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(REVIEW ARTICLE)

Bioactive flavonoid components in ethanolic extracts of *Marsilea Minuta*: An updated review of isolation techniques, structural elucidation and pharmacological properties

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Abstract

Marsilea Minuta (dwarf water clover) is a freshwater fern primarily from tropical and subtropical regions. Over the centuries it has remained a cornerstone of conventional systems of medicine for its wide range of healing properties. In recent times, scientific developments have brought forth the discovery of bioactive flavonoids in its ethanolic extracts which are the backbone of its pharmacological actions. This review article explains in detail the isolation techniques, structural determination methods and pharmacological actions of these flavonoids that may serve as promising agents for contemporary therapeutic uses. *Marsilea Minuta* contains flavonoids and other phenolic compound such as gallic acid, caffeic acid, rutin, quercetin and ferulic acid which have been affirmated through sophisticated HPLC-PFP procedures. The compounds have been shown to have antioxidant, antimicrobial and anti-inflammatory activities that make them interesting for treatments like anti-cancer, anti-coronary heart disease, anti-diabetes treatments. More recently, M. minuta has been proved to contain sedative, anticonvulsant and antidepressant-like Activities being associated with the presence of marsiline. From serving as antioxidants to fighting certain diseases, we can image the potential use of these flavonoids in a modern medication. Nevertheless, there remain difficulties in their isolation and identification, with further work needed to unlock their therapeutic potential.

Keywords: Flavonoids; Pharmacological Activities; *Marsilea Minuta*; Antioxidant Properties; Traditional Medicine

1. Introduction

1.1. Overview of *Marsilea Minuta* [1-3]

Marsilea Minuta, a very small aquatic fern that represents the family Marsileaceae, is noted for its morphology and ecological adaptability. Slender rhizomes support the floating plant whose clover-like four-angled leaves seem to appear on clean water surface or even emerge into damp terrestrial areas sitting in muddy sediment. Its sporocarps, water-resistant capsules that incorporate all reproductive structures as the plant alive thus underscores its tough adult survival in ephemeral aquatic habitats. A tropical and subtropical aquatic fern *Marsilea Minuta* grows preferentially in shallow stagnant or slow-flowing water, including marshes, rice fields, swamps etc. It occurs in South and Southeast Asia, some parts of Africa as well as Northern Australia, often forming dense mats that offer microhabitats for animals associated with freshwater and reduces soil erosion with its extensive underground root system. The small herb *Marsilea Minuta* is one of the Ayurvedic celebrated therapeutic genus back to ancient time in traditional medicinal practices. Prepared in decoction, paste and poultice leaves along with rhizome are used in the treatment of fever, rheumatic diseases; and skin infections including urinary tract infections. These include uses for its antipyretic properties to manage fever and analgesic mode of action in joint inflammation. In this region of Southeast Asia, the fern is part of topical ointments for treating wounds and eczema via its hypothesized antimicrobial and anti-inflammatory

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properties. This pattern is actually part of cultural pharmacopeias, a mix of empiric knowledge and the application of ecological wisdom over generations. Analysis of the botanical pharmacology of *Marsilea Minuta* has revealed not only a wealth of phytochemicals in keeping with its ethnomedicinal profile. The plant hosts flavonoids like quercetin and kaempferol – powerful antioxidants with amazing free-radical scavenging capabilities that help to prevent oxidative stress—underlying cause of many degenerative diseases. It also comprises polyphenols, alkaloids, saponins and steroidal compounds which together synergize for its bioactivity Improved. Flavonoids, in this regard have dual anti-inflammatory and anti-proliferative properties as they block pro-inflammatory cytokines such as TNF-α and interleukins and induce apoptosis in malignant cells. In presence of animal models, studies on mice have confirmed the efficiency of ferns in reducing both edema and hyperalgesia, especially the latter is comparable with conventional non-selective NSAIDs-like aspirin but much less associated to its negative gastrointestinal impact. Moreover, in vitro cell biology assays are also indicative of an anti-tumour role in which monolayer adherent human breast adenocarcinoma cells were exposed to DHP. Does an increased roles on neuroprotective and hepatoprotective sector flesh this out. An ethanolic extract of *Marsilea Minuta* demonstrated phromaptic activity against ethanol-induced hepatic damage in rats by promoting the antioxidant enzyme activity in hepatic tissue and if the bioactive fractions can mitigate neurodegenerative processes through inhibitory effects on acetylcholinesterase activity with in vitro positively identified suppression action against single dose experiments. This is in line with global uptake of plant-derived molecules for drug development (at least for some complex diseases like diabetes and Alzheimer's) and the importance to find new targets in these projects. However, it seems a long way in trials with humans and some speculations are needed about clinical utilization arising from pre-clinical evidence. Overharvest of *Marsilea Minuta* from wild habitats has already been exploited for its sustainable cultivation and agricultural expansion is destroying the last habitat fragments focused on MED supply as well. Controlled aquaculture and tissue culture along with the assistance for preservation of genetic diversity are greatly needed efforts to make supplies available both ecologically but also for pharmacological reasons. Biotechnological advancement confronted with traditional knowledge---the new techniques from nutraceuticals to a potential phytoremediator could utilize with some of the other purpose driven efforts. *Marsilea Minuta* being, one of the few genera being researched ethnobotanically is a prime example of the unexplored aquatic plant kingdom that could create a bridge between traditional and modern therapeutics as ethobiomedicines. Subsequently, direction line is from metabolomic profiling to lead compounds and community-based conservation towards securing this versatile fern for the coming generations.

Figure 1 Dwarf Water Clover (*Marsilea Minuta*)

1.2. Importance of Flavonoids in Medicinal Plants [4, 5]

Flavonoids is a large and diverse group of polyphenolic compounds found in a wide range of plant species due ubiquitously. These plant hormones are critical for many physiological processes taking place in plants (growth, pigmentation, UV-b screenings, pathogen defence and other stress responses). Alongside important roles in plant biology and basic biochemistry, flavonoids have been a focus of human health and medicine as result of the array of therapeutic properties attributed to its members. Broad, systematic scientific studies have revealed their strong antioxidant and anti-inflammatory anti-microbial, as well as anticancer activities which make them core in several natural/synthetic therapeutic agents. Flavonoid antioxidant capacity is particularly striking, free oxygen radicals and reactive oxygen species (ROS) which flavonoids neutralize leading to reduced oxidative stress, an established pathogenesis for many chronic diseases such as cardiovascular disorders, neurodegenerative illnesses and cancer. Furthermore, their anti-inflammatory effects are brought about by means of the regulation on molecular pathways such as the inhibition of pro-inflammatory cytokines and enzymes (e.g., cyclooxygenase COX, lipoxygenase LOX) involved in

inflammatory responses. Flavonoids show a huge assortment in antimicrobial capacity, such that they are active against broad range of bacterial, viral and fungal pathogens. As there is increasing concern with respect to the emergence of antibiotic resistance, this property makes flavonoids a promising alternative/adjunct to the current antimicrobial therapeutics. Moreover, anticancer properties are associated with inducing apoptosis, inhibiting tumor cell proliferation and blocking angiogenesis metastasis by targeting vessels formation in several types of malignancies. Interventions are executed via flavonoids interactions with different cellular signalling pathways such as the PI3K/AKT, MAPK and NF-kB pathways that are widely implicated in cancer. Flavonoids present in a number of plants, *Marsilea Minuta* is an outstanding plant containing flavonoids especially for semi-aquatic fern. The plant in question is used traditional to a number of medicinal systems and recent research has confirmed its flavonoid composition which gives rise to claim of its potential therapeutical effects. The flavonoids in *Marsilea Minuta* however supply antioxidant and anti-inflammatory effects and it can be one of the lead compounds for developing new phytopharmaceutical. Flavonoids in medicinal plants have a special importance and the transition from herbal to drug is met due to these medicinal properties by its bioactive compounds. Functional properties– ranging from antioxidant, anti-inflammatory and immune-modulating effects to triglyceride-lowering effects and effects on some immune-mediated disease– were related to their structural diversity and biological versatility which in turn determined their ability to interact with a wide variety of molecular targets to provide a wide range of health benefits. In addition, the increasing attention towards herbal medicine and natural products has motivated scientists to continue research on flavonoids to maximise its functions in order to benefit human health. With ongoing research revealing the complex mechanisms governing flavonoid action, the potential synergistic effects of flavonoids with other phytochemicals, and new utilization agents, their significance in both preventive and therapeutic medicine will continue to increase over the coming years. As a result, the vital role of flavonoids as flagship phytoconstituents in medicinal plants was rendered in this paper with *Marsilea Minuta* as shining example. The multi-dimensional biological nature of these compounds emphasizes their importance in plant metabolism and human health, thereby laying the foundation for pursuit of nature inspired and sustainable medicines.

Table 1 Biological Functions and Therapeutic Properties of Flavonoids in Plant Species and Human Health

Property/Function	**Details**	**Examples**
Plant Functions		
Growth and Pigmentation	Flavonoids regulate plant growth and contribute to pigmentation in flowers and fruits.	Found in many plant species.
UV Screening	Protect plants from harmful UV radiation by absorbing UV light.	Critical for survival in sunlight exposure.
Pathogen Defence and Stress Responses	Enhance plant defense mechanisms against pathogens and environmental stress.	Involved in pathogen defense.
Human Health Functions		
Antioxidant Activity	Neutralize free radicals and reactive oxygen species (ROS), reducing oxidative stress.	Prevention of cardiovascular disorders, cancer.
Anti-inflammatory Activity	Regulate inflammatory pathways, including the inhibition of COX and LOX enzymes and pro-inflammatory cytokines.	Treatment of chronic inflammatory diseases.
Antimicrobial Activity	Effective against bacterial, viral, and fungal pathogens.	Promising alternative to antibiotics.
Anticancer Activity	Induce apoptosis, inhibit tumor cell proliferation, and block angiogenesis/metastasis by targeting molecular pathways.	Targeting cancer via PI3K/AKT, MAPK, NF-kB.
Flavonoids in *Marsilea Minuta*		
Medicinal Use	*Marsilea Minuta* is traditionally used in various medicinal systems for its flavonoid composition and therapeutic effects.	Recent research supports its medicinal potential.

Phytopharmaceutical Potential	Provides antioxidant and anti-inflammatory effects, with potential as a lead compound for new drug development.	Can be used to develop new phytomedicines.
Transition from Herbal to Drug	Flavonoids in medicinal plants, such as *Marsilea Minuta*, support the development of drugs from herbal sources.	Flavonoids in *Marsilea Minuta*.
Future Research	Continued research focuses on understanding flavonoid mechanisms and potential synergistic effects with other phytochemicals.	Expected to play a role in preventive and therapeutic medicine.

2. Isolation Techniques for Flavonoids from Marsilea

2.1. Extraction Methods minuta [6, 7]

The extraction of flavonoids from *Marsilea Minuta* requires careful optimization to ensure high yield and purity. The process begins with sample preparation, including collection, washing, drying, and fine grinding to increase surface area for efficient solvent interaction. Solvent selection is critical, as polarity influences flavonoid solubility and stability. Common solvents include ethanol, methanol, and aqueous mixtures. Extraction techniques such as maceration, Soxhlet extraction, and ultrasonic-assisted extraction (UAE) are employed, each with distinct advantages and limitations. Key parameters—temperature, extraction time, and solvent-to-sample ratio—must be optimized to maximize flavonoid recovery while preserving their structural integrity and bioactivity.

2.1.1. Solvent Extraction

Solvent extraction is a widely used method for flavonoid isolation. Ethanol is a preferred solvent due to its ability to dissolve polyphenolic compounds while minimizing protein and polysaccharide co-extraction. Common ethanol-based techniques include:

- Maceration: Plant material is soaked in ethanol at room temperature for extended periods (hours to weeks). The solvent penetrates cells via diffusion, dissolving flavonoids, which are then separated by filtration and concentrated via evaporation. While simple and cost-effective, maceration is time-consuming and influenced by particle size, solvent ratio, and duration.
- Soxhlet Extraction: This method uses continuous solvent reflux for exhaustive extraction. Ground plant material is placed in a thimble, and heated ethanol cycles through the sample, dissolving flavonoids. Though efficient, prolonged heating may degrade thermolabile compounds, and high solvent consumption is a drawback.
- Ultrasonic-Assisted Extraction (UAE): UAE employs ultrasonic waves (20–100 kHz) to induce cavitation, disrupting cell walls and enhancing flavonoid release. Benefits include shorter extraction times, reduced solvent use, and lower temperatures, preserving heat-sensitive compounds. UAE is scalable and aligns with green chemistry principles.

2.1.2. Supercritical Fluid Extraction (SFE)

SFE uses supercritical carbon dioxide ($scCO_2$) as a solvent, which exhibits liquid-like solubility and gas-like diffusivity. Operating above CO_2's critical point (31.1°C, 7.39 MPa), SFE is ideal for thermolabile flavonoids. Advantages include:

- Selectivity: Adjustable pressure and temperature enhance compound specificity.
- Eco-friendliness: CO_2 is non-toxic and recyclable.
- Low-temperature operation: Preserves flavonoid stability.

Limitations include high equipment costs, technical expertise requirements, and reduced efficiency for polar flavonoids unless modified with co-solvents like ethanol.

2.1.3. Microwave-Assisted Extraction (MAE)

MAE utilizes microwave energy to heat solvents and plant matrices rapidly, improving extraction efficiency. Polar solvents (e.g., ethanol, water) absorb microwaves, generating heat that disrupts cell walls and releases flavonoids. Advantages include:

- Speed: Extractions complete in minutes versus hours.
- Reduced solvent use: Aligns with sustainable practices.
- Controlled conditions: Prevents thermal degradation.

MAE is versatile, applicable to various phytochemicals, and increasingly adopted in industrial and research settings. Flavonoid extraction from *Marsilea Minuta* depends on method selection and optimization. Traditional techniques (maceration, Soxhlet) remain relevant, while advanced methods (UAE, SFE, MAE) offer efficiency, sustainability, and improved compound stability. The choice of method should balance yield, cost, and environmental impact.

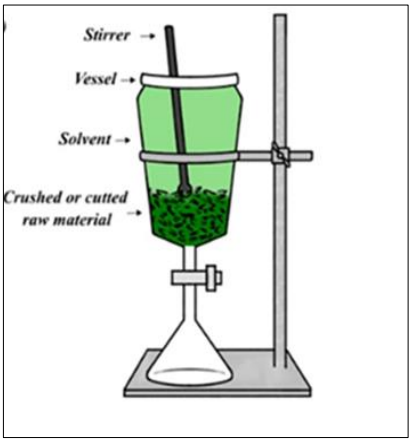

Figure 2 Methods of Maceration Extraction

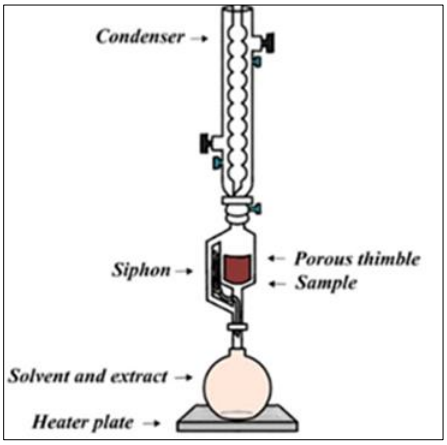

Figure 3 Methods of Soxhlet Extraction

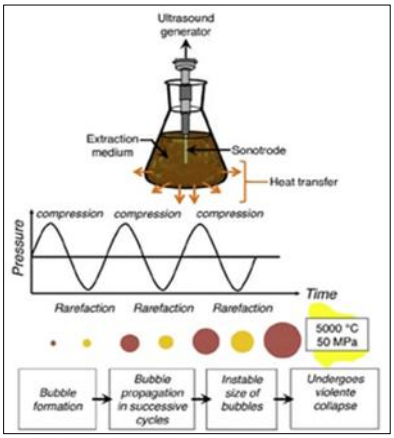

Figure 4 Methods of Ultrasonic Assisted Extraction

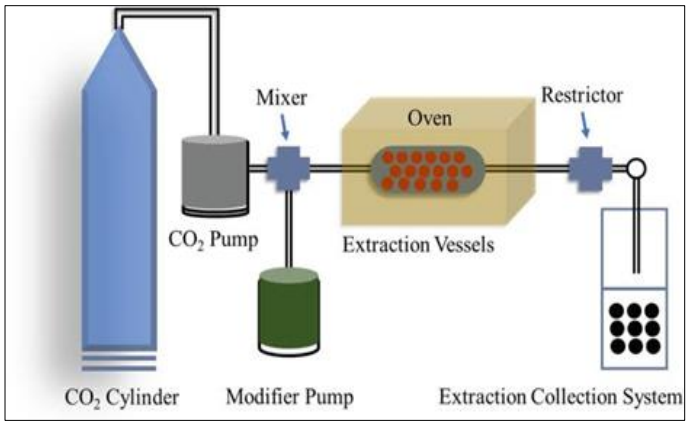

Figure 5 Methods of Supercritical Fluid Extraction

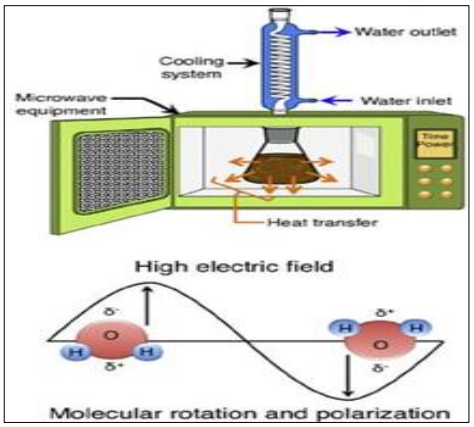

Figure 6 Methods of Microwave-Assisted Extraction (MAE)

Table 2 Isolation Techniques for Flavonoids from *Marsilea Minuta*

Extraction Method	Principle & Mechanism	Solvent Used	Advantages	Limitations
Maceration	Passive diffusion of solvent into plant matrix over an extended period, allowing solubilization of flavonoids.	Ethanol, Methanol, Aqueous blends	Simple, cost-effective, suitable for heat-sensitive compounds.	Long extraction time, risk of microbial contamination, low efficiency.
Soxhlet Extraction	Continuous solvent reflux and percolation through plant material for exhaustive extraction.	Ethanol, Methanol	Efficient for large-scale extractions, continuous process, ensures high yield.	High temperature may degrade thermolabile compounds, solvent-intensive.
Ultrasonic-Assisted	High-frequency ultrasonic waves generate cavitation bubbles, disrupting plant	Ethanol, Methanol, Water	Rapid, energy-efficient, enhances flavonoid yield,	Requires optimization of frequency and power, risk of compound

Extraction (UAE)	cells and enhancing solute diffusion.		preserves bioactivity.	degradation at high intensities.
Supercritical Fluid Extraction (SFE)	Utilizes supercritical CO_2 as a solvent to extract flavonoids under high pressure and temperature.	Supercritical CO_2 (with ethanol as a co-solvent)	High selectivity, eco-friendly, preserves thermolabile compounds.	High equipment cost, complex operation, limited solubility for highly polar compounds.
Microwave-Assisted Extraction (MAE)	Electromagnetic microwaves rapidly heat the solvent and plant matrix, improving mass transfer.	Ethanol, Methanol, Water	Fast, solvent-efficient, high extraction yield, reduced thermal degradation.	Requires specialized equipment, potential overheating risk, uneven heating distribution.

2.2. Purification Techniques for Flavonoid Isolation [8, 9]:

Following extraction, crude flavonoid extracts require purification to isolate individual flavonoid compounds. Several chromatographic techniques are employed for this purpose, including column chromatography, thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). These methods facilitate the separation, identification, and quantification of flavonoids based on their physicochemical properties.

2.2.1. Column Chromatography

Column chromatography is a widely used technique for the separation, isolation, and purification of polyphenolic compounds, including flavonoids. The method relies on differential affinities of flavonoids toward the stationary and mobile phases. Initially, a crude flavonoid extract is obtained through solvent-based extraction methods such as maceration, Soxhlet extraction, or ultrasonic-assisted extraction. The extract is then loaded onto a column packed with a stationary phase, commonly silica gel due to its high surface area and ability to interact with flavonoids via hydrogen bonding and van der Waals forces. Alternative stationary phases, such as alumina, cellulose, or polyamide, may be used depending on flavonoid properties.

Separation is achieved using an optimized solvent system, typically consisting of ethyl acetate, methanol, ethanol, acetone, or mixtures with water. Gradient elution, involving a gradual increase in solvent polarity, enhances the resolution of flavonoid separation. As flavonoids migrate through the column, they separate into distinct bands, which are collected as fractions using a fraction collector. These fractions are subsequently analyzed by TLC, HPLC, UV-Vis spectroscopy, or mass spectrometry (MS) for identification and purity assessment. Column chromatography is a scalable and cost-effective method suitable for both laboratory and industrial applications, making it fundamental in natural product chemistry.

2.2.2. Thin-Layer Chromatography (TLC)

Thin-layer chromatography (TLC) is a simple, rapid, and economical analytical method for flavonoid separation and preliminary identification. A crude extract is spotted onto a TLC plate coated with an adsorbent material such as silica gel, alumina, or cellulose. The plate is then placed in a developing chamber containing an appropriate mobile phase (e.g., organic solvents like chloroform-methanol mixtures). Flavonoids migrate along the plate based on their polarity, molecular size, and interaction with the stationary phase.

Following development, the plate is dried and visualized under UV light, as many flavonoids exhibit natural fluorescence. Alternatively, staining reagents such as vanillin-sulfuric acid or aluminum chloride may be used to enhance detection through color reactions. TLC provides qualitative information on flavonoid composition, including the number of components and their relative polarities. While TLC lacks the resolution and quantitative precision of HPLC, it serves as a valuable preliminary screening tool before more advanced analyses.

2.2.3. High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is a highly efficient and sensitive method for flavonoid separation, identification, and quantification. A crude extract is injected into an HPLC system equipped with a UV, diode array detector (DAD), or mass spectrometer. Separation occurs via interactions between flavonoids and a silica-based stationary phase modified with specific functional groups. A high-pressure mobile phase (e.g., aqueous-organic solvent mixtures) facilitates differential retention and elution of flavonoids.

Detection is typically performed at wavelengths between 200–400 nm, where flavonoids exhibit strong UV absorption due to their conjugated aromatic structures. DAD provides spectral data for structural elucidation, while comparison with known standards allows quantification. HPLC offers high resolution, reproducibility, and the ability to analyze complex mixtures. When coupled with mass spectrometry (HPLC-MS), it enables precise structural identification, making it indispensable for flavonoid research, quality control, and pharmacokinetic studies.

2.3. Challenges in Flavonoid Isolation

The isolation of flavonoids from plant matrices presents several challenges, including the complexity of the plant extract, structural similarities among flavonoids, and their susceptibility to degradation. Co-extracted compounds such as alkaloids, terpenoids, and phenolic acids can interfere with flavonoid separation. Additionally, structurally analogous flavonoids (e.g., isoflavonoids and anthocyanins) may co-elute, complicating identification.

Flavonoids are also prone to degradation under light, heat, oxygen, or extreme pH conditions. To mitigate these issues, extraction and purification parameters must be optimized, and protective measures (e.g., inert atmospheres or antioxidants) should be employed. Advanced techniques such as UPLC-MS and NMR spectroscopy enhance identification accuracy but require specialized expertise. Furthermore, biological variability in plant material (due to environmental factors) and scalability challenges must be considered for industrial applications.

3. Structural Elucidation of Flavonoids in *Marsilea Minuta* [10-15]

3.1. General Structure of Flavonoids

Flavonoids are a diverse and extensive group of naturally occurring polyphenolic compounds, characterized by a distinctive C6-C3-C6 carbon skeleton. This fundamental structure comprises two aromatic rings, designated as ring A and ring B, which are interconnected by a three-carbon bridge, forming a heterocyclic ring known as ring C. The aromatic ring A is typically derived from the shikimate pathway, while ring B originates from the acetate-malonate pathway, highlighting the biosynthetic complexity of these compounds. The structural versatility of flavonoids arises from the extensive modifications that can occur within this basic framework. These modifications include hydroxylation, methylation, glycosylation, and other substitution patterns, which significantly influence the chemical properties, biological activities, and functional roles of these compounds in both plants and humans. Hydroxylation, for instance, introduces hydroxyl groups at various positions on the aromatic rings, enhancing the compound's solubility and reactivity. Methylation, on the other hand, involves the addition of methyl groups, which can alter the compound's stability and interaction with biological targets. Glycosylation, a process where sugar moieties are attached to the flavonoid backbone, further diversifies the structure and affects the compound's bioavailability and metabolic fate. These structural variations give rise to a vast array of flavonoid subclasses, including flavones, flavonols, flavanones, isoflavones, anthocyanins, and chalcones, each with unique chemical and biological properties. Flavonoids are ubiquitously distributed in the plant kingdom, where they play crucial roles in plant physiology, such as pigmentation, UV protection, and defense against pathogens and herbivores. In addition to their ecological functions, flavonoids have garnered significant attention for their potential health benefits in humans. Epidemiological studies have consistently linked flavonoid-rich diets with reduced risks of chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and certain types of cancer. The mechanisms underlying these protective effects are multifaceted and include antioxidant activity, modulation of enzyme function, regulation of gene expression, and interaction with cellular signaling pathways. The antioxidant capacity of flavonoids, in particular, is attributed to their ability to scavenge free radicals and chelate metal ions, thereby mitigating oxidative stress, a key contributor to the pathogenesis of numerous diseases. Furthermore, flavonoids exhibit anti-inflammatory, anti-microbial, and anti-carcinogenic properties, making them promising candidates for therapeutic applications. Despite their potential, the bioavailability of flavonoids can be limited due to factors such as poor absorption, rapid metabolism, and excretion. Advances in nutraceutical and pharmaceutical research are focused on overcoming these challenges through the development of novel delivery systems and structural analogs with enhanced stability and efficacy. In summary, the structural diversity of flavonoids, driven by modifications such as hydroxylation, methylation, and glycosylation, underpins their wide-ranging biological activities and health benefits. Continued research into the biosynthesis, metabolism, and functional mechanisms of flavonoids holds promise for the development of new strategies to harness their therapeutic potential and improve human health.

3.2. Major Flavonoids Identified in *Marsilea Minuta*

Several flavonoids have been identified in the ethanolic extracts of *Marsilea Minuta*, including flavonols, flavones, and flavanones. The major flavonoids reported in *Marsilea Minuta* include quercetin, kaempferol, apigenin and luteolin.

3.2.1. Quercetin

Quercetin is a naturally occurring flavonol, characterized by the presence of a hydroxyl group at the 3-position of the C ring, which plays a crucial role in its biochemical activity. As one of the most widely distributed flavonoids in the plant kingdom, it is particularly abundant in species such as *Marsilea Minuta*, an aquatic fern known for its rich phytochemical profile. Quercetin is renowned for its exceptional antioxidant capabilities, which enable it to neutralize free radicals and reduce oxidative stress, a key factor in the pathogenesis of numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, and cancer. Its antioxidant activity is primarily attributed to its ability to donate electrons and chelate metal ions, thereby preventing the formation of reactive oxygen species (ROS) and protecting cellular components such as lipids, proteins, and DNA from oxidative damage. In addition to its antioxidant properties, quercetin exhibits significant anti-inflammatory effects, which are mediated through the modulation of various signaling pathways. It inhibits the production of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukins, and suppresses the activation of nuclear factor-kappa B (NF-κB), a transcription factor that regulates inflammation. These mechanisms make quercetin a promising therapeutic agent for managing inflammatory conditions, including arthritis, asthma, and inflammatory bowel disease. Furthermore, quercetin has been shown to enhance the body's immune response by promoting the activity of immune cells and reducing the release of histamine, which contributes to its anti-allergic properties. Its multifaceted biological activities, combined with its widespread availability in dietary sources such as apples, onions, and berries, underscore its potential as a valuable nutraceutical for promoting human health and preventing disease.

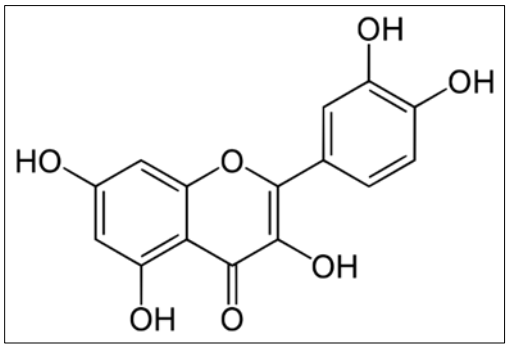

Figure 7 Structure of Quercetin

3.2.2. Kaempferol

Kaempferol is a prominent flavonol compound identified in *Marsilea Minuta*, a plant known for its medicinal properties. Structurally, kaempferol closely resembles quercetin, another well-studied flavonol, but differs in the absence of a hydroxyl group at the 3-position of its chemical structure. This subtle structural variation influences its biological activity and interaction with cellular targets. Kaempferol has garnered significant attention in scientific research due to its diverse pharmacological properties, including potent antioxidant, anticancer, and cardioprotective effects. As an antioxidant, kaempferol neutralizes free radicals and reduces oxidative stress by scavenging reactive oxygen species (ROS) and enhancing the activity of endogenous antioxidant enzymes. This mechanism not only protects cells from oxidative damage but also contributes to its anti-inflammatory properties, making it a potential therapeutic agent for chronic inflammatory conditions. In the context of cancer, kaempferol has demonstrated promising chemopreventive and chemotherapeutic potential. It modulates key signaling pathways involved in cell proliferation, apoptosis, and angiogenesis, thereby inhibiting the growth and metastasis of various cancer types. Additionally, kaempferol induces cell cycle arrest and promotes programmed cell death in malignant cells while sparing normal cells, highlighting its selective cytotoxicity. Furthermore, kaempferol exhibits cardioprotective effects by improving endothelial function, reducing blood pressure, and preventing the oxidation of low-density lipoprotein (LDL) cholesterol, which is a critical factor in the development of atherosclerosis. Its ability to enhance nitric oxide bioavailability and suppress inflammatory markers further underscores its role in cardiovascular health. Collectively, the multifaceted biological activities of kaempferol make it a valuable compound for further investigation in the development of nutraceuticals and therapeutic agents aimed at addressing oxidative stress-related diseases, cancer and cardiovascular disorders.

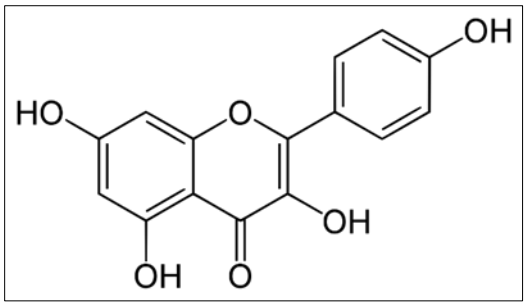

Figure 8 Structure of Quercetin

3.2.3. Apigenin

Naturally occurring flavone Apigenin exhibits a hydroxyl group attached at the 4-position of its C ring in its chemical structure. Scientific research has also focused heavily on this bioactive compound for its many other non-restriction pharmacological targets such as anti-inflammatory, anticancer and neuroprotective activities. As a member of the flavonoid family, ancient and common in many plant-based foods (parsley, celery, chamomile) and particular fruits apigenin could be easily incorporated to the diet with possible beneficial health attributes. Due primarily to its anti-inflammatory effects, apigenin is thought to be involved in the suppression of key signaling pathways, including NF-κBand mitogen-activated protein kinase pathways (MAPKs), as NF-κB plays an important role in regulation of cellular inflammation. Apigenin appears to attenuate cytokines and inflammatory enzymes (e.g., cyclooxygenase-2, COX-2; inducible nitric oxide synthase- iNOS) responsible for chronic inflammatory diseases by impairing production. Apigenin holds the capacity for chemopreventive and therapeutic effects in cancer through several mechanisms. By activation of both intrinsic and extrinsic apoptotic pathways it induces apoptosis in cancer cells, whereas it halts cell cycle at specific phases (e.g., G2/M phase) to block cell proliferation; Likewise, the ability of apigenin also suppressing angiogenesis and metastasis derive from down-regulating vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), which are pivotal factors for tumor progression via endothelial penetration. Modulation of oxidative stress and augmentation of standard chemotherapeutics further characterize its anticancer behavior, and its neuroprotective effects is particularly high in the realm of neurodegenerative disorders like Alzheimer's and Parkinson's.

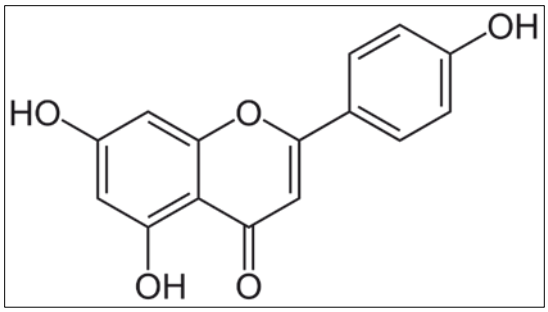

Figure 9 Structure of Apigenin

3.2.4. Luteolin

Luteolin is a naturally occurring flavone, a subclass of flavonoids, characterized by the presence of hydroxyl groups at the 3' and 4' positions on the B ring of its chemical structure. This specific arrangement of hydroxyl groups contributes to its diverse biological activities, making it a compound of significant interest in pharmacological and biomedical research. Luteolin has been extensively studied for its potent antioxidant properties, which enable it to neutralize reactive oxygen species (ROS) and mitigate oxidative stress, a key factor in the pathogenesis of numerous chronic diseases. By scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes, luteolin helps protect cellular components such as lipids, proteins, and DNA from oxidative damage. In addition to its antioxidant effects, luteolin demonstrates notable anti-inflammatory activity. It modulates various signaling pathways, including the suppression of nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinase (MAPK) pathways, which are central to the regulation of inflammatory responses. Through these mechanisms, luteolin inhibits the production of pro-inflammatory cytokines, chemokines, and enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), thereby attenuating inflammation at the molecular level. Furthermore, luteolin has garnered attention for its anticancer potential. It exerts its antitumor effects by inducing apoptosis, inhibiting cell proliferation, and

suppressing angiogenesis and metastasis. Luteolin achieves these outcomes through the regulation of multiple molecular targets, including the downregulation of oncogenic signaling pathways such as phosphatidylinositol 3-kinase (PI3K)/Akt and Wnt/β-catenin, as well as the upregulation of tumor suppressor genes. Its ability to selectively target cancer cells while sparing normal cells underscores its potential as a chemopreventive and therapeutic agent. Collectively, the multifaceted biological activities of luteolin, rooted in its unique chemical structure, highlight its promise as a natural compound for the prevention and treatment of oxidative stress-related disorders, inflammatory conditions, and various forms of cancer. Ongoing research continues to explore its mechanisms of action and potential applications in clinical settings.

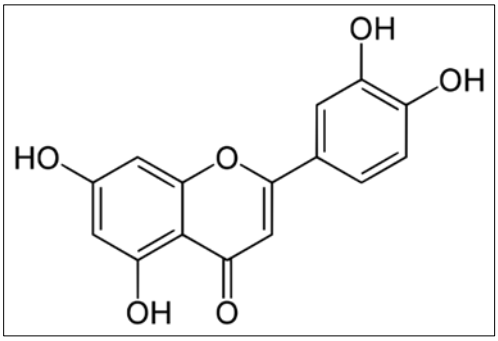

Figure 10 Structure of Luteolin

3.3. Techniques for Structural Elucidation [16-18]

The structural elucidation of flavonoids relies on advanced spectroscopic techniques, including UV-Vis spectroscopy, IR spectroscopy, NMR spectroscopy, and mass spectrometry (MS). Each method provides complementary information, enabling comprehensive characterization.

3.3.1. UV-Vis Spectroscopy

UV-Vi's spectroscopy analyzes electronic transitions in flavonoid molecules, primarily involving conjugated π-systems in aromatic rings. Flavonoids exhibit two major absorption bands:

- Band I (300–400 nm): Corresponds to the cinnamoyl system (B-ring).
- Band II (240–280 nm): Arises from the benzoyl system (A-ring). Substituents (e.g., hydroxyl or methoxyl groups) induce bathochromic shifts (longer λmax) and hyperchromic effects (increased absorbance). These spectral features help classify flavonoid subclasses and infer substitution patterns.

3.3.2. IR Spectroscopy

IR spectroscopy identifies functional groups via bond vibrational frequencies. Key absorptions include:

- O-H stretching (3200–3600 cm^{-1}): Broad band for hydroxyl groups.
- C=O stretching (1650–1750 cm^{-1}): Sharp peak for carbonyl groups.
- Aromatic C=C (1400–1600 cm^{-1}): Confirms aromatic rings.
- The spectrum serves as a molecular fingerprint, aiding in structural confirmation.

3.3.3. NMR Spectroscopy

NMR spectroscopy provides atomic-level structural details:

- ^{1}H NMR: Reveals proton environments, coupling patterns, and integration ratios. Hydroxyl and methoxyl substituents influence chemical shifts (δ).
- ^{13}C NMR: Identifies carbon types (aromatic, carbonyl, aliphatic) and symmetry. 2D NMR techniques (COSY, HSQC, HMBC) resolve connectivity and spatial arrangements.

3.3.4. Mass Spectrometry (MS)

MS determines molecular weight and fragmentation patterns:

- Electrospray Ionization (ESI): Generates intact molecular ions ($[M+H]^+$ or $[M-H]^-$).
- High-Resolution MS (HRMS): Provides exact mass for elemental composition.
- Tandem MS (MS/MS): Fragmentation pathways reveal glycosidic cleavages and ring modifications.

3.4. Challenges in Structural Elucidation

Flavonoid analysis in *Marsilea Minuta* is complicated by:

- Structural Similarity: Isomers (e.g., quercetin vs. kaempferol) require high-resolution techniques.
- Matrix Complexity: Co-extracted compounds (alkaloids, glycosides) interfere with isolation.
- Low Abundance: Minor flavonoids necessitate sensitive detection (e.g., LC-MS/MS).

4. Pharmacological Properties of Flavonoids in *Marsilea Minuta*

4.1. Antioxidant Activity

Flavonoids (e.g., quercetin, kaempferol) mitigate oxidative stress via:

- Radical scavenging (H^+/electron donation).
- Metal chelation (Fe^{2+}/Cu^{2+} binding).
- Enzyme inhibition (xanthine oxidase, NADPH oxidase).

In vitro studies (DPPH assay) confirm radical scavenging, while in vivo models show reduced lipid peroxidation.

4.2. Anti-Inflammatory Activity

Mechanisms include:

- Cytokine suppression (TNF-α, IL-6).
- Enzyme inhibition (COX-2, LOX).
- NF-κB pathway modulation.

Ethanolic extracts exhibit dose-dependent inflammation reduction in animal models.

4.3. Antimicrobial Activity

Flavonoids disrupt microbial growth by:

- Membrane destabilization.
- Enzyme inhibition (DNA gyrase).
- DNA intercalation.

In vitro tests show efficacy against *S. aureus* and *E. coli*; in vivo data remain limited.

4.4. Anticancer Activity

Flavonoids induce:

- Apoptosis (caspase activation).
- Cell cycle arrest (G2/M phase).
- PI3K/Akt pathway inhibition.

In vitro: Cytotoxicity in cancer cells. In vivo: Tumor growth suppression in xenografts.

Table 3 Mechanisms of Anti-Inflammatory Activity of Flavonoids

Mechanism	Description	Key Molecular Targets	Representative Flavonoids
Inhibition of Pro-Inflammatory Cytokines	Flavonoids suppress the production of pro-inflammatory cytokines, reducing the severity of inflammation.	TNF-α, IL-1β, IL-6, NF-κB, COX-2, LOX	Quercetin, Luteolin, Genistein
Inhibition of Enzymes	Flavonoids inhibit inflammatory enzymes, preventing the synthesis of pro-inflammatory mediators.	COX-1, COX-2, LOX, AP-1	Apigenin, Kaempferol, Rutin
Modulation of Signalling Pathways	Flavonoids regulate inflammatory pathways, balancing pro- and anti-inflammatory signals.	NF-κB, MAPK, JAK-STAT	Myricetin, Baicalein, Fisetin
Antioxidant Activity	Flavonoids neutralize reactive oxygen species (ROS) to prevent oxidative stress-induced inflammation.	ROS, Nrf2, HO-1	Catechin, Epicatechin, Hesperetin
Immune System Modulation	Flavonoids modulate immune responses by altering macrophage activity and cytokine release.	Macrophages, T-cells, B-cells	Chrysin, Naringenin, Isorhamnetin

4.5. Other Pharmacological Activities [19, 20]

In addition to the above-mentioned activities, flavonoids in *Marsilea Minuta* have been shown to exhibit other pharmacological activities, including:

- ***Anti-diabetic Activity****:* Flavonoids, a class of polyphenolic compounds, have been demonstrated to enhance insulin sensitivity and modulate blood glucose levels effectively. These natural compounds stimulate insulin secretion from pancreatic β-cells and activate the insulin signaling pathway, thereby maintaining glucose homeostasis. By promoting glucose uptake in insulin-sensitive tissues such as muscle and adipose tissue, flavonoids contribute to lowering blood glucose levels. Additionally, they modulate hepatic glucose output, further supporting glycemic control. The molecular mechanisms underlying these effects involve the activation of key signaling pathways, including the PI3K/AKT pathway, which facilitates GLUT4 translocation in adipocytes and skeletal muscle cells. This results in improved glucose utilization and reduced insulin resistance, a critical factor in the development of type 2 diabetes. Overall, the consumption of flavonoid-rich diets has been associated with enhanced insulin sensitivity and a reduced risk of type 2 diabetes, underscoring the potential therapeutic benefits of these compounds in managing metabolic disorders.
- ***Cardioprotective Activity****:* Flavonoids, a diverse group of plant-derived compounds, have been recognized for their potential in mitigating cardiovascular diseases. Their protective effects are primarily attributed to their ability to reduce oxidative stress and inflammation, which are key factors in the development of cardiovascular conditions. By scavenging free radicals, flavonoids can decrease the oxidation of low-density lipoproteins, thereby improving lipid profiles and vascular function. Additionally, flavonoids have been shown to induce vasodilation and modulate apoptotic processes in the endothelium, further contributing to their cardioprotective effects. The anti-inflammatory properties of flavonoids also play a crucial role in reducing the risk of cardiovascular morbidity and mortality. Studies have highlighted the benefits of consuming flavonoid-rich foods, such as tea, cocoa, and certain fruits, in lowering the risk of cardiovascular diseases. While the evidence supports the potential cardiovascular benefits of flavonoids, further research is needed to fully elucidate their mechanisms of action and to establish definitive guidelines for their dietary intake. Overall, the incorporation of flavonoid-rich foods into one's diet may be a valuable strategy for enhancing cardiovascular health.
- ***Neuroprotective Activity***: Flavonoids have been identified as potent neuroprotective agents, capable of mitigating the progression of neurodegenerative diseases by modulating oxidative stress and inflammation within the brain. These compounds, abundant in plant-based foods, exhibit antioxidant properties that counteract the detrimental effects of reactive oxygen species (ROS), thereby safeguarding neuronal integrity. By interacting with intracellular signaling pathways, flavonoids influence gene expression and mitochondrial function, which are crucial for neuronal survival and differentiation. Furthermore, flavonoids have been shown to modulate glutamate excitotoxicity, a mechanism implicated in neuronal death associated with various brain

disorders. Their ability to regulate inflammatory responses and suppress pro-apoptotic signaling cascades underscores their potential therapeutic value in neuroprotection. Overall, the neuroprotective effects of flavonoids highlight their promise as adjunctive treatments for neurodegenerative conditions, offering a natural and potentially effective strategy to enhance brain health.

4.6. Potential Applications in Modern Medicine [21-25]

Marsilea Minuta, an aquatic or sub-aquatic fern, is renowned for its diverse pharmacological properties, which make it a promising candidate for the development of novel therapeutic agents. This plant contains bioactive compounds, including flavonoids, that exhibit a wide range of biological activities. These compounds have potential applications in various therapeutic areas, including antioxidant, anti-inflammatory, antimicrobial, and anticancer therapies. In the realm of antioxidant therapy, the flavonoids present in *Marsilea Minuta* can serve as natural antioxidants, helping to mitigate oxidative stress-related diseases. Oxidative stress is a key factor in the pathogenesis of cardiovascular diseases, neurodegenerative diseases, and cancer. By scavenging free radicals, these flavonoids can potentially reduce the risk and progression of these conditions, thereby contributing to overall health and well-being. The anti-inflammatory properties of flavonoids in *Marsilea Minuta* make them suitable for anti-inflammatory therapy. Inflammatory diseases such as arthritis, asthma, and inflammatory bowel disease can benefit from these natural compounds, which may help reduce inflammation and alleviate symptoms associated with these conditions. This could provide an alternative or complementary approach to conventional anti-inflammatory treatments. Furthermore, the flavonoids in *Marsilea Minuta* have shown antimicrobial activity, which can be leveraged to develop treatments for infections caused by bacteria, fungi, and viruses. This is particularly significant given the rise of antibiotic resistance and the need for novel antimicrobial agents. By harnessing these natural compounds, it may be possible to create more effective and sustainable treatments for various infectious diseases. Lastly, the potential of flavonoids in *Marsilea Minuta* for anticancer therapy is noteworthy. These compounds have been studied for their ability to prevent and treat various types of cancer by modulating cellular pathways involved in tumor growth and metastasis. While further research is needed to fully explore their anticancer properties, the existing evidence suggests that these flavonoids could play a role in cancer prevention and treatment strategies.

Overall, *Marsilea Minuta* offers a rich source of bioactive compounds with diverse therapeutic potential, making it an attractive subject for further scientific investigation and drug development.

5. Discussion [26-30]

The ethanolic extract of *Marsilea Minuta* contains bioactive flavonoids, such as quercetin and kaempferol, known for their antioxidant and anti-inflammatory properties. These compounds mitigate oxidative stress and inflammation by scavenging free radicals and modulating pro-inflammatory signaling pathways. Traditional extraction methods (maceration, Soxhlet) are time- and solvent-intensive, whereas advanced techniques (ultrasonic-assisted extraction, supercritical fluid extraction) improve efficiency and sustainability. Subsequent purification via HPLC ensures high flavonoid purity. Preclinical studies support their therapeutic potential, aligning with traditional uses in treating fever, skin disorders, and sleep disturbances. However, challenges in scalability, stability, and sustainable sourcing must be addressed. Biotechnological approaches, such as metabolic engineering, could enhance production while conserving natural resources.

6. Conclusion

Flavonoids from *Marsilea Minuta* exhibit significant pharmacological potential, supported by their antioxidant, anti-inflammatory, and antimicrobial properties. While extraction and purification advancements enhance yield and purity, scalability and sustainability remain key challenges. Future research should optimize extraction protocols, validate clinical efficacy, and explore biotechnological production methods to ensure sustainable utilization. Rigorous clinical trials are essential to confirm safety and therapeutic efficacy, paving the way for their integration into modern medicine. Addressing these challenges will maximize the translational potential of *Marsilea Minuta* flavonoids in drug development.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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