized to laboratory conditions for one week and maintained under standard environmental conditions at a temperature of 22 ± 2°C, relative humidity of 55 ± 10%, and a 12 h light/12 h dark cycle. The animals were housed in clean polypropylene cages containing sterile paddy husk bedding and were provided with a standard pellet diet and water ad libitum throughout the study period.

All experimental procedures involving animals were reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the Advance Institute of Biotech and Paramedical Sciences, Kanpur, India, under approval protocol number (AIBS/IAEC/2025/04) on 6/12/2025. The study was conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India, under CCSEA registration number (1122/PO/Re/S/07/CCSEA). All efforts were made to minimize animal suffering (CCSEA guidelines) and to reduce the number of animals used in the study.

Acute and sub-acute oral toxicity studies

Test doses for acute and sub-acute studies were based on published safety data. For *Plumeria alba* hydroalcoholic root extract, acute oral administration in rats at 5000 mg/kg (single dose) and sub-acute at 250, 500, and 1000 mg/kg daily for 28 days showed no mortality or significant toxic effects (Tessou et al., 2013). Similarly, *Asparagus racemosus* root ethanol extract was well tolerated in mice at 2000 mg/kg for both acute and 28-day sub-acute administration, with no mortality or adverse effects (Bhandary et al., 2017). These findings guided safe starting doses for the current preclinical evaluation, ensuring ethical compliance and minimizing animal risk. Animals were randomly allocated to treatment groups using a simple randomization method. Doses of 100 mg/kg were selected based on published pharmacological studies.

Table 3. Experimental design and treatment groups

Group	Description	Treatment Administered
I	Normal Control	Vehicle (0.5% CMC, p.o.)
II	DEX Control	Dexamethasone (10 mg/kg, i.p.) + Vehicle
III	DEX + *P. alba*	Dexamethasone (10 mg/kg, i.p.) + *P. alba* leaf extract (100 mg/kg, p.o.)
IV	DEX + *A. racemosus*	Dexamethasone (10 mg/kg, i.p.) + *A. racemosus* root extract (100 mg/kg, p.o.)
V	DEX + Combination	Dexamethasone (10 mg/kg, i.p.) + *P. alba* (100 mg/kg, p.o.) + *A. racemosus* (100 mg/kg, p.o.)

Abbreviations: *DEX, dexamethasone; CMC, carboxymethyl cellulose; p.o., per oral; i.p., intraperitoneal*

(No standard control group was included, as there is currently no universally accepted pharmacological therapy for sarcopenia. In addition, animal use was minimized in accordance with the CPCSEA-approved protocol and the 3Rs principle of animal experimentation).

Induction of Sarcopenia

Secondary sarcopenia was induced by intraperitoneal administration of dexamethasone sodium phosphate at a dose of 10 mg/kg/day for 7 consecutive days. Dexamethasone-treated animals exhibited characteristic features of glucocorticoid-induced muscle wasting, including reductions in body and muscle weight, impaired muscle performance, oxidative stress, and upregulation of muscle atrophy-related markers. This model was used to evaluate the protective effects of *Plumeria alba, Asparagus racemosus*, and their combination against dexamethasone-induced skeletal muscle degeneration.

Functional Assessment

Grip Strength Test

Forelimb and hindlimb grip strength were assessed using a Yamto's grip strength meter (Model: YGS-X1) by Yamto Instruments Sales Corporation. Each rat was allowed to grasp the metal grid of the apparatus with its forelimbs or hindlimbs, and was gently pulled backward until it released the grid. The maximum force exerted before release was automatically recorded in grams (g). Three consecutive measurements were obtained for each animal, and the mean value was used for statistical analysis.

Rotarod Test

Motor coordination and muscular endurance were evaluated using a Rotarod (two compartment) apparatus consisting of a motorized rotating rod (3 cm diameter × 40 cm length) positioned 30 cm above the base. The rotational speed was gradually accelerated from 4 to 40 rpm over a period of 300 s. Each rat was placed individually on the rotating rod, and the latency to fall was recorded in seconds.

Forced Swim Test (Porsolt test)

Rats were individually placed in a transparent cylindrical tank (diameter 20–25 cm; height 40–50 cm) containing water maintained at a depth of 30–35 cm and temperature of 23–26°C. Each animal was allowed to swim freely for 6 min. Immobility time was recorded during the final 4 min of the test. A reduction in immobility time was considered indicative of improved endurance and resistance to fatigue.

Biochemical analysis

At the end of the experimental period, animals were deeply anesthetized with an overdose of ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Following confirmation of loss of reflexes and absence of response to external stimuli, the animals were euthanized, and blood and skeletal muscle tissues were collected for biochemical and histopathological analyses. All procedures were performed in accordance with CCSEA guidelines and the approved IAEC protocol.

Blood samples were collected by cardiac puncture. Serum was separated by centrifugation at 3000 rpm for 15 min and stored at −20°C until analysis. Gastrocnemius muscle tissues were excised, washed with ice-cold normal saline, and homogenized in chilled phosphate buffer (0.1 M, pH 7.4). The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C, and the resulting supernatants were used for biochemical estimations. Samples were analyzed within two weeks of collection.

Serum Creatine Kinase (CK)

Serum creatine kinase (CK) activity was estimated using a commercially available CK diagnostic kit according to the manufacturer's instructions. CK levels were expressed as U/L (Aleksovska et al., 2025).

Superoxide Dismutase (SOD)

Superoxide dismutase (SOD) activity in muscle homogenates was determined by the inhibition of nitro blue tetrazolium (NBT) reduction method. The enzyme activity was expressed as U/mg protein (Weydert and Cullen, 2010).

Catalase (CAT)

Catalase (CAT) activity was measured by monitoring the decomposition of hydrogen peroxide (H_2O_2) spectrophotometrically. The results were expressed as U/mg protein (Hadwan et al., 2024).

Malondialdehyde (MDA)

Lipid peroxidation was assessed by estimating malondialdehyde (MDA) levels using the thiobarbituric acid reactive substances (TBARS) assay. Briefly, muscle homogenates were reacted with thiobarbituric acid (TBA) reagent under acidic conditions and heated to develop a colored complex. Absorbance was measured spectrophotometrically, and MDA concentrations were expressed as nmol/mg protein (Aguilar Diaz & Borges, 2020).

Gene expression/Autophagy marker analysis

Total RNA was isolated from gastrocnemius muscle tissue and reverse-transcribed into complementary DNA (cDNA). Relative mRNA expression levels of MuRF-1, Atrogin-1, and LC3 were quantified by real-time polymerase chain reaction (RT-PCR) using gene-specific primers (Souza et al., 2025; Yokogawa et al., 2025). Gene expression levels were normalized against an internal housekeeping gene, and relative expression was calculated using the 2-ΔΔCt method, and results were expressed as fold change relative to the normal control group. Autophagy was evaluated by measuring the LC3-II/LC3-I ratio using Western blotting (Cao et al., 2025). The LC3-II/LC3-I ratio was calculated and

normalized to β-actin, with increased ratios indicating autophagy induction.

Histopathological evaluation

At the end of the experimental period, gastrocnemius muscle tissues were carefully excised, rinsed with normal saline to remove blood residues, and immediately fixed in 10% neutral buffered formalin for 24–48 h. The fixed tissues were processed through a graded series of alcohols for dehydration, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at a thickness of 5 µm using a rotary microtome and mounted on clean glass slides for histological examination. Histological analysis was performed by an investigator blinded to treatment allocation.

Hematoxylin and Eosin (H&E) Staining

For hematoxylin and eosin staining, sections were stained with hematoxylin, rinsed in water, differentiated and blued as required, counterstained with eosin, dehydrated, cleared, and mounted with a resinous medium for microscopic evaluation.

Picrosirius Red Staining

For Picrosirius Red staining, deparaffinized and rehydrated sections were stained with Weigert's hematoxylin, followed by incubation in Picrosirius Red solution to visualize collagen fibers. Excess stain was removed by acidified washing, after which the sections were dehydrated, cleared in xylene, and mounted.

Muscle Fiber Cross-Sectional Area (CSA) Analysis

Morphometric analysis of skeletal muscle fibers was performed using digital photomicrographs obtained from H&E-stained sections. Images were analyzed using ImageJ software (National Institutes of Health, Bethesda, USA). The cross-sectional area (CSA) of individual muscle fibers was measured from randomly selected microscopic fields at the same magnification. A minimum of 50–100 muscle fibers per animal were analyzed, and the mean CSA was calculated and expressed in µm².

Table 4. Methods and parameters for histological evaluation

Staining Method	Parameters Assessed
H&E Staining	Muscle fiber integrity, degeneration, inflammation, vacuolization, architecture
Picrosirius Red Staining	Collagen deposition, fibrosis, extracellular matrix accumulation
CSA Analysis	Muscle fiber size, degree of atrophy, muscle recovery

Statistical analysis

All experimental data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (Version 10.0, GraphPad Software Inc., San Diego, CA, USA). Comparisons among multiple experimental groups were carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test. Differences between groups were considered statistically significant when the p-value was less than 0.05 ($p < 0.05$).

RESULT & DISCUSSION

Percentage Yield

The hydroalcoholic extracts of powdered plant materials (100 g) yielded approximately 13.22 g of *Plumeria alba* extract presented a deep green-brown colour, and 10.14 g of *Asparagus racemosus* extract, appeared light brown via Soxhlet extraction (Table 5).

Table 5. Percentage yield of hydroalcoholic extracts via soxhlet extraction

Plant Material (part used)	Powdered Weight (g)	Solvent used (%)	Solvent Volume (L)	Soxhlet Temp (°C)	Extraction Time (h)	Rotary Evaporator Temp (°C)	Vacuum Pressure (mbar)	Rotation Speed (rpm)	Percentage Yield (%)
Plumeria alba (leaf)	100	Ethanol (95%)	1 – 1.5	60	48	40–50	100–200	100–150	13.22
Asparagus racemosus (root)	100	Ethanol (70%)	1 – 1.5	60	48	40–50	100–200	100–150	10.14

Phytochemical profiling

The alcoholic extracts of *Plumeria alba* leaves and *Asparagus racemosus* roots were screened for primary and secondary metabolites using standard qualitative tests. Analysis revealed the presence of saponins, tannins flavonoids, alkaloids, glycosides, phenolics, and terpenoids, indicating a rich spectrum of bioactive compounds responsible for their antioxidant and anti-sarcopenic effects (Table 6).

Table 6: Qualitative phytochemical profile of plant extracts

Phytochemical class	***Plumeria alba* (leaf)**	***Asparagus racemosus* (root)**
Alkaloids	±	+
Flavonoids	+++	++
Saponins	+	+++
Terpenoids	++	+
Tannins	+	+
Steroids	+	++
Phenolics	+++	+

where (±) trace, (+) low, (++) moderate, (+++) high concentration

Fourier Transform Infrared (FTIR) Spectroscopy

The FTIR spectrum of *Plumeria alba* leaf extract revealed characteristic peaks indicative of its phytochemical composition. The FTIR spectrum confirmed that the *Plumeria alba* leaf extract contains phenolics, flavonoids, polysaccharides, and glycosidic compounds, consistent with its reported antioxidant and bioactive properties (Figure 2).

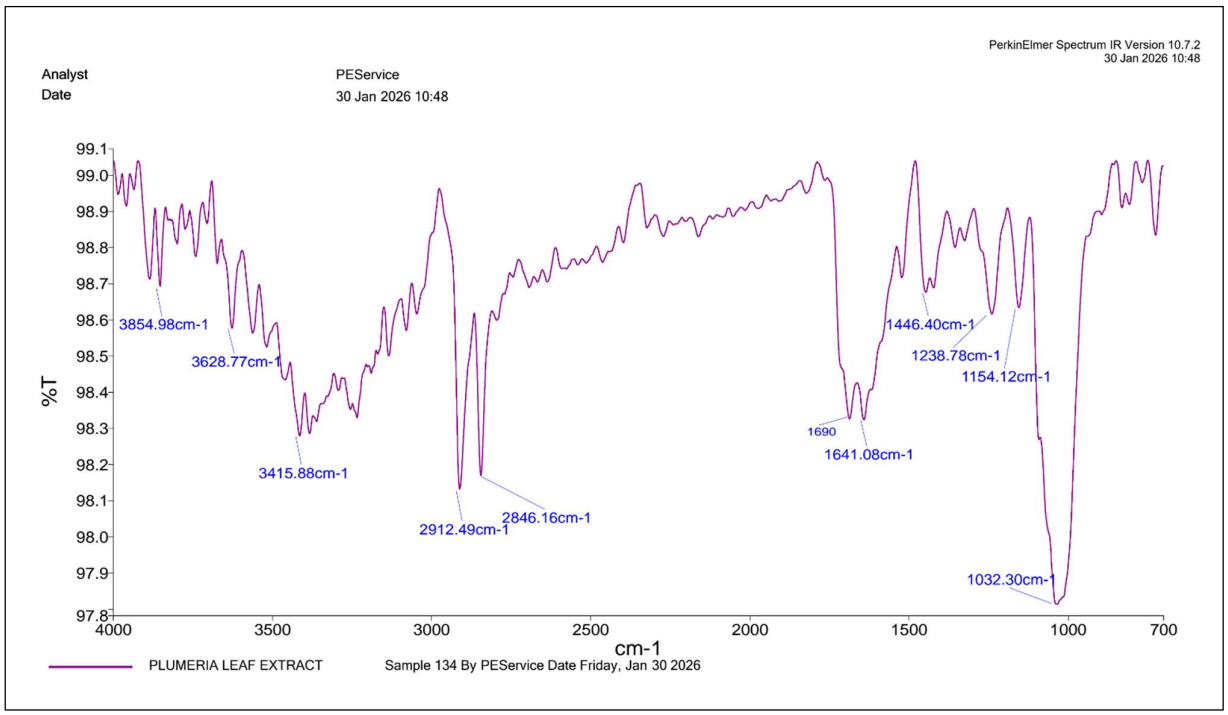


Figure 2: FTIR spectroscopy of *Plumeria alba*

Table 7. Major FTIR peaks of *Plumeria alba* leaf extract and associated bioactive functional groups

Wavenumber (cm^{-1})	Functional Groups	Possible Bioactive Compound
3855–3629	O–H stretching (broad)	Phenolics, flavonoids, alcohols
3416	O–H stretching	Hydrogen-bonded hydroxyls from polyphenols/flavonoids
2912–2846	C–H stretching	Aliphatic chains in fatty acids or terpenoids
1690–1641	C=O stretching	Carbonyls in flavonoids, ketones, carboxylic acids
1446	C–H bending	Methyl/methylene groups in secondary metabolites
1239	C–O stretching	Alcohols, ethers, phenolic –O– groups
1154	C–O–C stretching	Glycosidic linkages, polysaccharides
1032	C–O stretching / fingerprint	Sugars, polysaccharides, phenolic –OH

The FTIR spectrum of *Asparagus racemosus* root extract revealed characteristic peaks indicative of its phytochemical composition. The FTIR spectrum of *Asparagus racemosus* root extract confirmed the presence of saponins, glycosides, phenolics, and steroidal/triterpenoid compounds (Figure 3).

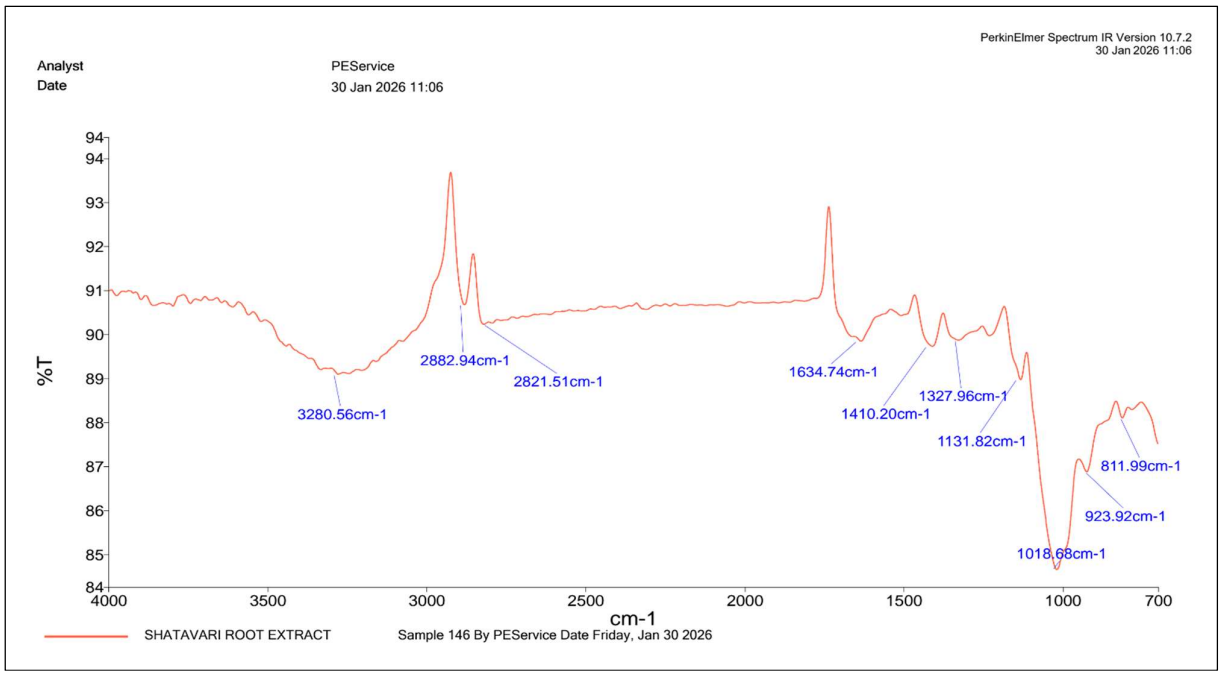


Figure 3: FTIR spectroscopy of *Asparagus racemosus*

Table 8. Major FTIR peaks of *Asparagus racemosus* root extract and associated bioactive functional groups

Wavenumber (cm^{-1})	Functional Group	Possible Bioactive Compound
3281	Broad O–H stretching	Hydroxyl groups in saponins and phenolics
2883–2822	C–H stretching	Aliphatic –CH2 / –CH3 in saponins or sterols
1635	C=O stretching / Amide I	Carbonyls in flavonoids, ketones, or conjugated acids
1410	C–H bending	Methyl/methylene groups

Wavenumber (cm^{-1})	Functional Group	Possible Bioactive Compound
1328	C–O stretching	Alcohols or glycosidic linkages
1132	C–O–C stretching	Glycosidic bonds in saponins
924	C–H bending (fingerprint)	Steroidal or triterpenoid skeletons
812	C–H out-of-plane bending	Aromatic or steroidal structures
1019	C–O stretching / fingerprint	Polysaccharide and sugar moieties

In-vitro Antioxidant Activity (DPPH Assay)

The DPPH radical scavenging activity of all extracts increased in a concentration-dependent manner (10–200 µg/mL). *P. alba* extract showed an IC_{50} value of 48.6 ± 2.3 µg/mL, while *A. racemosus* exhibited an IC_{50} of 62.4 ± 3.1 µg/mL. The combination extract (1:1) demonstrated significantly lower IC_{50} (31.2 ± 1.8 µg/mL, $p < 0.01$ vs. individual extracts), indicating superior antioxidant activity. At 100 µg/mL, the percentage scavenging was: *P. alba* 72.4%, *A. racemosus* 65.8%, combination 89.3%, compared to ascorbic acid standard (94.1%). The combination index (CI) calculated using Chou–Talalay method was 0.74 at IC_{50}, confirming synergy (CI < 1). Synergy was evaluated only at the IC50 level; further isobologram-based analyses are required to comprehensively characterize interaction profiles.

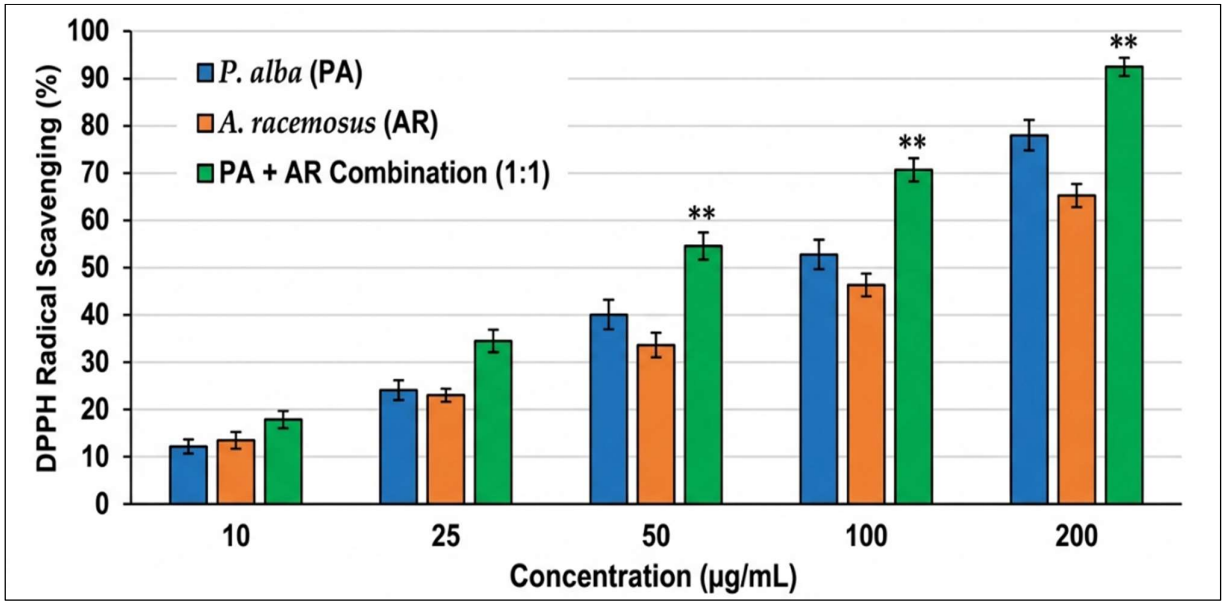


Figure 4: DPPH radical scavenging activity of P. alba, A. *racemosus*, and their combination (1:1). Values are mean ± SEM (n=3). $p < 0.001$ vs. individual extracts at same concentration. One-way ANOVA followed by Tukey's multiple comparison test.

Table 9: DPPH Radical Scavenging Activity of *Plumeria alba* (PA), *Asparagus racemosus* (AR), and Their Combination (1:1)

Concentration (µg/mL)	*Plumeria alba* (% Scavenging)	*Asparagus racemosus* (% Scavenging)	PA + AR Combination (% Scavenging)
10	12.3 ± 1.5	13.5 ± 1.7	18.2 ± 1.8
25	24.5 ± 2.0	23.1 ± 1.6	34.8 ± 2.1
50	40.2 ± 3.1	34.0 ± 2.5	54.8 ± 2.8**
100	52.8 ± 3.0	46.4 ± 2.4	70.8 ± 2.5**
200	78.0 ± 3.2	65.4 ± 2.3	92.5 ± 2.1**

Body weight and muscle wet weights

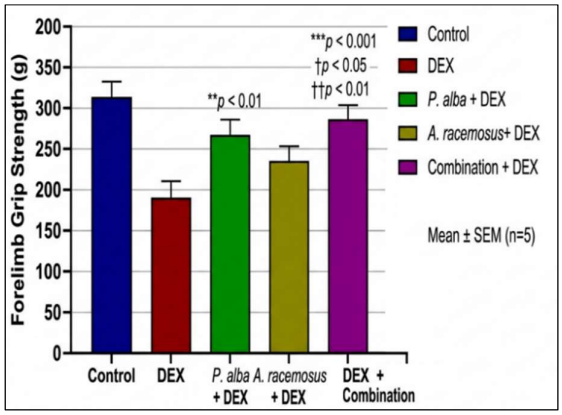
Dexamethasone (DEX) control (Group II) caused 18.2% body weight loss (final 162.4 ± 5.3 g vs. normal 214.5 ± 4.3 g, $p < 0.001$). *P. alba* (Group III) attenuated loss to 10.3% (178.2 ± 4.7 g), *A. racemosus* (Group IV) to 8.1% (182.5 ± 4.2 g), and combination (Group V) to only 3.2% (192.3 ± 3.9 g, $p < 0.01$ vs. monotherapies). Muscle weight (gastrocnemius, soleus, tibialis anterior and quadriceps) exhibited DEX-induced wasting: gastrocnemius, 42% reduction from normal. Combination restored gastrocnemius weight to 5.48 ± 0.10 mg/g (94% of normal) which was better than *P. alba* (4.61, 79% restoration) and *A. racemosus* (4.78, 82% restoration). The same pattern was followed in case of all muscles (Table 10).

Table 10: Effect of treatments on body weight and relative muscle weights

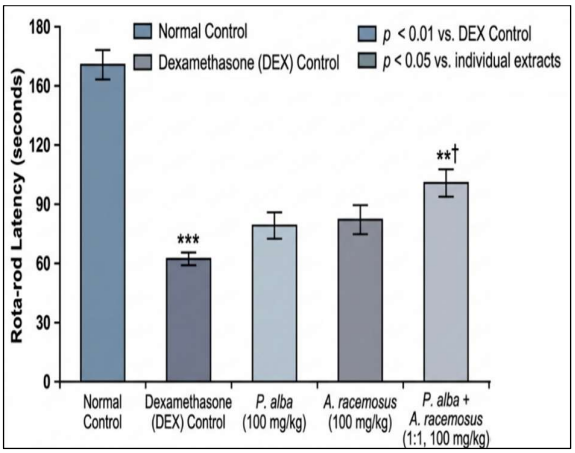
Parameters	Group I (Normal)	Group II (DEX)	Group III (*P. alba*)	Group IV (*A. racemosus*)	Group V (Combination)
Final body weight (g)	214.5 ± 4.3	162.4 ± 5.3	178.2 ± 4.7	182.5 ± 4.2	192.3 ± 3.9
Gastrocnemius (mg/g)	5.82 ± 0.14	3.38 ± 0.12	4.61 ± 0.13	4.78 ± 0.11	5.48 ± 0.10
Soleus (mg/g)	0.68 ± 0.03	0.41 ± 0.02	0.54 ± 0.02	0.56 ± 0.03	0.63 ± 0.02
Tibialis anterior (mg/g)	2.45 ± 0.08	1.52 ± 0.06	1.98 ± 0.07	2.04 ± 0.06	2.31 ± 0.05
Quadriceps (mg/g)	7.21 ± 0.21	4.45 ± 0.18	5.89 ± 0.19	6.02 ± 0.17	6.85 ± 0.15

Grip strength, rotarod, and forced swim test

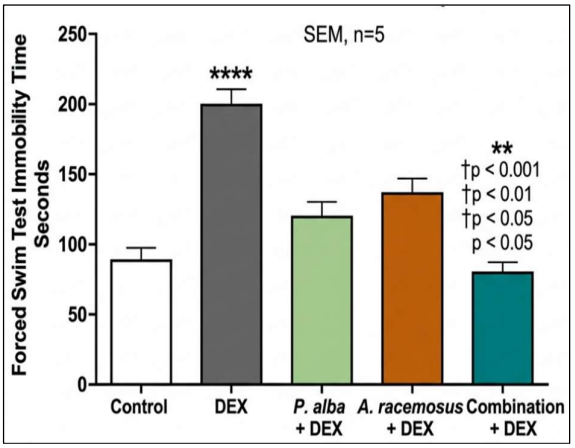
Forelimb grip strength in DEX control fell to 285 ± 12 g vs. normal 512 ± 15 g ($p < 0.001$). *P. alba* and *A. racemosus* restored strength to 386 ± 14 g and 402 ± 13 g, respectively ($p < 0.05$ vs. DEX). Combination achieved 478 ± 11 g (93% of normal, $p < 0.01$ vs. monotherapies). Synergy was found in hindlimb strength as well (89% restoration). Rotarod latency: DEX control fell to 48.3 ± 3.1 s vs. normal 132.5 ± 4.2 s. Combination, with a latency of 118.7 ± 3.8 s, was close to normal, whereas monotherapies yielded latencies between 82 and 91 s. Forced swim test immobility (behavioral despair/fatigue) increased to 198.4 ± 6.2 s in DEX vs. 68.3 ± 4.5 s normal. Combination reduced immobility to 89.6 ± 5.1 s ($p < 0.001$ vs. DEX, $p < 0.01$ vs. monotherapies) (Figure 5a–c).



(a)



(b)



(c)

Figure 5: Effect of *P. alba*, *A. racemosus*, and their combination on (a) forelimb grip strength, (b) rotarod latency, and (c) forced swim test immobility in dexamethasone-induced sarcopenic rats. Bars represent mean ± SEM (n=5). One-way ANOVA followed by Tukey's multiple comparison test.

Biochemical parameters (CK, SOD, CAT, MDA)

Serum creatine kinase (CK), a marker of muscle damage, rose dramatically in DEX control (1245 ± 86 U/L vs. normal 215 ± 18 U/L, $p < 0.001$). *P. alba* reduced CK to 627 ± 45 U/L (49.6% reduction), *A. racemosus* to 558 ± 42 U/L (55.2%), while combination lowered CK to 298 ± 31 U/L (76.1% reduction, $p < 0.001$ vs. DEX; $p < 0.01$ vs. monotherapies). Muscle antioxidant enzymes: DEX depleted SOD to 28.4 ± 2.1 U/mg (normal 78.5) and CAT to 12.3 ± 1.4 U/mg (normal 38.7). Combination restored SOD to 68.3 ± 2.5 U/mg (87% of normal) and CAT to 33.5 ± 1.9 U/mg (87%). Lipid peroxidation (MDA) increased 3.6-fold in DEX (9.82 ± 0.62 vs. normal 2.73 nmol/mg). Combination significantly decreased MDA to 3.86 ± 0.33 nmol/mg (60.7%), which is better than the monotherapies (6.18 and 5.47). (Table 11)

Table 11: Biochemical parameters in muscle homogenates and Serum

Parameter	Group I	Group II	Group III	Group IV	Group V
Serum CK (U/L)	215 ± 18	1245 ± 86	627 ± 45	558 ± 42	298 ± 31
SOD (U/mg protein)	78.5 ± 3.2	28.4 ± 2.1	51.6 ± 2.8	55.2 ± 2.6	68.3 ± 2.5
CAT (U/mg protein)	38.7 ± 2.1	12.3 ± 1.4	23.5 ± 1.7	25.8 ± 1.9	33.5 ± 1.9
MDA (nmol/mg protein)	2.73 ± 0.21	9.82 ± 0.62	6.18 ± 0.45	5.47 ± 0.41	3.86 ± 0.33

These data showed that dexamethasone markedly increased muscle injury and oxidative stress, whereas the combination of *Plumeria alba* and *Asparagus racemosus* restored antioxidant enzyme activities and reduced lipid peroxidation.

Molecular parameters (LC3-II, MuRF-1, Atrogin-1)

DEX treatment significantly led to an increase in the ratio of LC3-II/LC3-I by 3.2 fold compared with the control ($p < 0.001$) using western blotting, suggesting dysregulated autophagy. This was reduced by *P. alba* to 2.1 fold and by *A. racemosus* to 1.9 fold; the combination normalized to 1.2 fold (63% reduction from DEX, $p < 0.01$ vs. monotherapies) (Figure 6).

Similar trends were observed with LC3-II mRNA expression using RT PCR. Atrogenes: DEX induced an increase in the expression of MuRF-1 (5.6-fold), and Atrogin-1 (4.9-fold). MuRF-1 was decreased by ~3.0 fold and Atrogin-1 was decreased by ~2.8 fold with monotherapies. Combination suppressed MuRF-1 to 1.42 fold (75% reduction) and Atrogin-1 to 1.31 fold (73% reduction). The MuRF-1 and Atrogin-1 synergistic suppression indices were 1.52 (MuRF-1) and 1.48 (Atrogin-1), which indicated synergy (Table 4). The marked suppression of MuRF-1, Atrogin-1, and LC3 mRNA expression suggests that the combination treatment effectively attenuated protein degradation and excessive autophagic activity induced by dexamethasone.

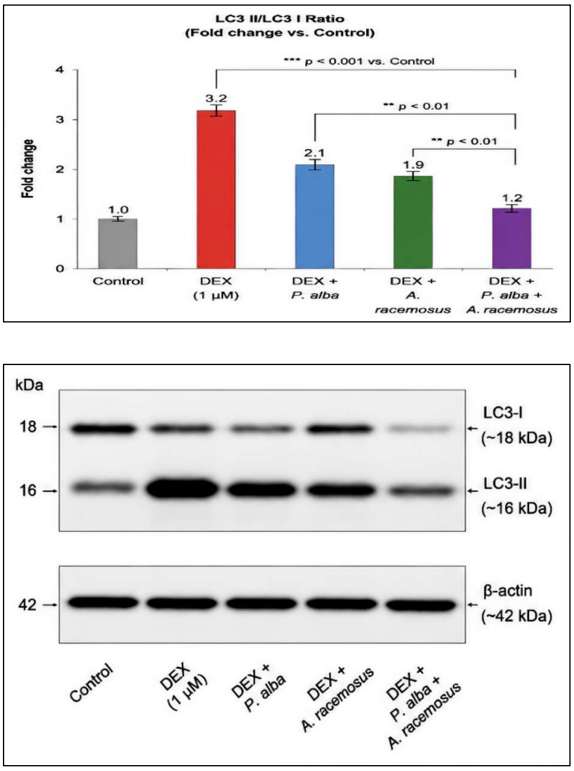


Figure 6: (a) DEX-induced autophagy increases LC3-II/LC3-I ratio, attenuated by *P. alba*, *A. racemosus*, or their combination. (b) Representative Western blot and quantification shown. One-way ANOVA followed by Tukey's multiple comparison test.

Table 12: Fold change in gene expression (relative to normal control)

Gene	Group I	Group II	Group III	Group IV	Group V
LC3-II (mRNA)	1.00 ± 0.08	3.24 ± 0.21	2.12 ± 0.15	1.94 ± 0.13	1.22 ± 0.09
MuRF-1	1.00 ± 0.07	5.61 ± 0.32	3.08 ± 0.22	2.81 ± 0.19	1.42 ± 0.11
Atrogin-1	1.00 ± 0.06	4.88 ± 0.28	2.93 ± 0.20	2.68 ± 0.18	1.31 ± 0.10

Comparison with Previous Phytotherapeutic Studies

The protective effects observed with *Plumeria alba* and *Asparagus racemosus* align with prior findings on plant-derived compounds against glucocorticoid-induced muscle atrophy. Resveratrol, a polyphenolic stilbene, has been studied in dexamethasone-induced muscle wasting. Alamdari et al. (2011) reported that resveratrol significantly reduced dexamethasone-induced myotube atrophy by suppressing MuRF-1 and Atrogin-1 via a SIRT1-dependent mechanism, reducing protein degradation and preserving muscle integrity. Resveratrol also improves mitochondrial function and inhibits AMPK/FOXO3-mediated catabolic signaling, underscoring antioxidant and mitochondrial protective pathways in preventing muscle loss (Liu et al., 2015). Similarly, curcumin from Curcuma longa shows significant anti-atrophic effects in glucocorticoid-induced muscle degeneration. Studies in dexamethasone-treated C2C12 myotubes showed curcuminoids downregulated MuRF-1 and Atrogin-1 while restoring Akt signaling, suppressing proteolysis, and promoting protein synthesis (Sani et al., 2020). Curcuma longa extracts in dexamethasone-treated mice significantly reduced ubiquitin–proteasome pathway activation and prevented muscle mass loss, supporting phytochemical antioxidants in mitigating muscle wasting (Kim et al., 2021; Furukawa et al., 2022). Ursolic acid, a pentacyclic triterpenoid, is a promising anti-sarcopenic compound. It suppresses muscle atrophy-associated genes like MuRF-1 and Atrogin-1 while enhancing IGF-1 and insulin pathways, increasing muscle mass and reducing proteolysis (Kunkel et al., 2011; Sakuma and Yamaguchi, 2012). Recent studies show ursolic acid attenuates sarcopenia through anti-inflammatory mechanisms, reduces TNF-α-induced myotube atrophy, and inhibits MuRF-1 and Atrogin-1 overexpression (She et al., 2025). These findings support oxidative stress, inflammation, and proteolytic signaling modulation as a strategy against muscle degeneration. The present study showed that *Plumeria alba* and *Asparagus racemosus* significantly improved muscle mass, grip strength, antioxidant enzyme activity, and histological architecture while suppressing MuRF-1, Atrogin-1, and LC3 mRNA expression. The polyherbal combination's superior efficacy may be due to the complementary actions of flavonoids, phenolic compounds, triterpenoids, and steroidal saponins in both extracts. Unlike studies on single phytochemicals like resveratrol, curcumin, or ursolic acid, this investigation suggests a multi-component phytotherapeutic approach offers broader protection against glucocorticoid-induced sarcopenia.

Histopathological observations further supported the biochemical and molecular findings. H&E staining demonstrated preservation of muscle fiber architecture and reduced myofiber degeneration in the combination-treated group, whereas Picrosirius Red staining revealed marked attenuation of collagen deposition and fibrosis.

Histopathology-H&E staining and muscle fiber CSA

This is a combination of histopathology and H&E staining of muscle fiber CSA. Histological scoring was performed by a blinded investigator. The normal control (Group I) had normal myofibers with peripheral nuclei, as revealed by the H&E stain (Figure 23). The severe atrophy, vacuolization, central nuclei, inflammation and disorganized fibers were seen in DEX control (Group II). *P. alba* and *A. racemosus* (Groups III & IV) demonstrated some improvement, but not significant, with a decrease in vacuolation and partial alignment of the fibers. In combination (Group V) the architecture was almost normal with little vacuolation and no central nuclei (Figure 7).

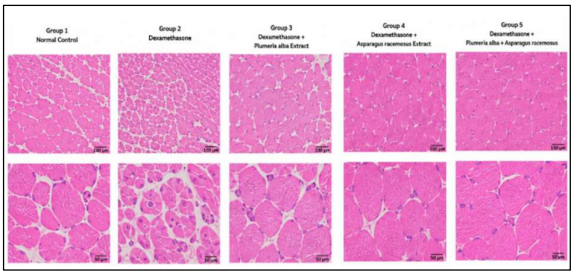


Figure 7: H&E staining: Representative photomicrographs of H&E-stained gastrocnemius muscle sections (20× magnification). (A) Normal control – regular fibers with peripheral nuclei. (B) DEX control – marked atrophy, vacuolization, central nuclei. (C) DEX+ *P. alba* – moderate improvement. (D) DEX+ *A. racemosus* – moderate improvement. (E) DEX + Combination – substantial restoration of muscle architecture. Scale bar = 100 µm.

Picrosirius red staining (fibrosis)

Picrosirius red staining (Figure 8) showed a large amount of collagen deposition (red fibers) in the gastrocnemius muscle of DEX control, which suggests that there is a high level of fibrosis. This fibrotic change leads to muscle stiffness and loss in function. Moderate but persistent fibrosis was seen with *P. alba* and *A. racemosus* monotherapies. However, the overall architecture of the skeletal muscle remained intact in the combination extract (Group V) where very little fibrotic area was observed and only a few collagen fibers appeared between myofibers (Figure 8).

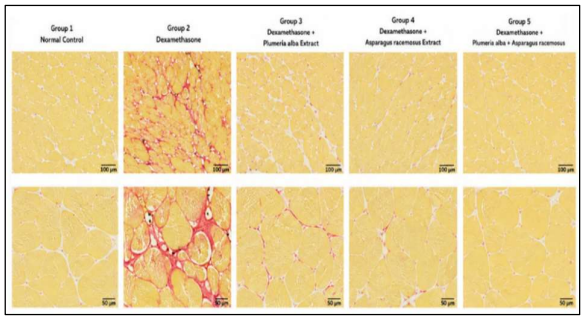


Figure 8: Picrosirius red staining of Gastrocnemius muscle section. Dexamethasone was shown to induce muscle fibrosis and the combination of *P. alba* and *A. racemosus* was showed to be very effective in greatly reducing the fibrosis while maintaining the overall structure of the skeletal muscle.

Collagen Deposition (% Fibrosis Area)

The normal control group showed minimal collagen deposition (approximately 2–3%), reflecting normal extracellular matrix organization. Dexamethasone administration resulted in a substantial increase in collagen accumulation (approximately 18–20%), indicative of severe skeletal muscle fibrosis ($p < 0.001$). Administration of *P. alba* significantly reduced collagen deposition to approximately 10–12% ($p = 0.005$), while *A. racemosus* further decreased fibrosis to approximately 7–8% ($p = 0.002$). The combination treatment produced the most pronounced reduction in collagen accumulation (approximately 3–5%; $p = 0.003$), restoring fibrosis levels close to those observed in the normal control group.

Muscle Fiber Cross-Sectional Area

The normal control group exhibited the highest muscle fiber cross-sectional area (approximately 3000–3500 µm²), indicating healthy muscle architecture. In contrast, the dexamethasone control group showed a marked reduction in muscle fiber area (approximately 1100–1300 µm²), confirming severe muscle atrophy induced by glucocorticoid treatment ($p < 0.001$). Treatment with *P. alba* significantly increased muscle fiber area compared with the dexamethasone control group ($p = 0.002$), suggesting partial restoration of muscle mass. Similarly, *A. racemosus* treatment produced a greater increase in muscle fiber size ($p = 0.003$), indicating stronger anti-atrophic activity. The combination-treated group exhibited the largest muscle fiber area among all treatment groups, approaching normal control values, suggesting a synergistic protective effect against dexamethasone-induced muscle wasting (Figure 9).

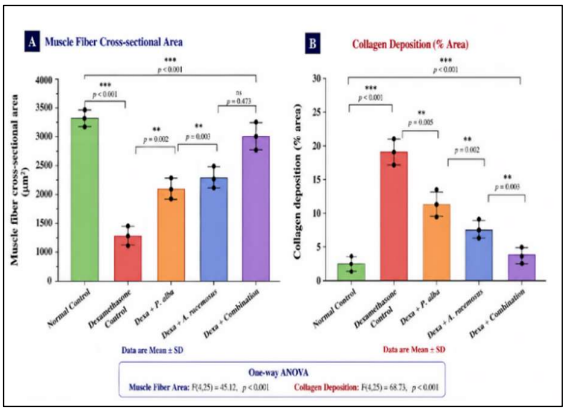


Figure 9. Effect of *Plumeria alba* and *Asparagus racemosus* on dexamethasone-induced skeletal muscle damage in Wistar rats. (A) Muscle fiber cross-sectional area and (B) collagen deposition quantified from Sirius Red-stained muscle sections. Data are expressed as Mean ± SD (n = 5). One-way ANOVA with Tukey's post hoc test; **p < 0.01, ***p < 0.001.

Table 13: Muscle fiber cross-sectional area

Group	Mean CSA (µm²)	% Restoration vs. Normal
Group I (Normal)	3278 ± 112	100%
Group II (DEX)	1245 ± 87	0% (baseline)
Group III (*P. alba*)	2187 ± 94	67%
Group IV (*A. racemosus*)	2312 ± 91	71%
Group V (Combination)	2976 ± 103	91%

Collectively, these findings indicate that the combination of *Plumeria alba* and *Asparagus racemosus* exerts protective effects through multiple complementary mechanisms including antioxidant activity, suppression of muscle proteolysis, modulation of autophagy, and preservation of skeletal muscle morphology. Such multitarget activity may provide an advantage over single-compound interventions in managing glucocorticoid-induced sarcopenia.

DISCUSSION

The study demonstrated that *Plumeria alba* and *Asparagus racemosus*, especially combined, effectively protected against dexamethasone-induced sarcopenia in Wistar rats. Dexamethasone treatment significantly reduced body weight, muscle mass, grip strength, motor performance, and endurance while increasing oxidative stress and muscle damage, consistent with glucocorticoid-induced muscle catabolism. Plant extracts significantly improved functional performance and restored muscle weight, likely due to antioxidant properties, evidenced by increased SOD and CAT activities and reduced MDA levels. By reducing oxidative stress, the extracts likely protected muscle tissue from degeneration and decline. Notably, suppression of MuRF-1, Atrogin-1, and LC3 expression in treated animals suggests attenuation of muscle protein degradation and autophagy pathways. Histopathological analysis showed improved muscle architecture, reduced fibrosis, and restored muscle fiber area. The combination of *Plumeria alba* and *Asparagus racemosus* produced the greatest protective effect across functional, biochemical, molecular, and histological parameters, likely due to the synergistic action of flavonoids, phenolic compounds, terpenoids, and steroidal saponins. Overall, the findings suggest the polyherbal combination mitigates dexamethasone-induced sarcopenia through antioxidant, anti-catabolic, and autophagy-modulating mechanisms.

CONCLUSION

The present study demonstrated that combined administration of *Plumeria alba* and *Asparagus racemosus* significantly attenuated dexamethasone-induced skeletal muscle wasting in Wistar rats. The combination improved muscle mass, grip strength, motor performance, antioxidant status, and histological architecture while suppressing the expression of muscle atrophy-related genes, including MuRF-1, Atrogin-1, and LC3. The superior efficacy observed with the combination compared with individual treatments suggests that multi-target phytotherapeutic interventions may represent a promising strategy for the management of glucocorticoid-induced sarcopenia. Further studies involving standardized extracts, protein-level validation, and clinical investigations are warranted.

Study Limitations

The present study has some limitations. First, the findings are based on a dexamethasone-induced sarcopenia model in rats and may not fully represent age-related sarcopenia in humans. Second, only selected molecular markers (MuRF-1, Atrogin-1, and LC3) were evaluated, while other pathways involved in muscle wasting were not investigated. Third, detailed phytochemical characterization of the extracts was not performed, limiting identification of the specific bioactive compounds responsible for the observed effects. Additionally, protein-level validation and inclusion of a pharmacological positive control were not undertaken. A limitation of the present study is the relatively small sample size (n = 5 per group). Although statistically significant differences were observed, further long-term studies, mechanistic investigations, and clinical evaluations are required to confirm the therapeutic potential of the polyherbal combination.

Ethical Approval Statement

All animal experimental procedures were approved (AIBS/IAEC/2025/04) by the IAEC, AIBPS, Kanpur, India, and were conducted in accordance with CPCSEA guidelines.

Conflict of Interest

The authors declare no conflicts of interest regarding the publication of this article.

Author Contributions

Conceptualization, Methodology, Writing & Editing: Arshad M.Z.; Investigation: Prajapati A.; Data Analysis: Kumar R.; Supervision: Mishra A.P.

Funding

The authors received no external funding for this research. The study was conducted using the facilities and resources provided by AIBPS, Kanpur, India.

Acknowledgements

The authors acknowledge the Advance Institute of Biotech and Paramedical Sciences (AIBPS), Kanpur, India, for providing research facilities and institutional support. The authors also thank the Central Instrumentation Facility, Pranveer Singh Institute of Technology (PSIT), Kanpur, India, for FTIR analysis, and Aakaash Biotechnologies Private Limited, Lucknow, India, for their technical support in biochemical and histopathological investigations.

REFERENCES

1. Agrawal, S., Chakole, S., Shetty, N., Prasad, R., Lohakare, T., & Wanjari, M. (2023). Exploring the Role of Oxidative Stress in Skeletal Muscle Atrophy: Mechanisms and Implications. Cureus, 15(7), e42178. https://doi.org/10.7759/cureus.42178

2. Aguilar Diaz De Leon, J., & Borges, C. R. (2020). Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. Journal of Visualized Experiments, 2020(159). https://doi.org/10.3791/61122

3. Alamdari, N., Aversa, Z., Castillero, E., Gurav, A., Petkova, V., Tizio, S., & Hasselgren, P.-O. (2011). Resveratrol prevents dexamethasone-induced expression of the muscle atrophy-related ubiquitin ligases atrogin-1 and MuRF1 in cultured myotubes through a SIRT1-dependent mechanism. Biochemical and Biophysical Research Communications, 417(1), 528–533. https://doi.org/10.1016/j.bbrc.2011.11.154

4. Aleksovska, K., Kyriakides, T., Angelini, C., Argov, Z., Claeys, K. G., De Visser, M., Filosto, M., Jovanovic, I., Kostera-Pruszczyk, A., Molnar, M. J., Sacconi, S., Schaefer, J., Siciliano, G., Vilchez, J. J., Toscano, A., & Schoser, B. (2025). What Are the Normal Serum Creatine Kinase Values for Skeletal Muscle? A Worldwide Systematic Review. European Journal of Neurology, 32(6), e70240. https://doi.org/10.1111/ene.70240

5. Alok, S., Jain, S. K., Verma, A., Kumar, M., Mahor, A., & Sabharwal, M. (2013). Plant profile, phytochemistry and pharmacology of Asparagus racemosus (Shatavari): A review. Asian Pacific Journal of Tropical Disease, 3(3), 242–251. https://doi.org/10.1016/S2222-1808(13)60049-3

6. Ankit, A., Kumar, H., Tiwari, K., Sharma, D., Kumar, A., Bhardwaj, S., Bhadauriya, T., Verma, A., Garg, A., & Kumar, B. (2025). The Legacy and Advancement of Phytotherapeutics. Asian Journal of Pharmaceutical Research, 333–336. https://doi.org/10.52711/2231-5691.2025.00052

7. Apriana, S., Maharani, F., & Riwayati, I. (2025). Effect of Extraction Variables on Phenolic Content and Antibacterial Activity of Frangipani (Plumeria alba) Leaf Extract by Microwave-Assisted Extraction (MAE). Journal of Chemical Process and Material Technology, 4(1), 25-35. https://doi.org/10.36499/jcpmt.v4i1.14534

8. Arshad MZ, Mishra AP, Rajpoot A, Prajapati A. (2026) Ursolic Acid Attenuates Glucocorticoid-Induced Sarcopenia: Molecular Mechanisms and Therapeutic Potential. Int J Drug Deliv Technol.;16(22s): 551-568. https://doi.org/10.25258/ijddt.16.22s.67

9. Barone, M., Baccaro, P., & Molfino, A. (2025). An Overview of Sarcopenia: Focusing on Nutritional Treatment Approaches. Nutrients, 17(7), 1237. https://doi.org/10.3390/nu17071237

10. Batsis, J. A., & Villareal, D. T. (2018). Sarcopenic obesity in older adults: aetiology, epidemiology and treatment strategies. Nature Reviews Endocrinology, 14(9), 513–537. Nature Portfolio. https://doi.org/10.1038/s41574-018-0062-9

11. Bopana, N., & Saxena, S. (2007). Asparagus racemosus—Ethnopharmacological evaluation and conservation needs. Journal of Ethnopharmacology, 110(1), 1–15. https://doi.org/10.1016/j.jep.2007.01.001

12. Buckland, J. (2013). Understanding the link between chronic inflammation and muscle degeneration in myositis. Nature Reviews Rheumatology, 10(2), 65. https://doi.org/10.1038/nrrheum.2013.201

13. Cairns, J., Frood, R., Scarsbrook, A., & Renehan, A. G. (2026). Sarcopenia and cancer outcomes: thresholds need to account for age-related changes in muscle mass: Clinical Studies. British Journal of Cancer, 1-2. https://doi.org/10.1038/s41416-026-03382-5

14. Campins, L., Camps, M., Riera, A., Pleguezuelos, E., Yébenes, J. C., & Serra-Prat, M. (2017). Oral Drugs Related with Muscle Wasting and Sarcopenia. A Review. Pharmacology, 99, 1–8. https://doi.org/10.1159/000448247

15. Cao, Y., Zhou, J., Quan, H., Li, W., Li, T., & Wang, L. (2025). Resistance training alleviates muscle atrophy and muscle dysfunction by reducing inflammation and regulating compromised autophagy in aged skeletal muscle. Frontiers in Immunology, 16, 1597222. https://doi.org/10.3389/fimmu.2025.1597222

16. Chaudhuri, S., Bakshi, S., Pande, J., & Biswas, M. (2015). Screening of in-vitroAntioxidant Profile of Different Extracts of the Leaves of Plumeria alba Linn. Journal of Advanced Pharmacy Education and Research, 5(2), 98–102. https://doi.org/10.51847/wi83eo5

17. Chen, M., Wang, Y., Deng, S., Lian, Z., & Yu, K. (2022). Skeletal muscle oxidative stress and inflammation in aging: Focus on antioxidant and anti-inflammatory therapy. Frontiers in Cell and Developmental Biology, 10(5), 964130. https://doi.org/10.3389/fcell.2022.964130

18. Chen, X., Ji, Y., Liu, R., Zhu, X., Wang, K., Yang, X., Liu, B., Gao, Z., Huang, Y., Shen, Y., Liu, H., & Sun, H. (2023). Mitochondrial dysfunction: roles in skeletal muscle atrophy. Journal of Translational Medicine, 21(1), 503. https://doi.org/10.1186/s12967-023-04369-z

19. Devendra, D., Girme, A., Salvankar, K., Chinchansure, A., Ranjan, R., NL, Y. K., & Mundkinajeddu, D. (2026). Scale-Up Isolation of High-Purity Shatavarins from Asparagus racemosus via Flash Chromatography with Bioavailability and Pharmacological Insights. ACS Omega, 11(13), 21068. https://doi.org/10.1021/acsomega.6c00145

20. Ding, S., Dai, Q., Huang, H., Xu, Y., Zhong, C. (2018). An Overview of Muscle Atrophy. In: Xiao, J. (eds) Muscle Atrophy. Advances in Experimental Medicine and Biology, vol 1088. Springer, Singapore. https://doi.org/10.1007/978-981-13-1435-3_1

21. Farhadi, A., Mohammadian, M., Afrashteh, S., Jorjani, G., Mousavi, S., Nabipour, I., & Larijani, B. (2026). Sarcopenia and its association with physical activity and sedentary time in older adults on the Bushehr Elderly Health program. Scientific Reports. https://doi.org/10.1038/s41598-026-39520-5

22. Frontera, W. R., Rodriguez Zayas, A., & Rodriguez, N. (2011). Aging of Human Muscle: Understanding Sarcopenia at the Single Muscle Cell Level. Physical Medicine and Rehabilitation Clinics of North America, 23(1), 201–207. https://doi.org/10.1016/j.pmr.2011.11.012

23. Furukawa, K., Kousaka, M., Jia, H., & Kato, H. (2022). Suppressive Effects of Turmeric Extract on Muscle Atrophy in Dexamethasone-Treated Mice and Myotubes. Nutrients, 14(19), 3979. https://doi.org/10.3390/nu14193979

24. Geevarghese, A. V., Ranganathan, H., Engels, S. M., & Ragul, A. (2026). Elucidating the Potential of Phytoconstituents from Plumeria alba leaves as SGLT-2 receptor antagonists: A Computational Approach. Pharmacological Research-Natural Products, 100521. https://doi.org/10.1016/j.prenap.2026.100521

25. Hadwan, M. H., Hussein, M. J., Mohammed, R. M., Hadwan, A. M., Saad Al-Kawaz, H., Al-Obaidy, S. S. M., & Al Talebi, Z. A. (2024). An improved method for measuring catalase activity in biological samples. Biology methods & protocols, 9(1), bpae015. https://doi.org/10.1093/biomethods/bpae015

26. Hopkins A. L. (2008). Network pharmacology: the next paradigm in drug discovery. Nature chemical biology, 4(11), 682–690. https://doi.org/10.1038/nchembio.118

27. Huang, K. C., Chiang, Y. F., Ali, M., & Hsia, S. M. (2025). Cisplatin-Induced Muscle Wasting and Atrophy: Molecular Mechanism and Potential Therapeutic Interventions. Journal of Cachexia, Sarcopenia and Muscle, 16(3), e13817. https://doi.org/10.1002/jcsm.13817

28. Imrana, M., & Asif, M. (2020). Morphological, ethnobotanical, Pharmacognostical and pharmacological studies on the medicinal plant Plumeria alba linn. (apocynaceae). Medicinal & Aromatic Plants, 6(1). https://doi.org/10.48347/imist.prsm/ajmap-v6i1.20372

29. Iwasa, M., Eguchi, A., Kohjima, M., Miyazaki, T., Kitamura, H., Takami, Y., Yamashita, N., Tempaku, M., Izuoka, K., & Kobayashi, Y. (2026). The Conjugated Bile Acids Profile Suggests a Novel Liver-Muscle Axis Associated With Sarcopenia in Chronic Liver Disease. Liver International, 46(5), e70612. https://doi.org/10.1111/liv.70612

30. Iyer, S. R., Shah, S. B., & Lovering, R. M. (2021). The Neuromuscular Junction: Roles in Aging and Neuromuscular Disease. International Journal of Molecular Sciences, 22(15), 8058. https://doi.org/10.3390/ijms22158058

31. Jin, K., Li, X., Ma, Y., Yang, D., Tan, X., Sun, Q., & Wang, R. (2026). Risk Factors Associated With Sarcopenia in Patients With Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Journal of Cachexia, Sarcopenia and Muscle, 17(1), e70166. https://doi.org/10.1002/jcsm.70166

32. Jun, L., Robinson, M., Geetha, T., Broderick, T. L., & Babu, J. R. (2023). Prevalence and mechanisms of skeletal muscle atrophy in metabolic conditions. International Journal of Molecular Sciences, 24(3), 2973. https://doi.org/10.3390/ijms24032973

33. Khalil, R. (2018). Ubiquitin-Proteasome Pathway and Muscle Atrophy. Advances in Experimental Medicine and Biology, 1088, 235–248. https://doi.org/10.1007/978-981-13-1435-3_10

34. Kim, J. W., Kim, R., Choi, H., Lee, S.-J., & Bae, G.-U. (2021). Understanding of sarcopenia: from definition to therapeutic strategies. Archives of Pharmacal Research, 44(9–10), 876–889. https://doi.org/10.1007/s12272-021-01349-z

35. Kim, S., Kim, K., Park, J., & Jun, W. (2021). Curcuma longa L. Water Extract Improves Dexamethasone-Induced Sarcopenia by Modulating the Muscle-Related Gene and Oxidative Stress in Mice. Antioxidants (Basel, Switzerland), 10(7), 1000. https://doi.org/10.3390/antiox10071000

36. Kunkel, S. D., Suneja, M., Ebert, S. M., Bongers, K. S., Fox, D. K., Malmberg, S. E., Alipour, F., Shields, R. K., & Adams, C. M. (2011). mRNA Expression Signatures of Human Skeletal Muscle Atrophy Identify a Natural Compound that Increases Muscle Mass. Cell Metabolism, 13(6), 627–638. https://doi.org/10.1016/j.cmet.2011.03.020

37. Lee, M. K., Jeong, H. H., Kim, M. J., Ryu, H., Baek, J., & Lee, B. (2022). Nutrients against Glucocorticoid-Induced Muscle Atrophy. Foods (Basel, Switzerland), 11(5), 687. https://doi.org/10.3390/foods11050687

38. Liu, J., Peng, Y., Wang, X., Fan, Y., Qin, C., Shi, L., Tang, Y., Cao, K., Li, H., Long, J., & Liu, J. (2015). Mitochondrial Dysfunction Launches Dexamethasone-Induced Skeletal Muscle Atrophy via AMPK/FOXO3 Signaling. Molecular Pharmaceutics, 13(1), 73–84. https://doi.org/10.1021/acs.molpharmaceut.5b00516

39. Ma, W., Xu, T., Wang, Y., Wu, C., Wang, L., Yang, X., & Sun, H. (2018). The role of inflammatory factors in skeletal muscle injury. Biotarget, 2(4), 7. https://doi.org/10.21037/biotarget.2018.04.01

40. Moriya, N., & Miyazaki, M. (2018). Akt1 deficiency diminishes skeletal muscle hypertrophy by reducing satellite cell proliferation. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 314(5), R741–R751. https://doi.org/10.1152/ajpregu.00336.2017

41. Nguyen, T. T., Dao, T., Nguyen, H. T., Park, J. H., Jeong, S. J., Kim, S., Jo, Y., Thieu, N. T. H., Zhao, J., Ding, F., Yu, Y., Dung, V. C., Gariani, K., Kim, B. J., & Ryu, D. (2026). Sarcopenia and Muscle Aging: Updated Insights into Molecular Mechanisms and Translational Therapeutics. Endocrinology and metabolism (Seoul, Korea), 41(1), 57–85. https://doi.org/10.3803/EnM.2025.2656

42. Oliveira, S. M. R., Girol, A. P., Nissapatorn, V., & Pereira, M. D. L. (2025). Bioactive Compounds Derived from Plants and Their Medicinal Potential. Pharmaceuticals, 18(11), 1732. https://doi.org/10.3390/ph18111732

43. Pandey, I., Vashishtha, S., & Chawla, S. (2022). Recent Investigations on Protective Mechanisms of Flavonoids against Sarcopenia (pp. 141–155). Crc. https://doi.org/10.1201/b22917-7

44. Pang X, Zhang P, Chen X and Liu W (2023) Ubiquitin-proteasome pathway in skeletal muscle atrophy. Front. Physiol. 14:1289537. https://doi.org/10.3389/fphys.2023.1289537

45. Permpoon, U., Moon, J., Kim, C. Y., & Nam, T.-g. (2025). Glucocorticoid-Mediated Skeletal Muscle Atrophy: Molecular Mechanisms and Potential Therapeutic Targets. International Journal of Molecular Sciences, 26(15), 7616. https://doi.org/10.3390/ijms26157616

46. Picca, A., & Calvani, R. (2021). Molecular Mechanism and Pathogenesis of Sarcopenia: An Overview. International Journal of Molecular Sciences, 22(6), 3032. https://doi.org/10.3390/ijms22063032

47. Rondanelli, M., Miccono, A., Peroni, G., Guerriero, F., Morazzoni, P., Riva, A., Guido, D., & Perna, S. (2016). A Systematic Review on the Effects of Botanicals on Skeletal Muscle Health in Order to Prevent Sarcopenia. Evidence-Based Complementary and Alternative Medicine, 2016(7), 1–23. https://doi.org/10.1155/2016/5970367

48. Sani, A., Hasegawa, K., Yamaguchi, Y., Panichayupakaranant, P., & Pengjam, Y. (2020). Inhibitory effects of curcuminoids on dexamethasone-induced muscle atrophy in differentiation of C2C12 cells. Phytomedicine Plus, 1(1), 100012. https://doi.org/10.1016/j.phyplu.2020.100012

49. Sakuma, K., & Yamaguchi, A. (2012). Novel intriguing strategies attenuating to sarcopenia. Journal of aging research, 2012, 251217. https://doi.org/10.1155/2012/251217

50. Seifi, S., Karimi, A., Marjani, A., Maftooh, M., Ghorbani, E., Fiuji, H., Ghayour-Mobarhan, M., Ferns, G. A., Khazaei, M., Hassanian, S. M., & Avan, A. (2024). Herbal Medicine and Skeletal Muscle Atrophy: From Basic Science to Translational Medicine. Current Traditional Medicine, 10(5). https://doi.org/10.2174/0122150838268183231121075627

51. She, M., Li, T., Zhou, L., Deng, Z., Huang, M., Yan, Y., Zhang, M., Yang, Y., & Wang, D. (2025). Ursolic acid attenuates sarcopenia through IL-17a-related gut-muscle axis in senile diabetic mice and myotube model. The Journal of Nutritional Biochemistry, 143, 109940. https://doi.org/10.1016/j.jnutbio.2025.109940

52. Souza, A. L. G. D., Alves, A. L. R., Martinez, C. G., Sousa, J. C. D., & Kurtenbach, E. (2025). Biomarkers of Skeletal Muscle Atrophy Based on Atrogenes Evaluation: A Systematic Review and Meta-Analysis Study. International Journal of Molecular Sciences, 26(8), 3516. https://doi.org/10.3390/ijms26083516

53. Tessou, K. Z., Lawson-Evi, P., Metowogo, K., Diallo, A., Eklu-Gadegkeku, K., Aklikokou, K., & Gbeassor, M. (2013). Acute and Sub-Acute Toxicity Studies of Plumeria alba Linn. (Apocynaceae) Hydroalcoholic Extract in Rat. International Journal of Biomedical Science : IJBS, 9(4). https://pmc.ncbi.nlm.nih.gov/articles/PMC3884797/

54. Tsai, S.-Y. (2024). Lost in translation: challenges of current pharmacotherapy for sarcopenia. Trends in Molecular Medicine, 30(11), 1047–1060. https://doi.org/10.1016/j.molmed.2024.05.016

55. Wang, X., Tang, X., Wang, Y., Zhao, S., Xu, N., Wang, H., Kuang, M., Han, S., Jiang, Z., & Zhang, W. (2025). Plant-Derived Treatments for Different Types of Muscle Atrophy. Phytotherapy Research : PTR, 39(2), 1107–1138. https://doi.org/10.1002/ptr.8420

56. Weydert, C. J., & Cullen, J. J. (2010). Measurement of superoxide dismutase, catalase and glutathione peroxidase in cultured cells and tissue.

Nature protocols, 5(1), 51–66. https://doi.org/10.1038/nprot.2009.197

57. Wiedmer, P., Jung, T., Castro, J. P., Pomatto, L. C. D., Sun, P. Y., Davies, K. J. A., & Grune, T. (2020). Sarcopenia – Molecular mechanisms and open questions. Ageing Research Reviews, 65, 101200. https://doi.org/10.1016/j.arr.2020.101200

58. Williams, D. M. (2018). Clinical Pharmacology of Corticosteroids. Respiratory Care, 63(6), 655–670. https://doi.org/10.4187/respcare.06314

59. Yokogawa, A., Kido, K., Miura, I., Oyama, E., Takakura, D., Tanaka, K., Wilkinson, D. J., Smith, K., Atherton, P. J., & Kawanaka, K. (2025). Low-Carbohydrate Diet Exacerbates Denervation-Induced Atrophy of Rat Skeletal Muscle Under the Condition of Identical Protein Intake. Journal of Cachexia, Sarcopenia and Muscle, 16(2), e13738. https://doi.org/10.1002/jcsm.13738

60. Zaufeen, M., & Rahim, S. (2025). Medicinal Potential of Plumeria Alba Linn: A Review of Its Phytochemistry and Pharmacological Activities. International Journal of Scientific Research in Science and Technology, 12(3), 1273–1275. https://doi.org/10.32628/ijsrst25123137

61. Zhang, H., Qi, G., Wang, K., Yang, J., Shen, Y., Yang, X., Chen, X., Yao, X., Gu, X., Qi, L., Zhou, C., & Sun, H. (2023). Oxidative stress: Roles in skeletal muscle atrophy. Biochemical pharmacology, 214, 115664. https://doi.org/10.1016/j.bcp.2023.115664