

STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF *DYNARIA QUERCIFOLIA* PORRIDGE MIX

PROJECT REPORT

Submitted in partial fulfilment of the requirements for the award of Bachelor of
Science degree in Clinical Nutrition & Dietetics

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**DEPARTMENT OF CLINICAL NUTRITION & DIETETICS
SCHOOL OF ALLIED HEALTH SCIENCES**

SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

Accredited with Grade "A⁺⁺" by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this project report is the Bonafide work of Swathika.V (41736016) and Poomuhi.D.S(41736012) who have done the project work entitled “**STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX**” under our supervision from October 2023 to April 2024.

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We, Poomuhi.D.S (41736012) and Swathika.V (41736016) hereby declare that the project entitled **"STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX"** done by us under the guidance of **Ms. Roshni .M** at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

DATE: 27.04.2024

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DECLARATION

We, Swathika. V (41736016) and Poomuhi.D.S (41736016) hereby declare that the project entitled **"STUDIES ON DEVELOPMENT AND NUTRITIONAL ANALYSIS OF DYNARIA QUERCIFOLIA PORRIDGE MIX"** done by us under the guidance of **Ms. Roshni .M** at the Department of Clinical Nutrition & Dietetics, Sathyabama Institute of Science and Technology, Chennai is submitted in partial fulfilment of the requirements for the award of Bachelor of Science degree in Clinical Nutrition & Dietetics.

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ABSTRACT

Autoimmune diseases, such as arthritis is now recognized as a common non-communicable disease worldwide, affecting around 1-2% of people globally. In recent years, studies suggested that diet play a major role in disease risk and progression. Several treatment strategies have evolved over time to enhance the quality of life for rheumatoid arthritis patients, yet a considerable number remain unresponsive to existing medications. Diet plays a major role in disease risk and progression. Hence, the present study aims to develop and evaluate the nutritional analysis of *Dynaria quercifolia* porridge mix for the benefit of arthritis patients. *D. quercifolia* was grated, shade dried while the other ingredients such as millets, grains, and nuts were roasted. Following this, all the ingredients were mixed together and ground. The optimized levels of all the ingredients were added for the development of porridge mix based on colour, appearance and taste. The proximate analysis of the product revealed that it comprises of 407g/100g of energy, 77.4g/100g of carbohydrates, 4.37g/100g of total fat, 14.4g/100g of protein, 13.1g/100g of dietary fibre with hedonic rating, the maximum and minimum overall acceptability is 8.1 and 7.2 respectively. During the study, it was observed that *D. quercifolia* had a higher calcium level of 297mg/100gm. Thus, incorporation of *D. quercifolia* in the porridge mix and including it in daily dietary habits could effectively manage, prevent and reduce the occurrence of arthritis.

Keywords: Arthritis, *Dynaria quercifolia*, porridge, Autoimmune, calcium.

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LIST OF ABBREVIATIONS

RA	Rheumatoid arthritis
QOL	Quality of life
ASEAN	Association of Southeast Asian Nations
DQR	<i>Dynaria quercifolia</i> rhizome
DQ	<i>Dynaria quercifolia</i>
AD	Autoimmune disease
M	Milk
W	Water
CFU	Colony forming units
WHO	World Health Organisation
NSAIDs	Nonsteroidal anti-inflammatory drugs
ALP	Alkaline phosphatase
SLE	Systemic Lupus Erythematosus
DL	Detection limit
%	percentage
kcal	kilocalorie
g	gram
mg	milligram

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CHAPTER 1

INTRODUCTION

Autoimmune diseases result from the immune system attacking the body's own cells and tissues. The diverse nature of these conditions makes them affect various organs, and they often show a higher prevalence in women (Davidson A. *et al.*, 2019).

Infections and microbiota play pivotal roles in triggering autoimmune diseases. The presence of autoantibodies before clinical signs, during the preclinical autoimmunity phase, underscores the potential for early detection and intervention in at-risk populations to mitigate tissue damage (Sethi S. *et al.*, 2022).

The autoimmune diseases manifest as advanced conditions where the immune system reacts against the host's own normal constituents, involving both B-cell and T-cell reactivity. These disorders often target specific organ systems and show a higher prevalence among women, emphasizing the complex interplay of factors in their development (Beck L H. *et al.*, 2019).

The autoimmune diseases exhibit clinical diversity. They can either manifest with organ-specific patterns or present in a generalized manner, affecting various systems throughout the body. The distinction between systemic and organ-specific involvement adds complexity to their clinical presentation and management (Pollak T A. *et al.*, 2020).

The perception of autoimmune diseases has shifted from being considered rare to now being recognized as prominent non-communicable diseases worldwide. The understanding of autoimmunity has advanced substantially, encompassing insights from gross structural levels to intricate micro-molecular details, contributing to a more comprehensive knowledge base in this field (Sakaguchi S *et al.*, 2000).

While significant progress has been made in understanding autoimmunology and autoimmune diseases, there is acknowledgment that the complete understanding of the ideology and causative mechanisms remains elusive. The complex pathological process involved have been unraveled to a considerable extent, and ongoing clinical observations continue to refine our understanding (Stojanorich L *et al.*, 2010).

Autoimmune diseases pose challenges in terms of preemptive strategies, and current treatment approaches often focus on managing symptoms rather than preventing onset. Drawing insights from protocols in infection, oncology, metabolic diseases, and transplantation might help develop more effective management models for autoimmune conditions (Chandrashekar, 2019).

Advancements in medical understanding and treatment strategies have significantly improved the survival rates of SLE over the past two decades. The increase in reported 90% survival beyond 5 years is a positive development in managing this autoimmune disease (Urowitz M B *et al.*, 2019).

The evolving understanding of autoimmune diseases has spurred the development of a wealth of information on their mechanisms. This knowledge has been instrumental in the creation of newer drugs and biologicals, opening up promising avenues for more effective treatments (Mc Gonagle D *et al.*, 2019).

The current approach in AD can be broadly categorized into two categories: (1) symptomatic or replacement therapy (a conservative approach) and (2) the immunosuppressive or immune-modulation therapy (aggressive therapy). Currently more target-specific drugs are available which have reduced the toxicity of immune suppressant drugs on other collateral systems and produces a more profound immunosuppression effect (Feldmann M *et al.*, 2005).

The current approach of immunosuppression resembles the concept of transplant rejection, but the immunosuppression used is much lesser in AD than what is normally prescribed in transplant rejection management. The immune homeostasis principle suggests that AD can be caused by lymphopenia and resurgence of the autoreactive clones (Dalakas MC *et al.*, 2006).

The diversity of autoimmune diseases poses a significant challenge in the medical field. Clinicians must navigate through a multitude of signs and symptoms to reach an accurate diagnosis, often requiring distinct and varied management approaches.

The clinical complexity and heterogeneity of autoimmune diseases, a focused search for aberrant B and T cell auto-reactivity becomes essential. Identifying these specific mechanisms is crucial for understanding how autoimmune processes drive the pathogenesis of these conditions (Den braanker *et al.*, 2021).

The exploring auto-reactivity becomes paramount in understanding poorly-understood autoimmune conditions with diverse clinical presentations. Investigating auto-antigens serves as the initial step, providing a foundation to assess the involvement of B or T cell auto-reactivity in the pathogenesis of these diseases (E K Mirmira *et al.*, 2020).

Autoimmune diseases like rheumatoid arthritis involve the immune system and targets various components of the joints, leading to the inflammation, tissue damage, and pain.

The global prevalence of the disease is estimated around 1–2%, with a large variation among different populations. In developed nations, osteoarthritis is considered to be one of the 10 most common disabilities in older individuals, especially those who remain active in the workforce (Palmer KT *et al.*, 2015).

The word arthritis is derived from the Greek words arthron for “joint” and itis for “inflammation”. Today, the term is used for hundreds of different varieties of joint problems that have specific symptoms, such as pain, swelling, and stiffness. Arthritis refers to a range of musculoskeletal conditions where a person’s joints become inflamed, which may result in pain, stiffness, disability, and deformity. These symptoms can often have a significant impact on a person’s everyday functioning life. There are more than 100 types of arthritis (Watson, 2019).

In 2019, 18 million people worldwide were living with rheumatoid arthritis. About 70% of people living with rheumatoid arthritis are women, and 55% are older than 55 years. 13 million people with rheumatoid arthritis experience severity levels (moderate or severe) that could benefit from rehabilitation. While rheumatoid arthritis is a systemic autoimmune disease that affects multiple body systems, the joints of hands, wrists, feet, ankles, knees, shoulders and elbows are most often affected (Koller smith L *et al.*, 2022).

Rheumatoid arthritis (RA) is a chronic, symmetrical, inflammatory autoimmune disease that initially affects small joints, progressing to larger joints, and eventually the skin, eyes, heart, kidneys, and lungs with varying severity among patients. Often, the bone and cartilage of joints are destroyed, tendons and ligaments are weakened. The risk factors include age, gender, genetics, and environmental exposure (cigarette smoking, air pollutants, and occupational) (Lee JE *et al.*, 2017).

Furthermore, patients with RA generally complain of gastrointestinal tract problems particularly dyspepsia (bloating, postprandial fullness, nausea, early satiety, epigastric pain, and burning and belching), mucosal ulceration, and altered bowel habits (constipation/diarrhoea) (Wolfe F *et al.*, 2016).

Certainly, environmental factors such as smoking, certain infections, and exposure to pollutants are known to contribute to the development of rheumatoid arthritis. These factors can have an impact before clinical symptoms appear, highlighting the complex interplay between genetics and the environment in autoimmune diseases (Edwards CJ *et al.*, 2008).

Common symptoms of RA include morning stiffness of the affected joints for >30 min, fatigue, fever, weight loss, joints that are tender, swollen and warm, and rheumatoid nodules under the skin (Chaudhari K *et al.*, 2016).

The goals of treatment for RA are to reduce joint inflammation and pain, maximize joint function, and prevent joint destruction and deformity (Staheli LT *et al.*, 1998).

The first line of treatment for RA includes disease-modifying antirheumatic drugs (DMARDs) that suppress disease activity and reduce joint damages. There are many other treatments like medications (NSAIDs: Nonsteroidal anti-inflammatory drugs; helps to reduce the pain and inflammation. Corticosteroids: provide relief from inflammation), Physical therapy (Exercises to improve joint mobility, strength, and flexibility, Lifestyle modifications (maintaining a healthy weight, avoiding stress on joints and other Alternative therapies (some people find relief from arthritis through massage therapy, dietary supplements) which can cure the arthritis including the diet part (Rubbert roth *et al.*, 2019)

Although a number of studies suggested associations between dietary habits, mainly regarding fruit, vegetables or meat intake and the disease's development, the results are still inconclusive (Pedersen M *et al.*, 2006).

Diet and nutrients have received considerable attention as potential environmental factors influencing the development and the course of the disease. Although a number of studies suggested associations between dietary habits, mainly regarding fruit, vegetables or meat intake and the disease's development, the results are still inconclusive (Grant W.B *et al.*, 2000).

In recent years, an increasing number of studies have investigated the role of diet and nutrition as potential tools for RA prevention and management (Philippou E *et al.*, 2018).

Dietary habits could represent both disease risk and protective factors based on the properties of specific food, dietary choices, or needs. Dietary habits show pro-inflammatory effects, e.g., red meat, salt, excessive caloric intake, or, on the contrary, radical consumption, oily, fatty fish, fruit, and others (Oliviero F *et al.*, 2015)

Excessive consumption of red meat and a high total protein supply have been associated with an increased risk of inflammatory polyarthritis (Pattison D.J *et al.*, 2004).

RA patients presented a lower intake of fish, potatoes, mushrooms and organ meats; mushrooms, citrus fruits and dairy products consumption showed a protective effect on RA, while potatoes and other fruits' consumption was associated with an increased risk (Wang Y *et al.*, 2016).

A high dietary sodium (salt) intake, common in Western countries, has been associated with an increased risk of RA (Salgado E *et al.*, 2015).

RA and other chronic diseases, including cardiovascular disease (CVD), type 2 diabetes mellitus. Indeed, C-reactive protein (CRP), a marker of inflammation, predicts future development of hypertension and diabetes mellitus better than body mass index.

Nutrients can modulate the inflammatory status of humans and consequently the pro- or anti-inflammatory properties of specific foods and components have emerged in nutrition sciences.

Millions of people suffer from painful and swollen joints associated with arthritis. In the past, many doctors reported dietary changes for an arthritis patient would not help them. However, this conclusion was based on older research with diets that included dairy products, oil, poultry, or meat. New research shows that foods may be a more frequent contributor to arthritis than is commonly recognized (Panush RS *et al.*, 1983).

For years, people have suspected that foods are an important factor in the development of rheumatoid arthritis. At present, 69% of patients with OA take some

form of dietary supplements for their condition. The *Spinacia oleracea* vegetable can mitigate OA effects by increasing bone volume to tissue volume, which results in a decrease of the trabecular pattern factor by more than 200% (Basedow M *et al.*, 2014).

Common forms of arthritis include osteoarthritis, caused by wear and tear on the joints; rheumatoid arthritis, an autoimmune disease in which the body attacks its own joints; and gout, caused by a buildup of uric acid in the body. Regardless of the type of arthritis you have, cutting back or eliminating foods that can aggravate inflammation may help alleviate the pain and swelling.

When a subject has arthritis, their body is in an inflammatory state. What the subject eat may not only increase inflammation, but it may also set up for other chronic conditions such as obesity, heart disease, and diabetes.

A survey conducted in 2015 by D. Sobel for more than 1000 arthritis patients, concluded that red meat, sugar, fats, salt, caffeine, and nightshade plants (e.g., tomatoes, eggplant) were most commonly believed to worsen the condition (Sobel D *et al.*, 1989).

On the other hand, certain foods can help alleviate arthritis symptoms and promote joint health. Some of foods like Fatty fish (salmon, mackerel, sardines are rich in omega 3 fatty acids), which have anti-inflammatory properties and may help reduce joint pain. Berries high in antioxidants, which helps in neutralize free radicals' leafy greens support overall joint health whole grains foods like brown rice, quinoa, barley provide fiber and nutrients that may reduce inflammation etc.

Certain dietary approaches, like anti-inflammatory diets or those rich in omega-3 fatty acids, are being explored for their potential benefits in mitigating symptoms and supporting overall health (Hermann, 2017).

Fruits, vegetables and olive oil could decrease RA risk, providing several nutrients with antioxidant properties, such as tocopherols contained in olive oil acting as free radical scavengers (Serra-majem *et al.*, 2003).

However, the already mentioned Danish study demonstrated no association between RA risk and the intake of citrus fruits, vegetables, retinol, beta-carotene, vitamins A, E, C, D, zinc selenium, iron and meat (Di Giuseppe D *et al.*, 2014).

Not only natural ingredients also medicinal plants have a history of being utilized for treating arthritis and various other conditions. They possess therapeutic properties that can alleviate inflammation, reduce pain, and improve joint mobility in arthritis sufferers. Furthermore, these plants are employed for diverse purposes beyond arthritis treatment, such as addressing digestive issues, enhancing immune function, and promoting overall well-being through their natural healing compounds (Sofowora A *et al.*, p. 1993).

Likewise, *D. quercifolia* known as 'Mudavattukal' in Tamil has gained popularity in recent times and being used in different group of people to cure various kinds of health problems.

So far *Dynaria quercifolia* has been mostly used in treating stomach ache, peptic ulcers, dysentery, gastro-intestinal disorders; skin problems, cold, cough, jaundice, fever, urinary complaints, fracture of bones and hypertension.

Ethnomedicinal information shows that the rhizome of plant DQ was traditionally used for the treatment of body pain, joint pain, dyspepsia, diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin diseases. Plants from the *Drynaria* genus can be used topically as a hair tonic to promote hair development and to improve the condition of the hair. *D. quercifolia* as a whole plant can be used to treat Tuberculosis, rheumatoid fever, dyspepsia, and cough. The plant's tonic has an astringent effect on the bowels. It is utilised in traditional medicine by a variety of people to address variety of health issues, including urinary tract infection. The rhizome is popularly used as a traditional soup condiment and analgesics by the people of Eastern Ghats, Tamil Nadu, India (Brahmam OM *et al.*, 2002).

It has also been used in Bangladesh by traditional healers for the treatment of jaundice, hepatitis, diabetes, gonorrhoea and malaria (Sen A *et al.*, 2001).

In Southeast Asia, rhizome decoction of *D. quercifolia* is used in antipyretic preparation and as poultice on swellings (Dixit RD *et al.*, 1984).

Based on the above evidence, our current study is focuses on development and nutritional analysis of *D. quercifolia* porridge mix which not only manage but prevent arthritis.

CHAPTER 2

REVIEW OF LITERATURE

The word arthritis is derived from the Greek words arthron for “joint” and its “inflammation.” Today, the term is used for hundreds of different varieties of joint problems that have specific symptoms, such as pain, swelling, and stiffness. Arthritis refers to a variety of musculoskeletal situations wherein a person’s joints grow to be inflamed, which can also additionally bring about pain, stiffness, disability, and deformity. These symptoms can often have a significant impact on a person’s everyday functioning of life. There are more than 100 types of arthritis (Watson R. r., 2019).

They are rheumatoid arthritis, osteoarthritis, childhood arthritis, reactive arthritis, fibromyalgia arthritis, systemic lupus erythematosus arthritis, spondyloarthropathies arthritis, etc.

Among the different types of arthritis research, the largest share of papers (49.35%) was published in rheumatoid arthritis, followed by adjuvant arthritis (10.70%), tuberculosis arthritis (8.39%), osteoarthritis (7.96%), bacterial arthritis (7.83%), lupus arthritis (7.64%), juvenile arthritis (7.60%), psoriatic arthritis (3.85%), hand arthritis (2.84%), polyarthritis (2.81%), gout arthritis (2.74%) and septic arthritis (2.20%) during 2007-16 (Gupta B.M, 2017).

Rheumatoid arthritis (RA) is a chronic, inflammatory autoimmune disorder characterized by the destruction of joints and persistent synovitis; it affects nearly one percent of the adult population worldwide (Paulraj, 2001).

The chronic inflammation leads to the development of pannus, an aggressive inflammatory state where activated T lymphocytes, macrophages and B cells together with the cytokines that they produce, as well as active angiogenesis, play a major part in the progression of the joint destruction (Paran. *et al.*, 2001).

Rheumatic Arthritis is the inflammatory disease of the immune system and it targets first the synovium or lining of the joint, resulting in pain, stiffness, swelling, joint damage, and loss of function of the joints. Inflammation most often affects joints of the hands and feet and tends to be symmetrical (Grapevine, 2017).

Rheumatoid arthritis (RA) is a systemic, debilitating, chronic inflammatory autoimmune disorder affecting approximately 1% of the world population (Gibofsky A., 2012).

The disease severely impacts quality of lifestyles with improved morbidity and decreased lifestyles expectancy. With the rapidly expanding population with RA, the disease has put a lot of economic burden on the society (Aggarawal A. *et al.*, 2006).

The global prevalence of the disease is estimated around 1–2%, with a large variation among different populations. Genetic and environmental factors interact during RA pathogenesis, a multistep process that begins years before clinical onset of the disease (Alamanosy Y. *et al.*, 2005).

Arthritis affects 15% of Indian population which is about 180 million people. There are more than 100 rheumatic diseases that collectively affect more than millions of people worldwide (Mela *et al.*, 2017).

In developed nations, osteoarthritis is taken into consideration to be one of the 10 most common not un usual place disabilities in older individuals, particularly individuals who stay energetic withinside the workforce.

Of persons with arthritis, more than half (58.9%) had osteoarthritis (deterioration of cartilage inside a joint), 11.5% had rheumatoid arthritis (an autoimmune disease in which the body is attacked by bacteria or viruses), and 34.8% had an unspecified type of arthritis (Masud parner GM. *et al.*, 2015).

In 2019, about 528 million people worldwide were living with osteoarthritis and 18 million people with rheumatoid arthritis; About 73% of people living with osteoarthritis are older than 55 years, and 60% are female whereas 70% of people living with rheumatoid arthritis are women, and 55% are older than 55 years (L K. s., 2022).

Musculoskeletal disorders are considered the second largest cause of disability globally, second but close only to behavioural and mental disorders (Vos T. *et al.*, 2012).

Furthermore, patients with RA generally complain of gastrointestinal tract problems particularly dyspepsia (bloating, postprandial fullness, nausea, early satiety,

epigastric pain, and burning and belching), mucosal ulceration, and altered bowel habits (constipation/diarrhoea) (Wolfe F. *et al.*, 2016).

Certainly, environmental factors such as smoking, certain infections, and exposure to pollutants are known to contribute to the development of rheumatoid arthritis. These factors can have an impact before clinical symptoms appear, highlighting the complex interplay between genetics and the environment in autoimmune diseases (Edwards CJ. *et al.*, 2008).

Many terms are used [excellent of life (QOL), fitness status, fitness-associated QOL] to talk about the impact of diseases, including arthritis, on functioning and experience of well-being. At the broadest level, QOL refers now no longer most effective to fitness reputation however additionally to environmental and monetary factors (e.g. profits and academic attainment that may drastically have an effect on well-being. Thus, different conceptual frameworks guide how quality of life is defined and measured (Wan G. *et al.*, 1997).

RA has major diverse effects on patients' quality of life, affecting both mental and physical do mains of well-being (Uhlir T. *et al.*, 2007).

The goals of remedy for RA are to lessen joint infection and pain, maximize joint function, and save you joint destruction and deformity. Treatment regimens consist of combinations of pharmaceuticals, weight bearing exercise, educating patients about the disease, and rest. Treatments are commonly custom designed to a patient's wishes and rely on their average health. This consists of elements which includes sickness progression, the joints involved, age, usual health, occupation, compliance, and training approximately the sickness. (Staheli LT. *et al.*, 1998). The intention of RA remedy is to obtain clinical, structural, and purposeful remission states (Van der Heijde DM. *et al.*, 1996).

In current years, the usefulness of phytopharmaceuticals, consisting of alisol-B, resveratrol, and arctigenin, has been suggested withinside the location of bone metabolism as a revolutionary adjuvant therapy. Resveratrol indeed shows promise in promoting osteoblast differentiation of mesenchymal stem cells, which are crucial for bone formation and repair. Its potential benefits in bone health have been studied extensively. The form of movement those drug treatments have on infection and bone metabolism warrants in addition research (Komori T. *et al.*, 2020).

Also, Medicinal plants have a history of being utilized for treating arthritis and various other conditions. They possess therapeutic properties that can alleviate inflammation, reduce pain, and improve joint mobility in arthritis sufferers. Furthermore, these plants are employed for diverse purposes beyond arthritis treatment, such as addressing digestive issues, enhancing immune function, and promoting overall well-being through their natural healing compounds (Sofowora A. *et al.*, p. 1993).

A medicinal plant is defined as any substance that can be employed for therapeutic purposes or as a precursor for the production of pharmaceuticals. In the last few years, researchers are becoming highly interested in the usage of physiologically active chemicals found in nature of therapeutic value. Medicinal plants encompass numerous compounds that may be used to deal with variety of ailments. Flavonoids, alkaloids, tannins, and different phytoconstituents saponins, quinines, terpenoids, glycosides, and polyphenols are a number of the compounds located in plants. Many plants are yet to be researched for their potential benefits and medicinal significance (Sofowora A. *et al.*, p. 1993).

Cinnamomum cassia Presl, also known as cassia or cinnamon, is a tropical aromatic evergreen tree, commonly used in traditional Oriental medicine. More than a hundred and sixty chemical compounds were recognized from *C. cassia*. The main constituents are terpenoids, phenylpropanoids, and glycosides (Zhang C. *et al.*, 2019).

The main components of *C. cassia* have a wide range of pharmacological effects, including anti-inflammatory and analgesic activity (Yang YL. *et al.*, 2020).

The dry root of *Aconitum kusnezoffii Reichb.* (caowu) has been used to deal with RA and joint ache for decades thanks to its anti-inflammatory properties. Pharmacological research has proven that diterpenoid alkaloids, along with aconitine, mesaconitine, hypaconitine, neoline, talatizamine beiwutine, and deoxyaconitine, are liable for the primary bioactive outcomes of *A. kusnezoffii* (Sun H. *et al.*, 2013).

According to the report of the W.H.O, about 80% of the world's populations rely mainly on traditional therapies which involve the use of plant extracts or their active substance. The use of medicinal plants as a source for relief from illness had been

recognized by early civilization in China, India and the other country (Yatskieych G *et al.*, 2002).

In ancient texts, about 500 plants have been indicated in the treatment of arthritis; however, only a small number of plants have been evaluated scientifically. In ancient Indian medicine, several ferns were also used, and in particular by Unani physicians in India and Western Asia. Ferns play a vital position in folklore medicine (Mondal AK *et al.*, 2011).

Accordingly, the ferns of *D. quercifolia* rhizome is used by local tribes in the rain forests of Western Ghats of Maharashtra, India. The rhizome is ground into a paste and used to treat diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin disease (Nejad B S *et al.*, 2009).

However, the rhizomes had been utilized in Indian machine of medication as anodyne, anti-inflammatory and for the remedy of arthralgia and migraine (Warrier *et al.*, 1995). Also used in the treatment of tuberculosis, loss of appetite, cough, and fever (Bhattacharya, 1990).

2.1 DYNARIA QUERCIFOLIA

D. quercifolia commonly known as “Oak Leaf Fern” is a medicinal pteridophyte belonging to the family Polypodiaceae. *Dynaria quercifolia* (L.) (Drynariaceae) is known as ‘Mudavaatukkal’ in Tamil.

In Ayurvedic system of medicine, it is called ‘Ashwakatri’ use by different tribal people for the treatment of arthritis (Nayar KB *et al.*, 1959).



Fig 2.1: Leaves of *D. quercifolia*

D.quercifolia is a medicinal fern with a huge variety of makes use of in treating a number of ailments. It is a terrestrial fern that can be found in fissures, shelves, or in the soil among boulders. They also can be discovered as epiphytic on trees, open forests and rainforest trunks (Cumming P *et al.*, 2011).

In Bangladesh, *D.quercifolia* and its several plant parts has been used by the local inhabitants, folk and tribal medicinal practitioners to treat jaundice, hepatitis, Chest pain, diabetes, gonorrhoea, debility and malaria (Mollik A H *et al.*, 2009).The above figure 2.1 shows the leaves of *D.quercifolia*.

The ASEAN Centre for Biodiversity mentions in its “Checklist of Medicinal Plant in Southeast Asia” that rhizome decoction or drink of *Drynaria quercifolia* is used as an antipyretic preparation (Chattopadhyay *et al.*, 2005).

D.quercifolia is an important ethnomedicinal plant used in traditional medicinal system by different group of people to cure various kinds of health problems. DQ along with other combination of herbs is used in pain from traumatic injury and also effective for lower back and ligament injuries (Dagar JC *et al.*, 1987).

Ethnomedicinal information shows that the rhizome of plant DQR was traditionally used for the treatment of body pain, joint pain, dyspepsia, diarrhoea, typhoid, cholera, chronic jaundice, fever, headache, and skin diseases.

2.2 RHIZOME

The plant part utilized in peoples remedy of *D.quercifolia* are rhizomes. (L, 2016). Traditionally, the soup organized from the rhizome of *D.quercifolia* will be very well-known among tribes of Eastern Ghats, Tamil Nadu and the soup drink enables to get treatment from rheumatic complaints (Samuydurai P *et al.*, 2012).



Fig 2.2: Rhizomes of *D. quercifolia*

The rhizomes, measuring 2cm in thickness, creep with a dense brown covering. Scales are 20-25mm long and 0.7-2.5mm broad and come in a variety of colours. The base of the rhizome is winged, but the lamina is not. Spores are 37.5-55 microns in length and 22.5-37.5 microns broad (Hooker W J *et al.*, 1862). The figure 2.1 below are the rhizomes of *D.quercifolia*.

The rhizomes are bitter, astringent and tonic. Rhizome paste is applied on the forehead to relieve head ache. As per the reports of Dagar (Dagar J C *et al.*, 1987).

The rhizomes have antibacterial and constriction properties. By mixing with the plant *Asparagus racemosus* Willd. (Liliaceae) then implemented on the top, it reasons coldness to the top and decreases alopecia (Hasan, 1993).

An herbal anti-pyretic agent with decreased or no toxicity is consequently essential. As rhizome of *D.quercifolia* is an old medicament used in ailments that caused fever (Bhattacharya *et al.*, 1990).

2.3 D.QUERCIFOLIA IN BONE HEALTH

D.quercifolia aids in the healing and strengthening of damaged bones. It promotes bone density and protects against osteoporosis and aids in the recovery of fractured bones ligaments (Brahmam O M *et al.*, 2002).

D.quercifolia has been shown in multiple studies to stimulate and osteoblasts to produce more healthy bone tissue (Wong K C *et al.*, 2012).

Anti-osteoporotic effects of extracts of *D.quercifolia* have been shown in female mammals following the loss of ovaries (Ma Z *et al.*, 1999).

An animal study done by one group of researchers revealed that the extract of *D.quercifolia* suppressed bone resorption and promoted bone (Chen L L *et al.*, 2004).

In 2011, scientists from School of Medicine in Hangzhou observed a significant decrease in the number of bone dissolving cells (Osteoclasts) in the *D.quercifolia* treated group as compared to the control animals. Their study has shown positive effects on improving alveolar bone remodelling and the study concluded that *D.quercifolia* could be an alternative medicine for periodontal therapy based on its

ability to reduce alveolar bone resorption while supporting the bone-building effects of osteoblasts (Ding P H *et al.*, 2011).

Naringin, one of the active ingredients in *D.quercifolia*, stimulates protein synthesis and metabolism of human periodontal ligament cells and naringin, also can promote proliferation of human periodontal ligament cells by enhancing ALP activity which is an important osteoblast function in bone building (Jiang J Q *et al.*, 2009).

The study done by Chinese researchers reported that total flavonoid content of *D.quercifolia* rhizomes has the ability to promote osteogenic differentiation of bone marrow cells into bone-building osteoblast cells at different concentrations of exposure to glucose (Shu X C. *et al.*, 2010).

2.4 OTHER PROPERTIES OF *D.QUERCIFOLIA*

The antifungal interest of the ethanolic extract of rhizome of *D.quercifolia* on dermatophytic species was first reported by Batool in 2009.

The methanol extracts of rhizome of *D.quercifolia* have effective against gram positive bacteria (*Bacillus subtilis*) and some gram-negative bacteria (Nath K. *et al.*, 2016).

The rhizome is reported to be used by tribal communities of Tamil Nadu and Kerala to cure various diseases and still in the regular practice of the local people of Kolli hills by consuming it as a soup for treating various ailments (Samydurai P. T. *et al.*, 2012).

So far, the market has exclusively offered *D.quercifolia* in form of a soup powder only. Being a functional ingredient, it has gained a demand in market in various other forms. Considering the above facts and need in the market our current study aims on the development and nutritional analysis of *D.quercifolia* porridge mix which not only aids in management but also in the prevention of arthritis.

CHAPTER 3

AIM AND OBJECTIVES

3.1 AIM

The aim of the project is to develop and analyse the nutrients of *D.quercifolia* porridge mix.

3.2 OBJECTIVES

1. To examine the nutritional analysis of *D.quercifolia*.
2. To examine the macronutrients of *D.quercifolia* porridge mix.
3. To examine the micronutrients of *D.quercifolia* porridge mix.
4. To analyse the sensory quality of *D.quercifolia* porridge mix.
5. To determine the shelf life of *D.quercifolia* porridge mix.

CHAPTER 4

MATERIALS AND METHODS

4.1 Locale of the study

The major part of the present study has been carried out in the Department of Clinical Nutrition and Dietetics at the Sathyabama Institute of Science and Technology. The nutritional analysis and shelf life was performed in the nearest lab.

4.2 Procurement of raw materials

Dynaria quercifolia was purchased from the markets of Yercaud and the other ingredients for the preparation of the porridge were purchased from the local markets of Chennai.

4.3 Development of *D.quercifolia* porridge mix

D.quercifolia, the rhizome was thoroughly washed under running tap water. The fur portion of the rhizome was removed. The flesh part was isolated, grated and subsequently shade-dried. Additionally, a variety of grains including finger millet, pearl millet, kodo millet, red rice, maize, white corn, black gram, whole wheat, barley, sago, green gram, badam and cashew were dry roasted. The ingredients were then combined and ground into a fine mixture.

The figure 4.1 and 4.2 summarizes the steps in preparation and the pictorial representation of preparation of *D.quercifolia* porridge mix.

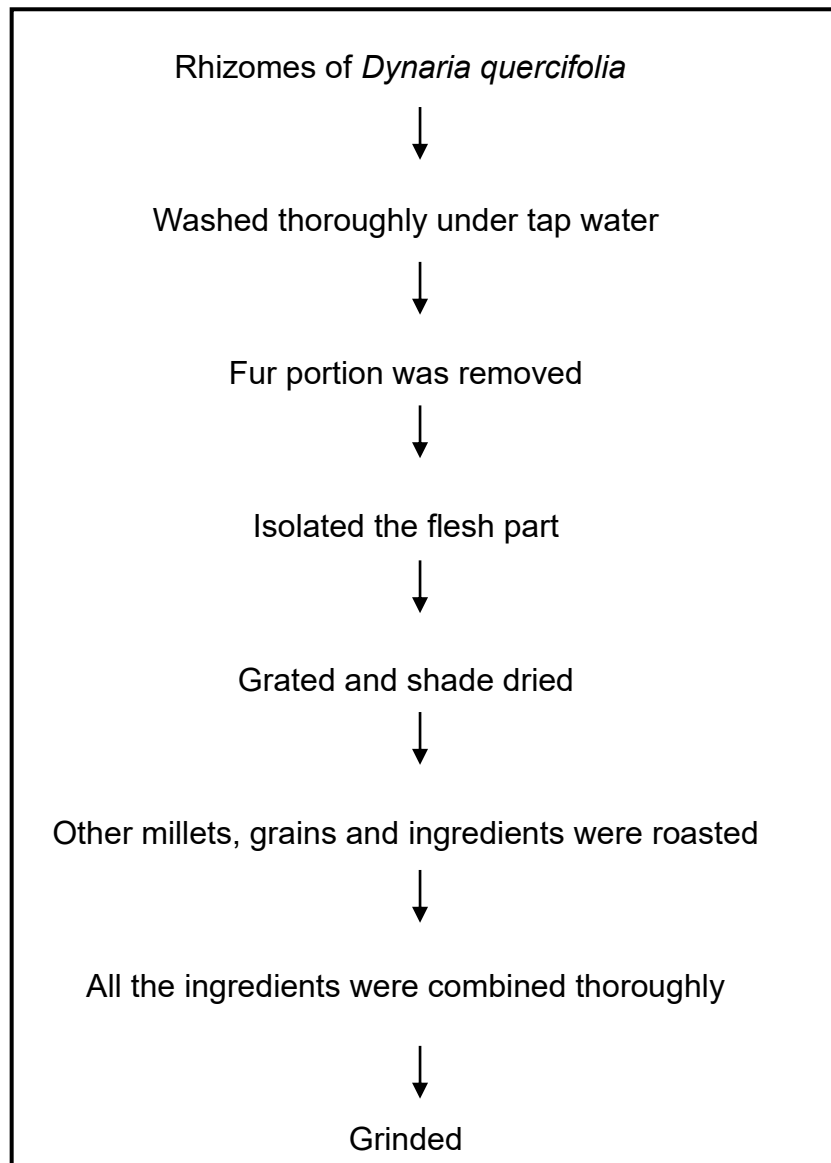


Fig 4.1: Steps in preparation of *D. quercifolia* porridge mix



Fig 4.2: Flow chart of pictorial representation in preparation of *Dynaria quercifolia* porridge mix

4.4 Nutritional analysis of *D.quercifolia*

4.4.1 Energy

Energy is calculated by the FAO method.

It is calculated by the following formula:

$$\text{Energy(kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

4.4.2 Total Carbohydrates

The total carbohydrate content in the porridge powder was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fibre, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate(g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat(g)} + \text{Protein(g)} + \text{Fibre(g)}]$$

4.4.3 Total fat

The total fat was determined by the method of AOAC 20th Edn.2016,920.39

Procedure:

Weigh accurately about 2g of sample in a fat extraction tube; add 2.0ml of alcohol to prevent lumping on the addition of acid, and shake to moisten all particles. Add 10ml HCl (25+11), mix well and set tube 30 to 40 mins in water bath at 70 °C and 80 °C, shaking frequently. Cool to room temperature and add alcohol. Add 25ml diethyl ether to the flask and shake for 1.0 mins. Then add 25ml petroleum ether to the flask and shake 1min. Decant ether layer into the pre-weighed dry flask through anhydrous sodium sulphate. Then, Decant ether layer into a pre-weighed dry flask through anhydrous sodium sulphate. Then add 25ml of 1:1 diethyl ether, and petroleum ether to the flask and shake for 1.0min. Ether layer can be evaporated under a water bath and keep the flask into the oven for further evaporation of ether residue. Remove the flask from the oven and place in desiccators to cool to room temperature and record the weight of the flask plus fat.

Calculation:

% of Fat = (Weight of flask + fat) – Empty weight of the flask × 100 / Sample weight

4.4.4 Protein

The protein($N \times 6.25$) was determined by the method of AOAC 20th Edn.2016, 986.25

Procedure:

Weigh suitable test portion of sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

%N (w/w) = (TV_s-TV_b) × Normality of standard HCl × 1.4007 / Sample weight

where, TV_b = Titer value of blank; TV_s = Titer value of sample

4.4.5 Dietary fibre

The dietary fibre was determined by the method of AOAC 20th Edn.2016,985.29

Procedure:

Weigh duplicate 1g test portion, accurate to 0.1mg into 400ml tall-form beakers. Add 0.1ml tetramethyl solution. Cover the beaker with Al foil and place in boiling water bath for 15mins. Add 5mg protease. Incubate 30 mins at 60°C with continuous agitation and cool down. Add 10ml 0.325M HCl solution. Measure pH and dropwise and add acid if necessary. Add 280ml 95% ethyl alcohol preheated to 60°C. Wash residue successfully with three 20ml, portions of 78%ethyl alcohol and two 10ml portions of acetone. Time for filtration and washing will vary from 0.1h to 6h. Dry crucible containing residue overnight in 70°C vacuum oven. Cool in desiccator and weigh to nearest 0.1mg.

Calculations:

Determination of blank:

B= blank, mg= weight residue- $P_B - A_B$

where,

weight residue = average of residue weights(mg) for duplicate blank determination;
and

$P_B - A_B$ = weights(mg)of protein and ash

Calculate TDF as follows:

$TDF (\%) = [(weight\ residue - P - A - B) / weight\ test\ portion] * 100$

where,

weight residue = average of weights (mg) for duplicate blank determination

P and A = weights(mg) of protein and ash;

weight test portion = average of two test portion weights(mg) takes.

4.4.6 Total sugars

The total sugars were determined by the method in FSSAI Manual (2015).

Procedure:

Weigh accurately about 5 g sample, transfer to a 200 ml volumetric flask dissolve in warm water, dilute to about 150 ml. In case solution is not clear, add 5 ml of Carrez 1 solution followed by 5 ml of Carrez 2 solution. Make up to 200 ml. Filter through a dry filter paper. Titrate the solution obtained as such to determine % Reducing sugars.

Preliminary Titration:

Pipet 5 ml each of Fehling A and B into 250 ml conical flask. Mix and add about 10 ml water and a few boiling chips or glass beads. Dispense solution. Heat the flask to boiling. Add 3 drops of methylene blue indicator. Continue the addition of solution dropwise until the blue colour disappears to a brick-red end point. (The concentration of the sample solution should be such that the titre value is between 15 and 50 ml). Note down the titre value.

Final Titration:

Pipet 5 ml each of Fehling A and B. Add sample solution about 2 ml less than titre value of the preliminary titration. Heat the flask to boiling within 3 minutes and complete the titration. Perform the titration duplicate and take the average.

Calculations:

Reducing sugars (%) = Dilution x Factor of Fehling (in gm) / Weight of sample x
Titre value x100

4.4.7 Sodium and potassium

The sodium and potassium were determined by the method of AOAC 20th
Edn.2016, 969.23

Procedure:

Weigh 4g test portion into the crucible, and char on electric hot plate or over low fame. Place on cold furnace and bring to 525°C and 2h to white ash. Add 15ml dil. HNO₃ (1-4) to the crucible, breaking up ash with a stirring rod if necessary. Filter through acid-washed quantitative paper into a 100ml volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume.

For flame emission- Read directly

For flame absorption- Dilute for direct readout as follows: Place 1ml aliquot in 100ml volumetric flask and dilute to volume with H₂O for Na; place 1ml aliquot in 25ml volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2ml HNO₃ to 100ml with H₂O.

Calculation:

For flame emission photometers:

$$\text{Na or K (100g)} = 100 \times F$$

where,

$$E = (\text{percent T of unknown}) - (\text{percent T of blank})$$

$$E = (\text{percent T of standard lower concentration than test solution}) - (\text{percent T of blank})$$

$E = (\text{percent T of standard of higher concentration than test solution}) - (\text{percent T of blank})$

$C = \text{mg in Na or K/ml in standard of lower concentration than test solution}$

$C = \text{mg in Na or K/ml in standard of higher concentration than test solution}$

$F = \text{dilution factor}$

For flame absorption photometers: Convert percent absorption to absorbance.

$\text{Mg in Na or K /100g} = (\text{concentration unknown} \times F \times 100) / \text{g test portion.}$

4.4.8 Calcium

Calcium was determined by titration method (IS 5949:1990 (RA.2010))

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 per cent sodium hydroxide solution (4.6) using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass $= V_1 \times 0.04008 / M$

Where V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

4.4.9 Iron

Iron was determined by the method of AOAC 20th Edn.2016,999.11

Procedure:

Homogenize the product if necessary, using non-contaminating equipment. In a crucible, weigh 10-20g test portion to nearest 0.1g. Dry in a drying oven or water bath at 100°C. Place dish in the furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C /h to 450°C. Let dish stand for at least 8 hours or overnight. Wet ash with 1-3ml water and evaporate on water bath. Put crucible in furnace at no more than 200°C and raise temperature (50°C -100°C /h) to 450°C. Proceed with ashing at 450°C for 1-2 hours or longer. Repeat the procedure until the product is completely ashed. Add 5ml 6M HCl to crucible ensuring that all ash comes into contact with the acid. Evaporate acid on water bath and dissolve residue in 10-30ml, to the nearest 0.1ml of 0.1M HNO₃. Cover with watch glass and lid for 1-2 hours. Then, it is determined by atomic absorption spectrometry (AAS).

Calculations:

Calculate the detection limit (DL) for Fe:

DL= 3 x standard deviation of the mean of the blank determination (greater than or equal to 20)

Calculate the concentration, c, of the metal in the test sample according to the formula:

$$c = (a-b) \times V / m$$

where,

c = concentration in the test sample (mg/kg)

a = concentration in the test solutions (mg/L)

b = mean concentration in the blank solution (mg/L)

V = Volume of the test solution (mL)

m = weight of the test portion (g)

If (a-b) is lower than the DL, then (a-b) is substituted with DL for calculation of the limit of detection in the test portion.

4.5 Evaluation of macronutrients in *D. quercifolia* porridge mix

4.5.1 Total Ash

The total ash was determined by the method of AOAC 20th Edn.2016,925.51

Procedure:

Weigh accurately about 5g of sample in a pre-weighed silica crucible. Ignite over the burner and complete the ignition by keeping in a muffle furnace at 550±20°C until carbon-free ash. Cool in desiccators and weigh. Ignite the crucible again in the muffle furnace for 30 minutes, cool, and weigh. Repeat the process until the difference between the successive weighing is less than 1mg. Use the ash for determination of minerals content.

Calculation:

The percentage of Total ash(w/w) = $100(M_2 - M) / M_1 - M$

Where,

M₂ = the lowest mass in g of the dish with ash

M₁ = mass in g of the empty dish, M = mass in g of the dish with the material taken for test

4.5.2 Moisture

The moisture was determined by the method of AOAC 20th Edn.2016,984.25

Procedure:

Thoroughly homogenize the test sample in the blender and weigh 10g in duplicate, using a balance accurate to 0.1mg. Use desiccated, tared weighing dishes, preferably made of Ni, Al stainless steel, or glass, 60-80mm diameter and 25mm deep, with well-fitting but easily removable lids. Place uncovered dish containing test portions with lid in convection drying oven at 103± 2°C for 16 hours. Replace the lid and transfer the desiccator to cool. When cool, weigh as quickly as possible to 0.1mg. Uncover and replace the dish and lid in the oven for an additional 2 hours. Replace the lid, cool in a desiccator and reweigh. Repeat 2 hours of drying,

desiccation and weighing steps until a decrease in mass between successive weightings does not exceed 0.5mg or until an increase in mass is recorded.

Calculations:

$$\% \text{ of Moisture(w/w)} = 100(M_1 - M_2) / M_1 - M$$

Where,

M₁ = mass in g of the dish with the material before drying

M₂ = mass in g of the dish with the material after drying, and M = mass in g of empty dish

4.5.3 Energy

Energy is calculated by the FAO method.

It is calculated by the following formula:

$$\text{Energy(kcal/100g)} = (\text{Carbohydrate} \times 4) + (\text{Protein} \times 4) + (\text{Fat} \times 9)$$

4.5.4 Carbohydrates

The total carbohydrate content in the porridge powder was calculated by difference method. This methodology separately assesses the quantities of various other constituents present in the food, including protein, fat, fibre, moisture, and ash. These values are then aggregated and subtracted from the overall weight of the food. This method is represented by the following formula:

$$\text{Carbohydrate (g/100g)} = 100 - [\text{Moisture (\%)} + \text{Ash (\%)} + \text{Total fat(g)} + \text{Protein(g)} + \text{Fibre(g)}]$$

4.5.5 Total fat

The total fat was determined by the method of AOAC 20th Edn.2016,920.39

Procedure:

Weigh accurately about 2g of sample in a fat extraction tube; add 2.0ml of alcohol to prevent lumping on addition of acid, and shake to moisten all particles. Add 10ml

HCl (25+11), mix well and set tube 30 to 40 mins in water bath at 70°C and 80°C, shaking frequently. Cool to room temperature and add alcohol. Add 25ml diethyl ether to the flask and shake for 1.0 mins. Then add 25ml petroleum ether to the flask and shake 1min. Decant ether layer into the pre-weighed dry flask through anhydrous sodium sulphate. Then, Decant ether layer into a pre-weighed dry flask through anhydrous sodium sulphate. Then add 25ml of 1:1 diethyl ether, and petroleum ether to the flask and shake for 1.0min. Ether layer can be evaporated under a water bath. Keep the flask into the oven for further evaporation of ether residue. Remove the flask from the oven and place in desiccators to cool to room temperature. Record the weight of the flask plus fat.

Calculation:

% of Fat = (Weight of flask + fat) – Empty weight of the flask ×100 / Sample weight

4.5.6 Protein

The protein(N×6.25) was determined by the method of AOAC 20th Edn.2016, 986.25

Procedure:

Weigh a suitable test portion of the sample into digestion flask. Add 10g of potassium sulphate or anhydrous sodium sulphate, about 0.4g CuSO₄ and 25mL or more, if necessary, of Conc.H₂SO₄ to the flask. Heat the flask for 90min for complete digestion. Cool, cautiously add 250mL H₂O and cool to room temperature. Add 100ml of 4% boric acid to the beaker, condenser tip will be sufficiently immersed. Add 3-4 drops of mixed indicator. Connect flask to distillation apparatus, add sufficient 45% NaOH solution, mix completely, such that mixture will be strongly alkaline and distil until 150ml distillate is collected in titration beaker. Titrate against 0.1N HCl solution. Correct for blank determination on reagents.

Calculation:

%N (w/w) = (TV_s-TV_b) x Normality of standard HCl ×1.4007 / Sample weight

where,

TV_b = Titer value of blank ; TV_s = Titer value of sample

4.5.7 Dietary fibre

The dietary fibre was determined by the method of AOAC 20th Edn.2016,985.29

Procedure:

Weigh duplicate 1g test portion, accurate to 0.1mg into 400ml tall-form beakers. Add 0.1ml tetramethyl solution. Cover the beaker with Al foil and place in boiling water bath for 15mins. Add 5mg protease. Incubate 30 mins at 60°C with continuous agitation and cool down. Add 10ml 0.325M HCl solution. Measure pH and dropwise and add acid if necessary. Add 280ml 95% ethyl alcohol preheated to 60°C. Wash residue successfully with three 20ml, portions of 78%ethyl alcohol and two 10ml portions of acetone. Time for filtration and washing will vary from 0.1h to 6h. Dry crucible containing residue overnight in 70°C vacuum oven. Cool in desiccator and weigh to nearest 0.1mg.

Calculations:

Determination of blank:

B= blank, mg= weight residue- $P_B - A_B$

Where,

Weight residue = average of residue weights(mg) for duplicate blank determination; and

$P_B - A_B$ = weights(mg)of protein and ash

Calculate TDF as follows:

$TDF (\%) = [(weight\ residue - P - A - B) / weight\ test\ portion] \times 100$

Where,

Weight residue = average of weights (mg) for duplicate blank determination

P and A = weights(mg) of protein and ash

Weight test portion = average of two test portion weights(mg) takes.

4.6 Evaluation of micronutrients in *D.quercifolia* porridge mix

4.6.1 Sodium and potassium

The sodium and potassium were determined by the method of AOAC 20th Edn.2016,969.23

Procedure:

Weigh 4g test portion into the crucible, and char on electric hot plate or over low flame. Place on cold furnace and bring to 525°C and 2h to white ash. Add 15ml dil. HNO₃ (1-4) to the crucible, breaking up ash with a stirring rod if necessary. Filter through acid-washed quantitative paper into a 100ml volumetric flask. Wash residue and paper three times with H₂O. Dilute to volume.

For flame emission- Read directly

For flame absorption- Dilute for direct readout as follows: Place 1ml aliquot in 100ml volumetric flask and dilute to volume with H₂O for Na; place 1ml aliquot in 25ml volumetric flask and dilute to volume with H₂O for K.

Prepare blank solution by diluting 2ml HNO₃ to 100ml with H₂O.

Calculation:**For flame emission photometers:**

Na or K (100g) = 100×F (in mg)

where,

E = (percent T of unknown) - (percent T of blank)

E = (percent T of standard lower concentration than test solution)- (percent T of blank)

E = (percent T of standard of higher concentration than test solution)- (percent T of blank)

C = mg in Na or K/ml in standard of lower concentration than test solution

C = mg in Na or K/ml in standard of higher concentration than test solution

F = dilution factor

For flame absorption photometers: Convert percent absorption to absorbance.

Mg in Na or K /100g = (concentration unknown ×F ×100) /g test portion.

4.6.2 Calcium

Calcium was determined by titration method (IS 5949:1990 (RA.2010))

Procedure:

Take a suitable aliquot of the sample solution prepared as prescribed in the material specification and expected to contain approximately 5 to 20 mg of calcium as CaO, in a 500 ml conical flask, Add 25 ml of triethanolamine solution, 10 ml of hydroxylamine hydrochloride solution and 2 ml of potassium cyanide solution. Dilute to about 150 to 200 ml with water and adjust the pH to 12.5 to 13 with 20 percent sodium hydroxide solution (4.6) using a pH indicator paper or preferably a pH meter. Add approximately 0.1 g of Patton and Reeder's indicator mixture and stir to dissolve. Titrate with 0.01 M EDTA solution from a burette, till the colour changes from red to pure blue free from any violet tinge. Towards the endpoint, the colour turns a violet which on further drop-wise addition gives the pure blue endpoint. It is easier to note the endpoint by comparing the colour with that of a previously titrated solution having a pure blue endpoint, where a slight excess of EDTA has been added.

Calculation:

Calcium (Ca) per cent by mass = $V_1 \times 0.04008/M$

where,

V_1 = Volume in ml of EDTX solution in titration; and

M = Mass in g of the sample in the solution for the test

4.6.3 Iron and Zinc

Iron and zinc were determined by the method of AOAC 20th Edn.2016,999.11

Procedure:

Homogenize the product if necessary, using non-contaminating equipment. In a crucible, weigh 10-20g test portion to nearest 0.1g. Dry in a drying oven or water bath at 100°C. Place dish in the furnace at initial temperature not higher than 100°C. Increase temperature at a maximum rate of 50°C /h to 450°C. Let dish stand for at least 8 hours or overnight. Wet ash with 1-3ml water and evaporate on water bath. Put crucible in furnace at no more than 200°C and raise temperature (50°C -100°C /h) to 450°C. Proceed with ashing at 450°C for 1-2 hours or longer. Repeat the procedure until the product is completely ashed. Add 5ml 6M HCl to crucible

ensuring that all ash comes into contact with the acid. Evaporate acid on water bath and dissolve residue in 10-30ml, to the nearest 0.1ml of 0.1M HNO₃. Cover with watch glass and lid for 1-2 hours. Then, it is determined by atomic absorption spectrometry (AAS).

Calculations:

Calculate the detection limit for Zn and Fe:

DL= 3 x standard deviation of the mean of the blank determination (greater than or equal to 20)

Calculate the concentration, c, of the metal in the test sample according to the formula:

$$c = (a-b) \times V / m$$

where, c = concentration in the test sample (mg/kg)

a = concentration in the test solutions (mg/L)

b = mean concentration in the blank solution (mg/L)

V = Volume of the test solution (mL)

m = weight of the test portion (g)

If (a-b) is lower than the DL, then (a-b) is substituted with DL for calculation of the limit of detection in the test portion.

4.7 Analysis of sensory quality of *D.quercifolia* porridge mix

The cooked *D.quercifolia* porridge was served hot and evaluated for sensory attributes (Appearance, consistency, aroma, mouthfeel, flavour and overall acceptability) through a panel of 10 subjects using a 9-point hedonic scale of scores from 1-dislike extremely to 9-like extremely.

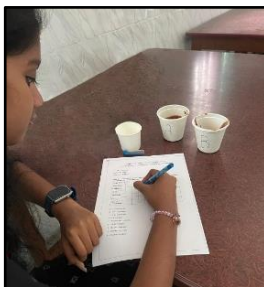


Fig 4.3: Sensory evaluation of the porridge

4.8 Determination of shelf life of *D.quercifolia* porridge mix

The chemical(moisture, acidity) and microbiological testing(Total pate count, Coliforms, Yeast and mould) was performed at the 12th, 24th, and 36th day of storage to determine the shelf life of *D.quercifolia* porridge mix.

CHAPTER 5

RESULTS AND DISCUSSION

5.1 Nutritional analysis of *Dynaria quercifolia*

Table 5.1: Nutritive content in *D.quercifolia* per 100g

S.No.	Nutrients	Results
1	Energy	357kcal
2	Carbohydrates	75.2g
3	Protein	7.01g
4	Total fat	3.13g
5	Dietary fibre	11.4g
6	Total sugars	13.1g
7	Sodium	246mg
8	Calcium	297mg
9	Iron	6.03mg

The data in Table 5.1 shows the nutritional analysis of *D.quercifolia* for different parameters. The nutritional analysis of the *D.quercifolia* porridge revealed that it comprises of 357g/100g of energy, 75.2g/100g of carbohydrates, 4.37g/100g of total fat, 7.01g/100g of protein, 11.4g/100g of dietary fibre, 13.1g of total sugars, 246mg of sodium, 297mg of calcium and iron of 6.03mg respectively.

The average daily requirement of a sedentary adult is 1885kcal, hence incorporating *D.quercifolia* in the diet gives 357kcal daily. The rhizome naturally has a low sugar content. There is a generous amount of calcium(297mg) which aids in reducing inflammation and also helps in the prevention of arthritis.

5.2 Evaluation of macronutrients in *D.quercifolia* porridge mix

Table 5.2: Macronutrients content in *D.quercifolia* porridge mix/100g

S.No.	Nutrients	Results
1	Moisture	4.59%
2	Total ash	2.83%
3	Energy	407kcal
4	Carbohydrates	77.4g
5	Protein	14.4g
6	Total fat	4.37g
7	Dietary fibre	13.1g

The macronutrients of *D.quercifolia* porridge mix is presented in Table 5.2 for different parameters. The macronutrient content in *D.quercifolia* porridge mix comprises of 4.59% of moisture, total ash content of 2.83%, energy of 407kcal, carbohydrates of 77.4g/100g, 77.4g/100g of carbohydrates, 14.4g of protein, 4.37g/100g of total fat, 13.1g/100g of dietary fibre.

The total ash content in the product is essential to analyse the amount of minerals present in it. The moisture content is a critical factor that directly impacts the physical and chemical properties of food. It is also useful for assessing the freshness, quality, and shelf life of food products. The moisture content in the porridge is 4.59%.

5.3 Evaluation of micronutrients in *D.quercifolia* porridge mix

Table 5.3: Micronutrients content in *D.quercifolia* porridge mix/100g

S.No.	Nutrients	Results
1	Sodium	16.4mg
2	Calcium	209mg
3	Iron	5.41mg
4	Potassium	757mg
5	Zinc	27.3mg

From the above table 5.3 the micronutrient content in the *D.quercifolia* porridge comprises of 16.4mg/100g of sodium, 209mg/100g of calcium, 5.41mg of iron, 757mg of potassium and 27.3mg/100g of zinc.

5.4 Analysis of sensory quality of *D.quercifolia* porridge mix

Table 5.4: Sensory evaluation of *D.quercifolia* porridge mix

Attributes	Quantity of DQ	Medium	Average scores
Appearance	25g	M	8.3
		W	7.2
	50g	M	7.8
		W	7.5
Consistency	25g	M	8.8
		W	8.3
	50g	M	8.1
		W	7.1
Aroma	25g	M	7.6
		W	7.2
	50g	M	6.2
		W	7.6
Mouthfeel	25g	M	8.1
		W	7.9
	50g	M	6.8
		W	7.5
Flavour	25g	M	8.8
		W	7.7
	50g	M	8.3
		W	7.4
Overall acceptability	25g	M	8.1
		W	7.6
	50g	M	7.2
		W	7.5

The table 5.4 shows the sensory evaluation of the porridge. The incorporation of 25g of *D.quercifolia* into porridge prepared with milk has been observed to yield superior taste profiles, as indicated by the average scores of overall acceptability. In assessing the sensory attributes of the porridge, the mean scores for appearance, consistency, aroma, mouthfeel, flavour, and overall acceptability were 8.3, 8.8, 7.6, 8.1, 8.8, and 8.1, respectively.

5.5 Determination of shelf life of *D.quercifolia* porridge mix

Table 5.5 : Determination of shelf life of *D.quercifolia* porridge mix (Chemical test)

S.No		1	2	3
Testing day from manufacturing		Day 12	Day 24	Day 36
Date of analysis		05.03.24	05.03.24	05.03.24
End date		11.03.24	11.03.24	11.03.24
Storage temperature		40°C	40°C	40°C
Storage		75%	75%	75%
Chemical test	Moisture (g/100g)	9.11	9.15	9.33
	Acidity (g/100g)	1.07	1.09	1.10

From the above table 5.5, the analysis of moisture and acidity in *D.quercifolia* porridge mix revealed an increase in both factors over consecutive days.

**Table 5.6: Determination of shelf life of *D.quercifolia* porridge mix
(Microbiological test)**

S.No		1	2	3
Testing day from manufacturing		Day 12	Day 24	Day 36
Date of analysis		05.03.24	05.03.24	05.03.24
End date		11.03.24	11.03.24	11.03.24
Storage temperature		40°C	40°C	40°C
Storage		75%	75%	75%
Microbiological test	Total plate count (CFU/g)	3,60,000	3,60,000	3,60,000
	Coliforms (CFU/g)	1,60,000	1,60,000	1,60,000
	Yeast and Mould (CFU/g)	1,20,000	1,20,000	1,20,000

The table 5.6 shows that there were no significant changes in the chemical and microbiological properties of *D.quercifolia* porridge mix upto 50 days of storage at 40°C ,75% storage humidity.

CHAPTER 6

SUMMARY AND CONCLUSION

Autoimmune diseases result from the immune system attacking the body's own cells and tissues. The autoimmune diseases exhibit clinical diversity. They can either manifest with organ-specific patterns or present in a generalized manner, affecting various systems throughout the body.

Autoimmune diseases like rheumatoid arthritis involve the immune system and target various components of the joints, leading to inflammation, tissue damage, and pain. The global prevalence of the disease is estimated at around 1–2%, with a large variation among different populations. In developed countries, osteoarthritis is recognized as one of the top 10 prevalent disabilities among older individuals, particularly those who continue to participate in the workforce. The objectives of rheumatoid arthritis treatment include minimizing joint inflammation and pain, optimizing joint functionality, and averting joint destruction and deformities.

Considerable focus has been placed on the role of diet and nutrients as potential environmental factors that may impact the development and progression of the disease. While several studies have suggested links between dietary habits, particularly regarding fruit, vegetable, or meat intake, and the development of the disease, the results remain inconclusive.

Hence, we have used an ingredient called *D.quercifolia* and formulated a porridge mix which not only helps in managing but also prevent arthritis.

The formulated porridge mix was evaluated for its nutritional quality, macronutrients and micronutrients, sensory analysis and shelf-life testing. The average daily requirement of a sedentary adult is 1885kcal, hence incorporating *D.quercifolia* in the diet gives 357kcal daily. The protein content in the porridge is notably low 7.01g/100g, hence it is safe to consume.

D.quercifolia has significant nutritional benefits. This porridge is suitable not only for arthritis patients but also for healthy individuals due to its various health benefits. Consuming a glass of porridge four times per week is recommended to assess the efficacy of the improvement in recovery. Taking all these factors into account, its

nutritional richness adds variety to patients diets and makes it a nutritious breakfast option for people of all ages, including those who are healthy.

Further it can be recommended that besides soup and porridge, other recipes such as biscuits, energetic bars can be formulated. The *D.quercifolia* porridge mix can be given to arthritis patients to determine the efficiency of the porridge in arthritis management and conducting a comparative study on Mudavattukal kilangu bought from various regions.

CHAPTER 7

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