EFFECTIVENESS OF *COSMOS CAUDATUS* EXTRACT IN IMPROVING SARCOPENIA INDICES AND DIETARY INTAKE AMONG COMMUNITY-DWELLING OLDER ADULTS WITH POSSIBLE SARCOPENIA AND SARCOPENIA IN KELANTAN

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by

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ASEAN	Association of Southeast Asian Nations
AUC	Area under the curve
AWGS	Asian Working Group for Sarcopenia
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BMR	Basal metabolic rate
CC	Cosmos caudatus
CDC	Centers for Disease Control and Prevention
CDR	Centre for Drug Research, Universiti Sains Malaysia
CI	Confidence interval
CFU	Colony forming unit
CT	Computed tomography
DAD	Diode array detector
DHQ	Diet history questionnaire
DOSM	Department of Statistics Malaysia
DPPH	1,1-diphenyl-2-picrylhydrazyl
DXA	Dual x-ray absorptiometry
EtOH	Ethanol
EWGSOP	European Working Group for Sarcopenia in Older People
FRAP	Ferric reducing antioxidant power
FRGS	Fundamental Research Grant Scheme
GCMS	Gas chromatography and mass spectrophotometry
GMP	Good manufacturing practice
HREC	Human Research Ethics Committee
HGS	Handgrip strength
IQR	Interquartile range
IWGS	International Working Group on Sarcopenia
MDG	Malaysian Dietary Guidelines
MeOH	Methanol
MOH	Ministry of Health, Malaysia

MOHE	Ministry of Higher Education, Malaysia
MPN	Most probable number
MRI	Magnetic resonance imaging
MUAC	Mid-upper arm circumference
MYR	Malaysian Ringgit
NMT	Not more than
OR	Odds ratio
PAL	Physical activity level
PLI	Poverty line income
PPM	Parts per million
RAPA	Rapid assessment of physical activity
RCT	Randomized controlled trial
RP-HPLC	Reverse-phase high-performance liquid chromatography
RPM	Rotation per minute
RT	Retention time
UN	United Nations
UV	Ultraviolet
SD	Standard deviation
SMD	Standard mean difference
SMI	Skeletal muscle index
SPPB	Short physical performance battery
TAMC	Total aerobic microbial count
US	The United States
W/V	Weight for volume
W/W	Weight for weight
WHO	World Health Organization

LIST OF APPENDICES

KEBERKESANAN EKSTRAK *COSMOS CAUDATUS* DALAM MENINGKATKAN INDEKS SARKOPENIA DAN PENGAMBILAN DIETARI DALAM KALANGAN WARGA EMAS YANG TINGGAL DI KOMUNITI DENGAN KEMUNGKINAN SARKOPENIA DAN SARKOPENIA DI KELANTAN

ABSTRAK

Sarkopenia, dicirikan oleh kehilangan progresif jisim otot dan fungsi, kebanyakannya menjejaskan warga emas dan boleh mengakibatkan peningkatan risiko jatuh, kerapuhan dan kualiti hidup yang berkurangan. Oleh itu, kajian ini bertujuan untuk menilai keberkesanan ekstrak *Cosmos caudatus* (CC) dalam meningkatkan indeks sarkopenia dan pengambilan dietari pemakanan dalam kalangan warga emas yang tinggal di komuniti dengan kemungkinan sarkopenia dan sarkopenia. Fasa I melibatkan penghasilan suplemen CC, dimana CC segar diekstrak melalui kaedah ekstraksi sejuk, seterusnya melalui kaedah pengeringan semburan (*Inlet*: 180°C, *Outlet*: 110°C, 1,000 rpm) dan dianalisis menggunakan kromatografi cecair prestasi tinggi fasa terbalik. Profil keselamatan dianalisis mengikut *British Pharmacopoeia*, 2008. Profil kromatografi *flavonoid rutin*, *quercitrin*, dan *quercetin* dalam sampel CC yang dirumuskan dielusi pada minit 14.98 (0.33% w/w), 19.52 (2.86% w/w), dan 28.70 (0.03% w/w). Suplemen tersebut selamat untuk dimakan dengan kesan logam berat dan pencemaran mikrobiologi yang minimum. Fasa II merangkumi kajian keratan rentas untuk mengenalpasti dan mencirikan warga emas yang tinggal dalam komuniti dengan kemungkinan sarkopenia dan sarkopenia berdasarkan kriteria *Asian Working Group for Sarcopenia* (AWGS) 2019. Seramai 92 individu dengan kemungkinan sarkopenia dan sarkopenia telah mengambil bahagian dalam ujian *double-blinded*,

placebo-controlled dan dibahagikan secara rawak kepada kumpulan intervensi (n=47) dan kumpulan kawalan (n=45). Setiap seorang daripada mereka mengambil 500 mg kapsul ekstrak CC setiap hari atau *maltodextrin* sebagai plasebo. Selepas 12 minggu supplemen diambil, ANOVA ukuran berulang mendedahkan bahawa ekstrak CC yang diformulasikan tidak meningkatkan jisim otot, kekuatan, dan prestasi secara signifikan berbanding plasebo, seperti yang dinilai menggunakan definisi *Asian Working Group for Sarcopenia* (AWGS) 2019. Pengurangan yang signifikan diperhatikan dalam pengambilan serat pemakanan yang dinilai menggunakan Soal Selidik Sejarah Diet dari garis asas [3.55 mg/hari, 95% CI: 2.9-4.2] hingga minggu ke-12 [3.07 mg/hari, 95% CI: 2.4-3.8], dihipotesiskan akibat faedah yang dirasakan dari pengambilan CC telah mendorong peserta untuk mengurangkan pengambilan serat. Kesimpulannya, kekurangan peningkatan yang signifikan dalam indeks otot mencadangkan bahawa pengambilan supplemen CC sahaja tidak mencukupi sebagai intervensi untuk sarkopenia. Penyelidikan akan datang harus menggabungkan ekstrak CC dengan strategi terapeutik lain untuk melihat kesan sinergistik, menyediakan dos yang berbeza untuk melihat potensi hubungan tindak balas, atau melanjutkan tempoh intervensi dengan saiz populasi yang lebih besar untuk memperlihatkan kesan terapeutiknya.

EFFECTIVENESS OF *COSMOS CAUDATUS* EXTRACT IN IMPROVING SARCOPENIA INDICES AND DIETARY INTAKE AMONG COMMUNITY-DWELLING OLDER ADULTS WITH POSSIBLE SARCOPENIA AND SARCOPENIA IN KELANTAN

ABSTRACT

Sarcopenia, characterized by the progressive loss of muscle mass and function, mostly affects older adults, leading to increased risks of falls, frailty, and reduced quality of life. Thus, this study aimed to formulate and evaluate the efficacy of *Cosmos caudatus* (CC) extract in improving sarcopenia indices and dietary intake among community-dwelling older adults. Phase I involved the formulation of a CC supplement, where fresh CC was obtained by cold extraction, spray-dried (Inlet: 180°C, Outlet: 110°C, 1,000 rpm) and analyzed by reverse-phase high-performance liquid chromatography. The safety profile was analyzed according to the British Pharmacopoeia, 2008. Chromatographic profiles of flavonoids rutin, quercitrin, and quercetin in the formulated CC sample eluted at minutes 14.98 (0.33% w/w), 19.52 (2.86% w/w), and 28.70 (0.03% w/w), respectively. The supplement was safe for consumption with minimal heavy metal traces and microbiological contamination. Phase II comprised a cross-sectional study to identify and characterize community-dwelling older adults with possible sarcopenia and sarcopenia based on the Asian Working Group for Sarcopenia (AWGS) 2019 criteria. A total of 92 individuals with possible sarcopenia and sarcopenia were recruited for Phase III, a double-blinded, placebo-controlled trial and were randomized into the intervention (n=47) and control (n=45) group. Each of them consumed 500 mg of capsuled CC extract daily or maltodextrin as a placebo. After 12 weeks of supplementation, repeated measures

ANOVA revealed that the formulated CC extract did not significantly improve muscle mass, strength, and performance compared to placebo, as assessed using the Asian Working Group for Sarcopenia (AWGS) 2019 definition. A significant reduction was observed in dietary fiber intake assessed by the Diet History Questionnaire from baseline [3.55 mg/day, 95% CI: 2.9-4.2] to week 12 at [3.07 mg/day, 95% CI: 2.4-3.8], hypothesized to be due to the perceived benefits of CC supplementation, prompting the participants to reduce their intake. In conclusion, the lack of significant improvement in muscle indices suggests that CC supplementation alone is not a sufficient intervention for sarcopenia. Future research should combine CC extract with other therapeutic strategies to observe potential synergistic effects, provide different dosages to observe potential dose-response relationship, or extend intervention duration with a larger population size to clarify its therapeutic effects.

CHAPTER 1

INTRODUCTION

1.1 Study Background

In recent years, more emphasis has been placed on expanding the scope of health science practices as various elements can influence scientific research direction. Age is a significant demographic indicator as the human body continuously undergoes complex biological changes over time. Globally, nearly every country is witnessing an increase in the older adult population from 6% in 1990 to 9% in 2019 (UN, 2019). By 2050, this proportion is expected to rise to 16%, signifying that one in six individuals worldwide will be 65 or older. In Malaysia, the Department of Statistics (DOSM) estimated a 0.4% increase from 2020 to 2021 in the older adult population, translating roughly to an additional one million individuals (DOSM, 2021). This shift demonstrated the evolving age structure towards a higher proportion of older adults, attributable to longer life expectancies and declining birth and fertility rates.

Owing to the increase in the aging population, age-related or time-progressive diseases are becoming more prevalent. One such issue is sarcopenia, characterized as a degenerative skeletal muscle condition that causes an accelerated loss of muscle mass and function (A. J. Cruz-Jentoft et al., 2019). The concept of possible sarcopenia has also been discussed by the Asian Working Group for Sarcopenia (AWGS), referring to individuals at risk for sarcopenia before they meet the full requirement for the diagnosis, allowing for earlier intervention (Chen et al., 2020). A meta-analysis conducted by a group of researchers revealed a significant association between sarcopenia and fractures among community-dwelling older adults, particularly among men as they often lose muscle strength twice as fast as women (Rivera et al., 2016; Zhang et al., 2018). Significant reduction in quality of life may be observed through disruptions in mobility and daily living activities; thus, effective treatments are crucial to alleviate symptoms and improve life satisfaction (Hiligsmann et al., 2020).

A more direct approach to improve muscle protein synthesis and reduce muscle protein breakdown, such as amino acids, magnesium, and calcium supplementation may yield better increments in muscle mass than plant-based interventions, especially with short durations of clinical trials. However, individuals with digestive issues or dietary restrictions due to certain conditions (chronic kidney disease) or personal preferences (vegetarian, vegan) may have limited protein intake and might choose herbal supplementation over conventional medicine to maintain nitrogen balance. Thus, several nutraceutical studies in Asia have shown that dietary supplementation using herbs such as *Astragalus membranaceus*, *Atractylodes macrocephala*, and *Curcuma longa* were protective against sarcopenia (Liu et al., 2021; Varma et al., 2021). Active components of the herbs were capable of significantly increasing catalase, superoxide dismutase, and glutathione peroxidase activity in skeletal muscles, further delaying muscle atrophy (Liu et al., 2021).

The herb of interest in this study is *Cosmos caudatus* (CC) or *Ulam raja*, an edible plant native to Mexico, the Caribbean, Central America, and South America, though naturalized in tropical parts of Asia, Africa, and Australia (Moshawih et al., 2017). The genus name '*Cosmos*' originated from Greek, meaning order or ornaments, whereas the species epithet '*caudatus*' is derived from a Latin word that means having a tail. In Malaysia, CC is either eaten raw as a salad or with condiments such as chili paste and fermented shrimp paste, often served with rice. It has the highest overall phenolic content compared to nine other typical Malaysian vegetables, especially in aqueous solution (Sumazian et al., 2010). CC also proved beneficial in human clinical trials, demonstrating beneficial glucose control and cognitive effects (Moshawih et al., 2017; You et al., 2021). However, its potential for managing sarcopenia has not yet been explored. Thus, a randomized, double-blind, placebo-controlled clinical trial was conducted to examine the effects of a 12-week CC supplementation on sarcopenia and dietary intake.

1.2 Problem Statement and Study Rationale

Sarcopenia, a crucial age-associated muscle atrophy, gained initial recognition in clinical diagnosis in the 1980s (Liu et al., 2024). In 2016, sarcopenia was officially recognized as a disease according to the International Classification of Diseases, Tenth Revision, Clinical Modification (ICD-10-CM) (Anker et al., 2016). Individuals with sarcopenia have a significantly higher risk of falls, fractures, and hospitalization compared to those without the condition (Huang et al., 2021; Yeung et al., 2019; Zhang et al., 2018). The condition is especially exacerbated following hospitalization, given long bed rest and physical inactivity (Wan et al., 2023), prompting more rigorous interventional strategies. Additionally, older adults may have reduced intake of beneficial nutrients including protein due to chronic kidney disease, veganism, or mastication and deglutition issues. Since no prescription medications were prescribed to specifically target sarcopenia, disease management strategies such as dietary supplementation and exercise play important key roles in reducing sarcopenia risk and potentially reversing the condition.

Patients with sarcopenia prioritized the maintenance of mobility and capacity to handle domestic duties as the most important outcomes (Hiligsmann et al., 2020). Therefore, this study explored the use of CC extract supplementation to improve sarcopenia and dietary indices for an enhanced quality of life, especially chosen for its green, economically friendly, and ubiquitous nature, allowing for widespread consumption and commercialization. CC also offers various beneficial effects as it exhibits potent antioxidant, anti-inflammatory, anti-cancer, and anti-bacterial properties in pre-clinical studies due to its high phenolic content (Barakatun-Nisak, et al., 2015). Given muscle atrophy highly correlates with oxidative stress and inflammation (Nikawa et al., 2021), phenolic compounds in CC can mitigate the antioxidant imbalance by reversing mitochondrial dysfunction, subsequently reducing muscle protein breakdown and muscle damage (Salucci & Falcieri, 2020). In the current study, CC was formulated and administered as a powdered extract in gelatin capsules for ease of consumption, promoting broader acceptance across ethnicities as CC is less common among ethnicities other than the Malays. The method of delivery is especially beneficial for older adults with mastication or deglutition difficulties who may experience a significant reduction in daily dietary intake.

The study participants consumed approximately 500 mg/day of spray-dried CC extract for twelve consecutive weeks, with sarcopenia and dietary indices measured at baseline, week six, and week twelve. This dosage aligns with an in-vivo safety study examining acute oral toxicity in Wistar rats, indicating that CC is safe for consumption up to 500 mg/kg of body weight (Norazlina et al., 2013). Previous clinical trials administering 500 mg/day of CC supplement and 15 g/day of raw CC among older adults reported significant benefits on cognitive ability and glucose control, respectively (Cheng et al., 2015; You et al., 2021). Thus, CC supplementation shows potential for sarcopenia management.

The study also explored the potential efficacy of CC supplementation in increasing dietary intake. The participants might experience improved perception and preference towards vegetables following supplementation, especially as the study recruited possibly sarcopenic and sarcopenic older adults with inadequate fruit and vegetable intake as outlined by the 2020 Malaysian Food Pyramid.

1.3 Research Questions

1. How to formulate a supplement obtained from the CC extract?
2. How to assess the presence and quantify flavonoid content and the safety profiles of the formulated CC supplement?
3. Is the formulated CC extract effective in improving sarcopenia indices among community-dwelling older adults with possible sarcopenia and sarcopenia in Kelantan?
4. Is the formulated CC extract effective in improving dietary intake among community-dwelling older adults with possible sarcopenia and sarcopenia in Kelantan?

1.4 Research Hypothesis

The formulated CC extract supplement is efficacious in improving sarcopenia indices and dietary intake among community-dwelling older adults with possible sarcopenia and sarcopenia in Kelantan.

1.5 Research Objectives

1.5.1 General Objective

To develop and evaluate the efficacy of a supplement derived from CC extract in improving sarcopenia indices and dietary intake among older adults with possible sarcopenia and sarcopenia.

1.5.2 Specific Objectives

1. To develop a formulated CC extract supplement (Phase I).
2. To characterize the formulated CC supplements' flavonoid content and assess its safety through heavy metal and microbiological analyses (Phase I).
3. To determine the prevalence of possible sarcopenia and sarcopenia among community-dwelling older adults in Kelantan (Phase II).
4. To determine the efficacy of the formulated CC extract supplementation in improving sarcopenia indices among community-dwelling older adults with possible sarcopenia and sarcopenia in Kelantan (Phase III).
5. To determine the efficacy of the formulated CC extract supplementation in improving dietary intake among community-dwelling older adults with possible sarcopenia and sarcopenia in Kelantan (Phase III).

1.6 Conceptual Framework

Figure 1.1 illustrates the overall concept associating the potential therapeutic benefits of CC in improving sarcopenia status and dietary intake. CC contains a complex array of bioactive constituents with numerous secondary metabolites, necessitating precise identification and analysis. Thus, high-performance liquid chromatography (HPLC) was chosen as the principal analysis method as it allows for systematic profiling of complex plant samples and focuses on the identification, separation, characterization, and consistent evaluation of the detected chemicals (Kumar, 2017).

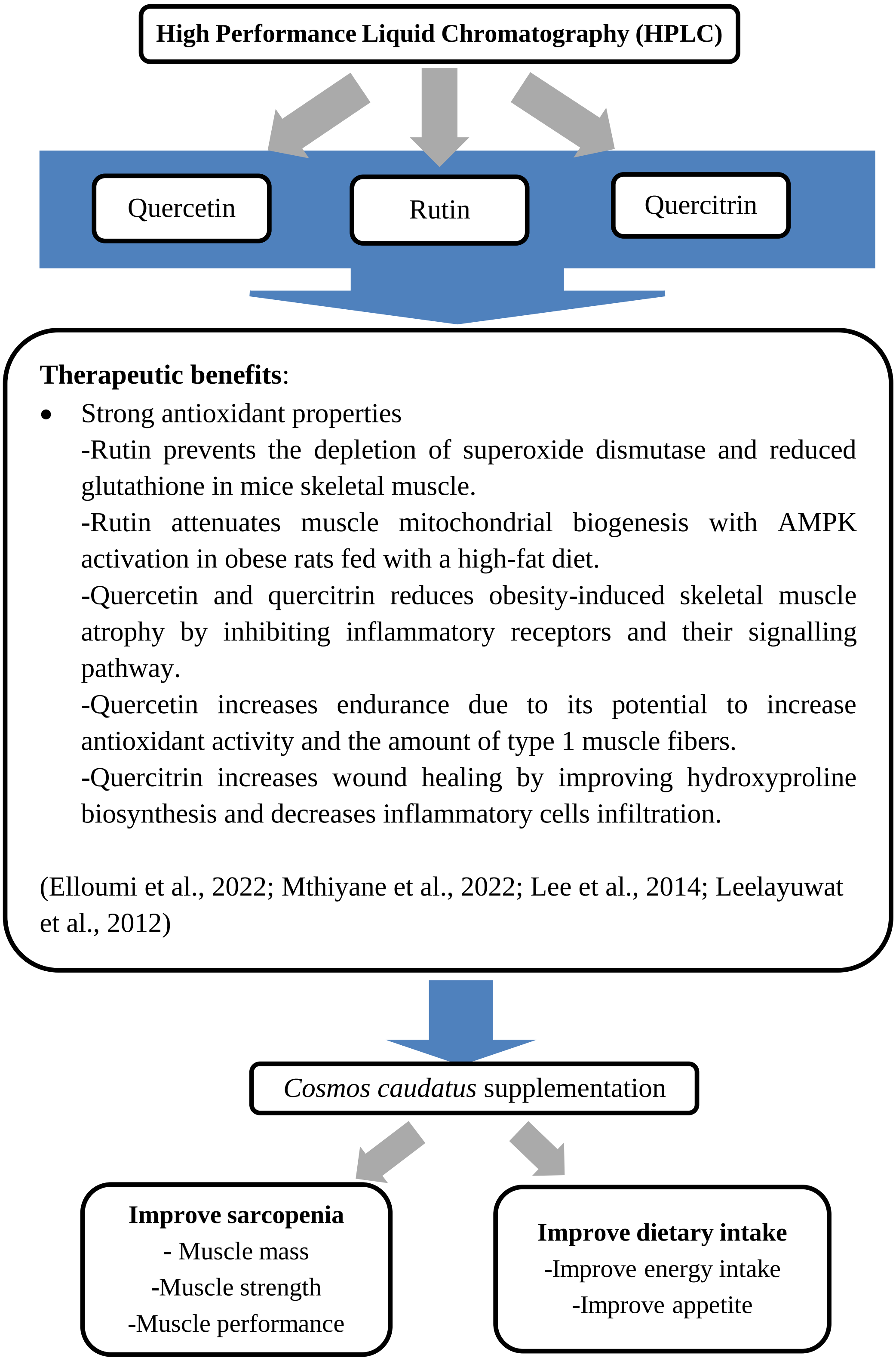

Figure 1.1: Conceptual framework

Naturally available flavonoids in CC, such as quercetin, rutin, and quercitrin, have demonstrated significant therapeutic benefits in pre-clinical studies. The primary mechanism for skeletal muscle preservation involves cell atrophy deceleration and improvement in cell regeneration through anti-oxidative pathways. Quercetin was capable of enhancing endurance through an increase in type I muscle fibers (Lee et al., 2014; Leelayuwat et al., 2012), while rutin prevents superoxide dismutase and glutathione depletion in mice (Mthiyane et al., 2022). Rutin was also thought to promote mitochondrial biogenesis activity in muscle cells through the AMP-activated protein kinase (AMPK) pathway (Seo et al., 2015). These findings highlighted the efficacy of the flavonoids, encouraging more researchers to expand the investigation towards human subjects in improving muscle-related conditions, including sarcopenia. The current study also observed changes in nutrient intake and the link between CC supplementation and dietary intake. As aforementioned, the participants' perception of healthier food options might be altered, specifically by the increase in favorable nutrient intake or, by the reduction in appetite, reducing high-fat or high-carbohydrate food due to changes in intestinal digestion influenced by the formulated CC extract.

1.7 Operational Definitions

i. Sarcopenia

Defined by the Asian Working Group for Sarcopenia (AWGS) as an "Age-related loss of muscle mass, plus low muscle strength, and/or low physical performance" (Chen et al., 2020).

ii. Possible sarcopenia

Defined by the AWGS as an "Age-related loss of muscle strength and/or low physical performance" (Chen et al., 2020).

iii. Community-dwelling older adults

Non-institutionalised individuals aged 60 years and older (UN, 2019), residing in urban and semi-urban areas of Kelantan.

iv. *Cosmos caudatus*

Known locally as *ulam raja*, an edible herbal plant native to Mexico, the Caribbean, Central America, and South America, though naturalised in tropical parts of Asia, Africa, and Australia (Moshawih et al., 2017).

v. Sarcopenia indices

Include the parameters such as muscle mass, muscle strength, and physical performance.

vi. Dietary intake

Defined as the average daily intake of a nutrient in a week (NIH, 2010) assessed using the Diet History Questionnaire.

CHAPTER 2

LITERATURE REVIEW

2.1 Sarcopenia: A Global Issue

2.1.1 Definition

Sarcopenia refers to a degenerative skeletal muscle condition characterized by accelerated loss of muscle mass and function and is linked to a higher risk of adverse outcomes, including falls, functional decline, frailty, and mortality (A. J. Cruz-Jentoft et al., 2019). The term, proposed by Rosenberg, derives from the Greek word '*sarx*,' meaning flesh, and '*penia*,' meaning loss (Rosenberg, 1997). Sarcopenia is more commonly diagnosed among older adults as muscle loss accumulates over time, although it can occur earlier in life (Yuan & Larsson, 2023).

Figure 2.1 illustrates the cross-sectional magnetic resonance imaging comparing a healthy thigh muscle of a 25-year-old with a sarcopenic thigh muscle of a 65-year-old (Sergi et al., 2016). Significant muscle fiber reduction and fat infiltration have occurred in the muscle matrix, evident in the sarcopenic thigh muscle.

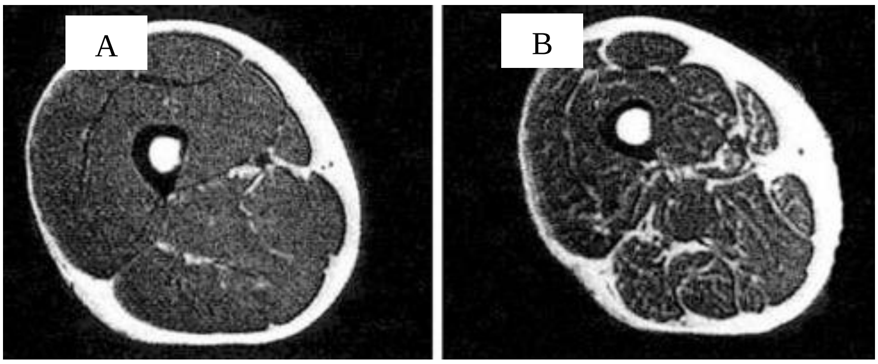

Figure 2.1: A: MRI of healthy thigh muscle and B: MRI of sarcopenic thigh muscle

Source: Sergi et al. (2016)

Age-related increases in oxidized proteins in skeletal muscle have led to the accumulation of lipofuscin and cross-linked proteins that are insufficiently eliminated by the proteolysis system (Dhillon & Hasni, 2017). Lipofuscin is an auto-fluorescent pigment particularly prevalent in nerve cells, cardiac muscle cells, and skin, which consists of lipids, metals, and misfolded proteins. The skeletal muscles are thought to accumulate non-contractile malfunctioning protein, contributing to the dramatic decline in muscular strength observed in sarcopenia (Dhillon & Hasni, 2017).

Several clinical and operational definitions with population-specific cut-offs have been established given the geographical and racial differences, as shown in Table 2.1. Multiple operational working groups including the AWGS 2014 (Chen et al., 2016), revised AWGS2 in 2019 (Chen et al., 2020), the European Working Group on Sarcopenia in Older People (EWGSOP 2010) (Cruz-Jentoft et al., 2010), revised EWGSOP2 (Cruz-Jentoft et al., 2019), and the International Working Group on Sarcopenia (IWGS) (Chumlea et al., 2011), among others, have proposed sarcopenia definitions tailored to better diagnose sarcopenia and possible sarcopenia.

Table 2.1: Sarcopenia cut-offs

Consensus	**Muscle mass index (kg/m²)**		**Muscle strength**	
	BIA	**DXA**	**HGS (kg)**	**GS (m/s)**
AWGS	Men < 7.0 Women < 5.7	Men < 7.0 Women < 5.7	Men < 26 Women < 18	Both < 0.8
AWGS2	Men < 7.0 Women < 5.7	Men < 7.0 Women < 5.4	Men <28 Women < 18	Both < 1.0
EWGSOP	Men: 8.31 - 10.75 Women: 6.42 - 6.75	Men: 7.23 - 7.26 Women 5.45 - 5.67	Men < 30 Women < 20	Both < 0.8
EWGSOP2	-	Men < 7.0 Women < 5.7	Men < 27 Women < 16	Both < 0.8

Table 2.1 - Continued

IWGS	Men < 7.23 Women < 5.67	-	Both < 1.0

Abbreviations: AWGS: Asian Working Group on Sarcopenia; EWGSOP: European Working Group on Sarcopenia; IWGS: International Working Group on Sarcopenia; BIA: Bioelectrical impedance analysis; DXA; Dual x-ray absorptiometry; HGS: Handgrip strength; GS: Gait speed

The EWGSOP and AWGS consensus categorized sarcopenia based on stages; ‘normal,’ ‘probable/possible sarcopenia,’ ‘sarcopenia,’ and ‘severe sarcopenia.’ They emphasize reduced muscular strength as a significant sign of sarcopenia, while the addition of poor physical performance is indicative of severe sarcopenia. The IWGS consensus only involved two of those criteria through the premise that sarcopenia is linked with a loss in muscle mass or an increase in fat mass (Fielding et al., 2011). Diagnosing sarcopenia through muscle mass is crucial since the size and number of skeletal muscle fibers, particularly type two or fast-twitch fibers, decrease with age, reducing power and force (Walston, 2012). However, a study revealed an increased risk of disability and death due to the loss of muscle strength, which happens two to five times faster than the loss of muscle mass (Mitchell et al., 2012). Thus, these insights emphasize the need for early detection and intervention strategies focused on preserving muscle strength to mitigate adverse outcomes. Additionally, satellite cells, essential for muscle repair and regeneration, also have significant age-related changes. These cells are activated from intensive muscle activity, such as during weight-bearing exercises or in response to trauma inflicted during injury (Patel & Zwibel, 2022). Over time, cellular-level muscle changes in adults may potentially amount to the geriatric syndrome, as muscle mass was found to decline at an annual rate of 1% to 2% at the age of 50, while muscle strength declines by 1.5% in the next decade, accelerating to 3% thereafter (Liu et al., 2024).

Validated assessments with various cut-offs were developed across the population to screen and diagnose sarcopenia. Although anthropometric measurements are relatively easy to perform, they are less reliable as age-related changes in body composition may cause variations in skin elasticity or mass, reducing the accuracy of the technique (Guttikonda & Smith, 2021). Thus, a cocktail of assessments of muscle mass, strength, and performance tests is recommended to better identify sarcopenia. Muscle mass can be assessed through dual-energy x-ray absorptiometry (DXA), computed tomography (CT), MRI, and more commonly through bioelectrical impedance analysis (BIA) due to its feasibility for epidemiological research (Verstraeten et al., 2023). Additionally, despite the lack of established cut-offs, high cost, and lack of portability, the MRI and CT scan remains the gold standard for sarcopenia diagnosis given the high sensitivity in precisely evaluating muscle mass and quality (Voulgaridou et al., 2024). Upper muscle strength is typically assessed through a handgrip strength (HGS) test, whereas lower muscle strength can be measured with a knee strength test or gait speed test (Swan et al., 2022). Muscle performance is measured by observing walking speed or muscle power, with the most common being the short physical performance battery, usual walking speed, and timed-up and-go test (Voulgaridou et al., 2024).

2.1.2 Sarcopenia Prevalence

Estimating the prevalence of sarcopenia is challenging due to varying cut-off points in studies and the difficulties in measuring muscle mass (Petermann-Rocha et al., 2022). A meta-analysis using the EWGSOP, IWGS, and AWGS definitions reported a global prevalence of sarcopenia at 10% for both genders globally, despite high heterogeneity across studies (Shafiee et al., 2017). The finding was similar to another meta-analysis, which reported the overall prevalence of sarcopenia for elderly

60 years old and above at 10% (EWGSOP), 14% (IWGS), and 14% (AWGS) (Petermann-Rocha et al., 2022). A more recent systematic review found that sarcopenia affects 10% to 16% of older adults worldwide, with higher rates in patients compared to the general population (Yuan & Larsson, 2023). Among those hospitalized patients, sarcopenia is linked to a wide range of unfavorable health outcomes, including reduced survival rate, postoperative complications, and prolonged hospital stays. In contrast, the general population is linked to risks of falls, fractures, metabolic disorders, cognitive impairment, and mortality. However, the aforementioned meta-analyses predate the revised AWGS2, suggesting potential changes in the current prevalence estimates. A recent study among post-acute hospitalized older adults reported sarcopenia prevalence at 54%, with a prevalence of severe sarcopenia at 38.9% (Tan et al., 2024). Older men with low physical activity were more susceptible, whereas those with larger hip circumference and a body mass index (BMI) of ≥27.5 kg/m² were less likely to have sarcopenia (Tan et al., 2024). With evolving diagnostic procedures and definitions, these statistics are crucial for guiding clinical practice and research in sarcopenia intervention and treatment.

In Malaysia, a study in Selangor found that 20.4% of community-dwelling older adults were at risk for sarcopenia, with most being female, older, and with a history of falls (Iskandar et al., 2021). Another study in Klang Valley using the AWGS diagnostic criteria among a similar population reported a sarcopenia prevalence of 33.6%, with females more affected than males (Ranee et al., 2022). The lower prevalence in males may be due to their proportionately higher skeletal muscle mass compared to their female counterparts (Stewart & Pell, 2010). In Kelantan, a study among 230 community-dwelling Chinese older adults reported the prevalence of sarcopenia at 12.5% with no significant difference between gender (Foo et al., 2023).

Those aged 70 and older with increased pro-inflammatory cytokine (Interleukin-6) were found to be at increased risk of sarcopenia, suggesting a modulation of inflammation signaling in muscle deterioration, despite adjusting for adiposity (Foo et al., 2023). The finding highlights the potential interaction between chronic low-grade inflammation on the onset of sarcopenia, with possible influence from lifestyle and environmental factors.

In Singapore, the population-adjusted prevalence of sarcopenia among people 60 years of age and older was 32.2% (33.7% for men, 30.9% for women), according to AWGS 2019 (Pang et al., 2021). They also noted an increase in prevalence from 6.7% to 13.6% for AWGS 2014 and AWGS 2019 due to the difference in diagnostic criteria. The increase indicates that sarcopenia diagnosis is highly dependent on the diagnostic criteria used. The current study used the latest AWGS 2019 criteria (AWGS2) due to its compatibility with the study population, allowing for better comparison with other studies in the Asian population.

2.1.3 Sarcopenia Risk Factors

2.1.3(a) Age

Age plays the most significant role in predicting the probability of sarcopenia, as older adults are more susceptible to the condition. In community-dwelling older adults, sarcopenia was thought to be a mortality predictor, which suggested that those with severe sarcopenia had a higher short-term risk of death (Liu et al., 2017). The condition is more prevalent in the aging population than the younger counterpart due to increased muscle loss, leading to decreased mobility. A study in Taiwan found that aging increases the likelihood of reduced muscle mass by 1.19 times, with older adults aged 70 years old and above having significantly higher sarcopenia rates (Therakomen et al., 2020). Studies in Japan and China supported the findings, as age was also identified as an independent risk factor for sarcopenia (Cheng et al., 2021; Kurose et al., 2020). Alongside the reduction in muscle mass, a progressive increase in fat mass can lead to changes in body composition and a higher incidence of insulin resistance in older individuals (Boccardi, 2024). Despite muscle loss being part of the aging process, it can be accelerated by various factors such as age-related inflammation (Dalle et al., 2017), inactivity (Seo & Lee, 2022), poor nutrition (Wang et al., 2023), and catabolic crises (Arentson-Lantz et al., 2023).

2.1.3(b) Body composition

Body composition, referring to the chemical or physical components that systematically make up a person's body, is another indicator of sarcopenia (Stewart & Sutton, 2012). The bi-compartmental model, which splits the body into fat and fat-free mass, is usually used to measure body composition (Marra et al., 2019). Underweight elderly have a higher risk of developing sarcopenia than those with normal weight (Cheng et al., 2021). A study among patients with secondary sarcopenia also suggested that decreased BMI may be used as a sign of sarcopenia (Sugawara et al., 2019). Muscle growth is regulated by the Akt kinase protein, the central component in protein synthesis via the mTOR (mammalian target of rapamycin), and protein degradation via the FoxO (Forkhead box O) family's transcription factors. Underweight individuals with undernutrition may have impaired mTOR pathways, reducing ribosome efficiency and/or capacity leading to decreased protein expression and subsequently sarcopenia (Bilski et al., 2022). In community-dwelling older adults, obesity, categorized using BMI, was found to be protective against sarcopenia [Odds Ratio, OR= 0.35, (95% Confidence Interval, CI: 0.11-1.05, $p = 0.062$)] (Therakomen et al., 2020). The results align with the findings suggesting the likelihood of developing sarcopenia decreases by 0.45 for every 1 kg/m^2 increase in BMI (Wu et al., 2021).

However, when adjusted for muscle quantity, a higher BMI was linked to an elevated risk of sarcopenia (Yuan & Larsson, 2023). The linkage follows the positive correlation observed between the visceral fat area and the risk of sarcopenia, suggesting that excessive fat alone does not protect against sarcopenia (Feng et al., 2022). Instead, sarcopenic obesity, affecting 11% of older adults globally, has been associated with various adverse outcomes (Gao et al., 2021). In sarcopenic obese individuals, adipose tissue accumulates ectopically in skeletal muscle, inducing mitochondrial dysfunction, further impairing β-oxidation capacity, and increasing the formation of reactive oxygen species (Kalinkovich & Livshits, 2017). The myokines released by these cells exacerbate skeletal muscle inflammation, thus, supporting and maintaining the sarcopenic obesity environment (Kalinkovich & Livshits, 2017). However, despite growing evidence indicating sarcopenic obesity may increase the risk of comorbidity, falls, disability, and long-term care placement, the relationship between being overweight and the development of sarcopenia is still up for debate. The various findings point to the two extremes in BMI potentially increasing the risk of sarcopenia among older adults, prompting research to focus on BMI as a risk factor.

2.1.3(c) Dietary habits

Dietary factors can also influence sarcopenia risk through various components and are responsible for muscle strength and the development of sarcopenia. Protein is one of the main dietary components essential for muscle protein synthesis. Leucine, essential amino acids, beta-hydroxy-beta-methyl butyrate, cheese and milk protein, and vitamin D have all been studied as prospective protein supplements to enhance muscle quality in sarcopenic elderly (Yanai, 2015). Consuming enough protein-rich meals, greens and legumes, vegetables, and whole fruits has been found to decrease the likelihood of sarcopenia by 18 to 23%, according to a study in the United States

(Bigman & Ryan, 2021). Additionally, the study reported an association between low dietary iron intake and sarcopenia risk. This finding is in agreement with another study among older adults in Malaysia, which found that the likelihood of developing sarcopenia increases by 0.93 times for older adults with each milligram of habitual dietary iron consumption (Ranee et al., 2022). Iron constitutes 10 to 15% of the muscle tissue; thus, the depletion of these iron stores can reduce mitochondrial aerobic metabolism. This condition may result in a structural change and a reduction in the number of mitochondria, potentially causing skeletal muscle loss (Polonifi et al., 2010). Meanwhile, in a study among community-dwelling Japanese elderly, sarcopenia was found to be inversely correlated with a dietary pattern defined by high intakes of fish, soybean products, potatoes, most vegetables, mushrooms, and fruits and low rice intake (Yokoyama et al., 2021). Low rice intake may have an inverse association with sarcopenia due to the imbalance in food intake, where consuming a large amount of rice could lead to reduced consumption of foods beneficial for sarcopenia (Yokoyama et al., 2021). The findings are slightly different from a meta-analysis by (Granic et al., 2020) as lean red meat has been shown to increase muscle mass in both observational and intervention trials while eating more fruit and vegetables was linked to improved muscular function. Thus, older adults should consume a variety of wholesome foods within the recommended servings, as suggested in Key Message 1 by the Malaysian Dietary Guidelines (MDG) for Older Persons (MOH, 2023). The message includes consuming adequate protein, calcium, vitamin D, iron-rich foods, and more vegetables (including *ulam*), fruits, and enough legumes.

2.1.3(d) Physical Activity Level

Physical inactivity is a major risk factor for sarcopenia. The results of a meta-analysis showed that physical activity lowers the risk of developing sarcopenia later in life [OR =0.45, (95% CI: 0.37-0.55)] (Steffl et al., 2017). Given that physical inactivity or the absence of physical stimulation of the muscle may lead to more rapid muscle atrophy, the risk of sarcopenia is further increased. A longitudinal study among type 2 diabetes mellitus elderly in Malaysia revealed a significant association between low physical activity and sarcopenia (Sazlina et al., 2020). This study is congruent with the Korean National Health and Nutrition Examination Survey, observing a positive correlation between physical activity intensity and both skeletal muscle index (SMI) and handgrip strength (HGS) in older adults (Seo & Lee, 2022). Conversely, a study among rural older adults in Taiwan suggested that physical inactivity was not independently related to probable sarcopenia once potential confounders were considered; instead, they reported a significant association between poor lower limb body function and sarcopenia (Wu et al., 2022). Thus, a sedentary lifestyle or muscle disuse became a point of concern for individuals at risk for sarcopenia.

2.2 Dietary Intake

Diet refers to a pattern of consumption or a measure of the variation among essential food groups compared to those advised by dietary guidelines (Wirt & Collins, 2009). It alludes to a varied, balanced, healthy diet encompassing all the nutrients needed for growth, health, and energy. Dietary habits vary across the human lifespan and can be determined by various causes such as demographics, religious beliefs, cultural foods, appetite or personal taste, season change, financial status, and age. The risk for unfavorable health conditions such as cardiovascular disease, sarcopenia, and death was linked to poor diet quality (Bloom et al., 2018; Long et al., 2022). A recent study among 2,299 older adults in Peninsular and East Malaysia reported significant deficiency in magnesium (100.0%), vitamin B6 (98.4%), manganese (97.9%), zinc (95.6%), vitamin E (91.2%), potassium (91.0%), calcium (89.3%), vitamin K (81.5%)

and vitamin B12 (80.2%) intake (Ja’afar et al., 2024). Malaysian older adults were observed to consume energy-dense diet with moderate carbohydrate and high proteins and fats despite being insufficient in essential macro and micronutrients (Ja’afar et al., 2024).

A study in Kuala Lumpur, Malaysia, found that older adults’ diet quality significantly decreased with rising age among older adults, with Malay individuals having poorer diet quality compared to Indian and Chinese groups (Nohan et al., 2020). The study also noted insufficient consumption of fruits, fiber, and calcium, despite meeting protein guidelines, primarily from legumes (69.6%) rather than fish (10.9%) or meat (30.4%). Given red meat is relatively more expensive than legumes and fish, it is evident that aside from age and ethnicity, financial constraints can impact purchasing power and subsequently food choices. Thus, older adults with low financial standing due to retirement, inability to work due to sickness, or receiving inadequate allowances from familial or social support might have reduced diet intake and, subsequently, poor nutritional status (Bardon et al., 2021).

Older adults’ food choices may also be influenced by functional limitations. Due to the functional and muscular deterioration of aging, older persons are typically more prone to losing their independence (Nohan et al., 2020). Malaysian older adults also iterated the convenience of food preparation and access to convenient food products as the second most common consideration influencing their dietary habit (Shahrin et al., 2019). The increased reliance on others and focus on convenience suggests that older adults might have a higher reliance on ready-to-eat or minimally prepared foods varying in quality. For instance, some may be fortified with vitamins and minerals while some are high in sodium and added sugars (Ja’afar et al., 2024).

A Brazilian National Health Survey involving 11,177 older adults revealed that those with functional limitations consumed less meat, fruit, vegetables, and legumes independent of age, sex, marital status, housing arrangements, and education (Alves Valle et al., 2016), leading to the exacerbation of diseases. Another study among Spanish Mediterranean older adults reported insufficient vitamin D and iodine intake, in contrast to the excessive intakes of proteins, lipids, mono and polyunsaturated fatty acids, fiber, iron, and vitamins B1, B2, B6, B12, C, and E (Zaragoza-Martí et al., 2020). These differences might be attributable to the Mediterranean dietary pattern consisting of high monounsaturated fatty acids and vitamin E from the ubiquitous use of extra virgin olive oil (Zaragoza-Martí et al., 2018). The increased fiber and protein intake were likely due to high intake of fruits and vegetables and proteins with high biological value, respectively. Additionally, they also reported a significant difference dietary cholesterol and potassium intake according to the area of residence (Zaragoza-Martí et al., 2020), which might indicate differences in food preparation and preferences between urban and non-urban area.

Research on the association between diet quality and sarcopenia indices varies across the population around the world. A prospective cohort study in Hong Kong suggested that higher DQI-International score among community-dwelling older adults is inversely associated with a four-year risk of frailty incidence (Chan et al., 2015). The Italian TRELONG study reported a significant correlation between Mediterranean diet adherence and better lower limb performance (SPPB score >7) (Fougère et al., 2015). Conversely, an Australian study found no association between lean muscle mass and Short Physical Performance Battery (SPPB) and HEI score among community-dwelling older adults (Bloom et al., 2018). Contrastingly, Malaysian older adults had a significantly better diet quality as assessed using an M-

HEI score (70.2 ± 12.0) compared to younger adults (59.7 ± 9.9) (Ramadas et al., 2021). The variations and impact in diet quality across regions and age groups on disease risks and functional performance stipulate nutrient intake's modifiable and malleable nature, improving disease progression. Thus, dietary assessment in Malaysian older adults is essential in ensuring appropriate intervention and improvement for healthy aging.

2.3 Cosmos caudatus

Figure 2.2: *Cosmos caudatus* plant

Source: National Parks Board (2023)

2.3.1 Taxonomy

Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Asterales
Family:	Asteraceae
Genus:	Cosmos Cav
Species:	*Cosmos caudatus* Kunth

(Integrated Taxonomic Information System ITIS)

Cosmos caudatus or *Ulam raja* is a perennial, scented herb that can grow up to two meters tall, as shown in Figure 2.2. It features pinnate leaves and is segmented into leaflets and pink or violet composite flowers with a cluster of yellow florets in the center, hence its use as ornamental shrubs in the European region (Chan et al., 2016). The lower surface of the leaf lamina is light green with tiny hairs, while the upper surface is dark green and soft and tastes pungent when consumed (Chan et al., 2016). In Malaysia, the leafy aerial portion is utilized as a side dish and eaten raw as *ulam*, or salad, often paired with other foods and condiments. It is more commonly consumed among the Malay ethnicity due to its distinctive minty flavor.

2.3.2 Phytochemical Composition and Antioxidant Properties

Through various phytochemical screening and pre-clinical studies, CC has been found to contain phytochemicals such as phenolic acids, flavonoids, tannins, carotenoids, terpenes, saponins, sesquiterpene lactones, sterols, and phenylpropanoids (Moshawih et al., 2017). However, phenylpropanoids can only be found in the roots, limiting their nutritional efficacy.

Polyphenols, aromatic compounds with hydroxyl groups attached to benzene rings, are among the numerous naturally occurring substances in CC. They exhibit potent antioxidant, anti-inflammatory, antibacterial, and anti-aging actions (Phong et al., 2022). The high phenolic content augments antioxidant effects by limiting auto-oxidation, neutralizing electrophiles and reactive oxygen species, reducing nitrosation, and chelating metal ions (Mediani et al., 2013). The most prevalent secondary metabolites of the phenolic chemicals involved in the antioxidant activity of herbs are flavonoids and esters. Numerous flavonoids were discovered in CC, including quercetin rhamnoside (quercitrin), quercetin glucoside (isoquercetin), quercetin, rutin, and chlorogenic acid (Mediani et al., 2013; Sharifuldin, 2014; Sukrasno et al., 2011). Studies consistently identify quercitrin as the primary compound in CC (Seyedreihani et al., 2016; Yusoff et al., 2021).

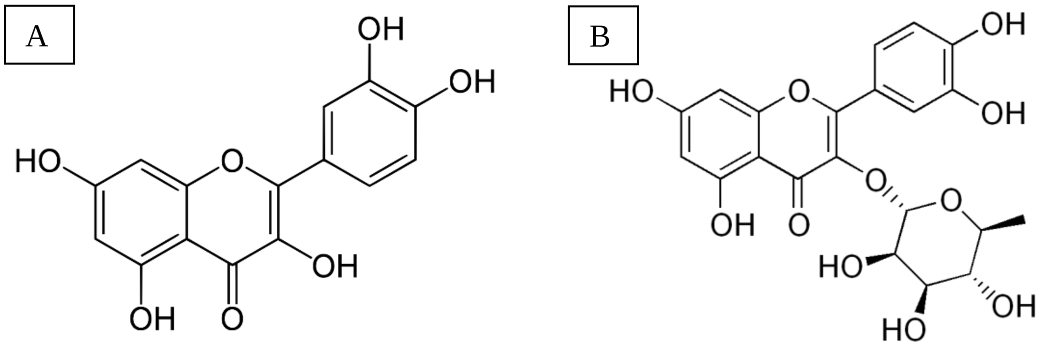

Figure 2.3: Chemical structures of A: quercetin and B: quercitrin

Quercetin is a yellow, crystalline flavonoid with a bitter taste, completely insoluble in cold water but soluble in lipids and alcohol. The term quercetin, in use since 1857, originates from the word '*quercetum*' (oak woodland), paying tribute to Quercus. Quercetin is predominantly found in its glycoside form quercitrin as presented in Figure 2.3, and stands as the most widely distributed flavonoid in nature. It is found in flowers, stems, bark, roots, tea, vegetables, fruits including apples, dill, berries, cilantro, and onions (Aghababaei & Hadidi, 2023). A pre-clinical study utilizing both quercetin and quercitrin demonstrated protection against cytokine-induced cell death and reduced reactive oxygen species and nitric oxide accumulation (Dai et al., 2013). These effects were linked to decreased expression of inducible nitric oxide and inhibition of nuclear factor-κB (NF-κB) translocation. Additionally, quercetin/quercitrin suppressed cytochrome-c release from mitochondria and subsequent alterations in downstream proteins, suggesting quercetin as a mitigation factor for mitochondrial apoptosis (Dai et al., 2013).

In skeletal muscle health, an in vitro study suggested that at doses attainable through dietary and dietary supplement intake, quercetin inhibited the development of muscle-derived PDGFR+/CD201+ cells into adipocytes and fibroblasts, indicating its preventative impact on the deterioration of muscle quality (Ohmae et al., 2022). This