The Potential Role of Flavonoids in Mitigating Inflammatory Disorders

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Edited by

Anuradha Mishra, Narhari N. Palei,
Sumel Ashique and Satya Prakash Singh

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CHAPTER 1

FLAVONOIDS: AN INTRODUCTION AND EXPLORATION OF THEIR IMPACT ON THE MANAGEMENT OF NEURODEGENERATIVE DISORDERS

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Abstract

Neurodegenerative disorders (NDs) are characterized by progressive nerve cell loss, affecting millions worldwide. Ethnopharmacology offers potential for novel therapeutic approaches in NDs. Traditional medicinal plants and their bioactive compounds address key pathological processes in inflammatory neurodegenerative diseases like Alzheimer's and Parkinson's. Three major compound classes - polyphenols, alkaloids, and terpenoids - demonstrate neuroprotective effects. Specific compounds, including catalpol, geniposide, hesperetin, and hispidulin, are evaluated for their ability to target various inflammatory aspects of neurodegeneration. These compounds exhibit diverse mechanisms of action, including antioxidant properties, modulation of neuroinflammatory pathways, and regulation of neurotransmitter systems. The review emphasizes the promising role of ethnopharmacology in identifying novel therapeutic approaches for neurodegenerative disorders while highlighting the need for further research to fully harness its potential in clinical applications.

Keywords: Inflammatory disorders; Ethnopharmacology; Polyphenols; Alkaloids; Terpenoids; Neuroprotection; Alzheimer's disease; Parkinson's disease; Catalpol; Geniposide; Hesperetin; Hispidulin

1. Introduction

1.1 Overview of Neurodegenerative Disorders

Neurodegenerative diseases (NDs) involve the gradual destruction of nerve cells, which may result in their eventual death. Worldwide, neurodegenerative diseases impact millions of individuals. While age remains the main cause of risk for NDs, new research indicates that individual genetic composition & environmental factors can both increase the main cause for NDs. Furthermore, the duration and degree of neurodegeneration primarily depend on the surrounding environment even while specific genes responsible for NDs are expressed (within an individual) [1-3]. Recent research indicates that a single neurodegenerative illness may be underlined by several disorders. As a result, NDs can be extremely dangerous or even fatal in certain cases, this all depends on the kind & stage of the disease [4-7]. Since the brain regulates several bodily functions, neurodegenerative diseases impact a variety of aspects of human functioning and impair the capacity to carry out both simple (such as speech, movement, stability, and balance) & complex (such as cognitive functions and bladder and bowel functions) tasks. While in some cases treatments focus on improving symptoms, relieving pain if it is present, and restoring movement and balance, most NDs develop without remission. Various alternative approaches are available for the treatment of NDs, such as immunotherapy and nanotechnology. Nanotechnology, in particular, modulates immune responses to protect neurons from damage, potentially slowing disease progression. Nanoparticles also enhance drug delivery across the blood-brain barrier, improving the efficacy of treatments for NDs [8,9]. However, these approaches can lead to side effects, including inflammatory reactions and autoimmune responses in the brain. Given these challenges, ethnopharmacology presents a promising alternative. By exploring traditional remedies used for centuries, ethnopharmacology offers a rich source for discovering new drugs that may be effective in treating NDs with fewer side effects.

1.2. Importance of ethnopharmacology in drug discovery

Ethnopharmacology identifies botanicals and natural remedies that have traditionally been utilized to treat neurological conditions. Using this method, scientists can find therapeutic compounds that could have gone unnoticed in the current wave of pharmaceutical research [10]. A wide range of bioactive chemicals found in medicinal plants have the ability to affect several cellular pathways linked to neurodegenerative disorders. These substances have the ability to target important pathological processes that are essential to the development of disorders like Alzheimer's, including tau hyperphosphorylation, oxidative stress, amyloid-beta aggregation & neuroinflammation, according to ethnopharmacological studies [11,12]. In the current target-rich, lead-poor situation, ethnopharmacology & natural product drug development remain a major hope [13]. While the industrial drug discovery process typically uses high-throughput and medium-throughput bioassay screening platforms to identify promising compounds for a given target, ethnopharmacology takes a different approach, testing the informal efficacy of medicinal plants in a laboratory setting in an effort to comprehend the pharmacological underpinnings of historically significant plants [14].

2. Natural Compound with Neuroprotective Potential

Natural compounds, such as polyphenols, alkaloids, terpenoids, and flavonoids, have shown considerable neuroprotective potential in combating neurodegenerative diseases. These compounds influence various signaling pathways and enzymes, offering a broad spectrum of therapeutic benefits. Polyphenols possess strong antioxidant and anti-inflammatory effects and are known to regulate key signaling pathways like nuclear factor kappa B (NF-κB), contributing to neuroprotection. Alkaloids can modulate neurotransmitter systems and ion channels, offering potential benefits in slowing the progression of diseases like Alzheimer’s and Parkinson’s. Terpenoids are effective in reducing oxidative stress and neuroinflammation. Flavonoids, a type of polyphenol, promote neuronal survival by activating pro-survival kinases and modulating apoptotic mechanisms. They also influence key enzymes involved in amyloid precursor protein processing and tau phosphorylation. These natural compounds exhibit multi-functional, pleiotropic effects, making them promising for the development of multi-target therapies for neurodegenerative conditions.

2.1. Polyphenols

Polyphenols are a class of antioxidants that show a protective effect against neuronal oxidative stress which is an important characteristic in neurodegenerative diseases Polyphenols also have an effect on specific signaling pathways associated with processes of neuronal survival and inflammation crucial to neurodegenerative diseases such as Alzheimer's and Parkinsonism. For example, quercetin and resveratrol compounds show protective potential in multiple in vitro and animal models of neurodegeneration [15]. Increased levels of MOA -A and MAO-B are mainly responsible for neurodegenerative diseases so this monoamine oxidase-A and B can be inhibited by polyphenols and show their therapeutic action in neurodegenerative diseases [16].

By influencing many signaling route points, polyphenols enhance the regulation of neuronal survival; this could be a useful treatment strategy for disorders of the central nervous system [29]. Members of the neurotrophin family, brain-derived neurotrophic factor (BDNF) & nerve growth factor (NGF), are linked to neurogenesis, the growth and regeneration of neurons, and long-term potentiation in the hippocampus. They also cause structural alterations in synapses, as well as survival and resistance to neuronal damage, all of which are commonly regarded as essential goals in neurodegenerative potential through the presence of polyphenols [17-19].

The reduction of ROS in the brain is another neurodegenerative effect of polyphenols. By initiating protein kinases signaling molecular pathways like Keap1/Nrf-2/ARE, the primary defense pathways against endogenous and external ROS, polyphenols produce an antioxidant action [20-22]. In order to disrupt the Keap1/Nrf2 complex and enable the translocation of nuclear factor 2 (Nrf2)-related transcription factor to the nucleus, where it binds to receptors rich in adenylate and uridylate (ARE) and promotes the expression of antioxidant proteins and enzymes (GSH, GST, GPX, SOD, and CAT), polyphenols interact and activate receptor kinases ERK1/2, JNK, and p38 protein. By inhibiting the generation of NO and PGE2, polyphenols also have antioxidant qualities that lower the activation of NADPH oxidase and prevent the formation of ROS in the brain.

2.2 Alkaloid

Alkaloids and their phytoconstituent have the ability to protect neurons in various ways such as by inhibiting the Acetylcholinesterase (AChE) enzyme that breaks down acetylcholine, a neurotransmitter crucial for memory and cognitive function. In Alzheimer's disease, there is a deficit of acetylcholine, so by inhibition of Ach Enzyme, alkaloids can help preserve acetylcholine levels in the synaptic cleft, allowing for prolonged neurotransmission. It can potentially improve cognitive functions like memory and attention. For example, galantamine, an alkaloid from snowdrop flowers, is used as an AChE inhibitor in Alzheimer's treatment [23,24]. MAO-B is an enzyme that breaks down dopamine, a neurotransmitter crucial for motor control and reward. In Parkinson's disease, where dopamine levels are depleted, inhibiting MAO-B can help maintain higher dopamine levels. Alkaloids will inhibit MAO-B and maintain the dopamine level. For example, β-carbolines, found in various plants, have shown MAO-B inhibitory activity and potential neuroprotective effects in Parkinson's disease models [25]. Many alkaloids possess antioxidant properties, either by directly scavenging free radicals or by enhancing the body's own antioxidant defenderships can help protect neurons from oxidative damage [26]. Gamma-aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the central nervous system. It counterbalances excitatory neurotransmission, preventing overstimulation of neurons. Alkaloids can enhance GABA activity, either by increasing its release, slowing its reuptake, or directly activating GABA receptors, this can help reduce excitotoxicity (neuronal death due to overstimulation) and may have neuroprotective effects. For instance, the alkaloid muscimol from Amanita mushrooms is a potent GABA agonist [25].

2.3 Terpenoids

2.3.1 Catalpol

Catalpol is a monoterpene that is an iridoid and has an aldehyde acetal. Since its isolation from plants in 1888, it has undergone a great deal of research to determine its biological qualities, including its ability to reduce inflammation and act as an antioxidant [26,25]. Catalpol is the main active component of *Rehmanniae Radix*, and it protects several disorders affecting the central nervous system [26]. It has been discovered that catalpol has neuroprotective and antioxidant effects on depression-stricken mice models. By upregulating the PI3K/Akt/Nrf2/HO-1 signaling pathway, catalpol improves the depressive behavior of these mice, suggesting that this pathway may be a potential target and biomarker for catalpol-assisted depression treatment. Furthermore, according to certain research, catalpol triggered the PI3K/Akt/mTOR pathway improving in vivo axon development and neuronal survival in stroke models by downregulating miR-124 expression and upregulating downstream protein S6 expression [27].

Derivatives compounds of catalpols such as 6-O-acyl catalpols, and 6-O-glycosyl catalpols, showed a broad variety of pharmacological activity and exhibited neuroprotective properties [28]. Therefore, chemical modification of its derivatives must result in improved bioavailability. Liu et al. (2020)

used computer-aided drug design (CADD) to dock catalpol crotonates with glutathione peroxidase (GSH-Px) which has neuroprotective properties. By using microwave-assisted synthesis (MWAS) as a synthesis method, they were successful in designing and synthesizing catalpol hexacrotonate (CC-6) which shows their action as neuroprotective potential [28].

2.3.2 Geniposide

Geniposide is a bioactive iridoid glycoside that has been identified in a variety of plant species, including those in the Rubiaceae family [29]. The studies have demonstrated that geniposide activated Akt and then improved by upregulating the levels of GSK-3β and PI3K. Additionally, Wang and colleagues have verified that geniposide may prevent the death of hippocampus neurons, leading to antidepressant characteristics of the brain [30].

It has been shown that geniposide dramatically lowers the amount of amyloid-beta (Aβ) plaques that form in the brains of animal models. Research shows that geniposide reduces the amount of soluble and insoluble types of Aβ, which helps to decrease the cognitive impairment by Alzheimer's disease. As a neuroprotective drug, geniposide shows great promise when used for neurodegenerative illnesses. It acts by several mechanisms such as inhibiting the release of inflammatory cytokines (such as TNF-α, IL-6, and IL-1β) and modulating inflammatory signaling pathways (e.g., MAPK signaling), tau phosphorylation inhibition, amyloid plaque reduction, oxidative stress alleviation benefits emphasize its promise as a therapeutic option [31,32].

2.4 Flavonoids

2.4.1 Hesperetin

The citrus flavonoid Hesperetin possesses a variety of preventive qualities of interest in neurons and glial cells relating to CNS diseases. Hesperetin is a bioactive molecule used traditionally in Chinese medicine having antioxidant, anti-inflammatory, and anticarcinogenic activities [33]. Hesperetin is an aglycone of hesperidin, demonstrating diverse biological actions.

Hesperetin has been found to penetrate the central nervous system (CNS), where it may exert neuroprotective effects by neutralizing free radicals generated during cellular metabolism [35]. Research indicates that hesperetin protects neurons from damage caused by oxidative stress and inflammation. Studies using animal models and brain endothelial cells have shown that hesperetin, along with other flavonoids, is taken up by brain cells [36]. Flavonoids enhance neuronal survival by activating the phosphatidylinositol 3-kinase (PI3K) and Akt protein kinase B (Akt) pathways, as well as the MAPK pathway. They also promote the recruitment of neural progenitor cells, which influences astrocyte function. Hesperetin has demonstrated anti-inflammatory, antioxidant, and neuroprotective effects in various neurodegenerative diseases [34].

Hesperetin has also been shown to reduce the overexpression of inducible nitric oxide (NO) synthase and proinflammatory cytokines, including IL-1β, TNF-α, and IL-6, as well as MAPK signaling molecules ERK1/2 and p38, in LPS-stimulated BV-2 murine cell lines [37]. The modulation of signaling pathways, along with its antioxidant and anti-inflammatory properties, may contribute to the cognitive and motor improvements observed in Alzheimer's disease animal models treated with hesperetin. In a similar study investigating hesperetin's effects against Aβ-induced Alzheimer's disease, it was found that hesperetin significantly reduced oxidative stress-mediated neuroinflammation, apoptosis, and neurodegeneration. The study specifically targeted endogenous antioxidant systems, such as TLR4-mediated glial cell neuroinflammation. Further findings showed that hesperetin prevented cognitive and memory decline in mice [38].

2.4.2 Baicalein

Baicalein is a flavonoid extracted from the roots of *Scutellaria baicalensis*. In MPTP-treated mice, baicalein reduces cytokine upregulation in the substantia nigra & striatum, including tumor necrosis factor-a and interleukin-1b. Baicalein inhibits the activation of microglia, astrocytes, JNK, and ERK in MPTP mice. Baicalein reduced dopaminergic neuron loss and improved motor function in MPTP-treated rats [39,40]. In an Alzheimer's disease rat model, baicalein reduced Ab1-40-induced cognitive function and impacted protein expression levels related to energy metabolism, neurotransmission, anti-apoptosis, anti-oxidation, stress response, protein phosphorylation, cytoskeleton, phospholipid metabolism, and cell signaling. Baicalein reduced beta-amyloid and enhanced non-amyloidogenic amyloid precursor protein processing in an Alzheimer's disease transgenic mice model [41,42]. As a powerful antioxidant baicalein scavenges reactive oxygen species (ROS) & reduces oxidative stress, which is a key role in the pathophysiology of neurodegenerative illnesses like Alzheimer's and Parkinson's. Baicalein protects neuronal function and integrity by reducing oxidative damage [43].

3. Conclusion

Ethnopharmacology presents a promising avenue for uncovering potential treatments for neurodegenerative disorders. The rich tapestry of traditional medicinal plants offers a wealth of bioactive compounds, including polyphenols, alkaloids, and terpenoids, which have proven neuroprotective properties in preliminary studies. Compounds such as catalpol and hesperetin exemplify this potential, warranting further investigation. Priority areas might include well-designed clinical trials, the development of standardized quality control measures, the exploration of synergistic combination therapies, innovative approaches to enhance bioavailability, and the establishment of ethical guidelines that respect and protect traditional knowledge in the context of modern drug discovery. By addressing these considerations with sensitivity and diligence, ethnopharmacology can continue to ancient wisdom with contemporary science, potentially yielding valuable insights and therapies for neurodegenerative disorders.

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CHAPTER 2

FLAVONOIDS: SOURCES, EXTRACTION, PURIFICATION AND CHARACTERIZATION

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Abstract

Flavonoid is a major class of polyphenols included in natural products. They are often found in plants as a group of analogues with comparable physico-chemical characteristics, and structures and have an elevated antioxidant capacity. Flavonoids constitute a diverse class of compounds of considerable importance to human health, primarily attributable to their anti-inflammatory and antibacterial properties, which enable them to neutralize ROS upon their formation. Advanced approaches are replacing traditional flavonoid extraction methods to fulfill environmental requirements, meet growing market demand, and boost efficiency and selectivity while consuming less energy and solvent. Alternatives now in use include advanced technology including pressurized liquids, electric fields, ultrasound, supercritical fluids, and microwaves. Compared to traditional extraction methods, these contemporary methods are often quicker, more ecologically friendly, and more automated. Along with providing insight into their potential, a comprehensive examination of current advancements and their industrial significance is also covered. This examines the developments in compound extraction techniques, describing the advantages and disadvantages of each to give a more thorough

considerate of flavonoid separation from diverse plant matrices. This chapter outlines the developments in flavonoid detection, chromatographic separation, sample extraction, and structural analysis in dietary and medicinal plants.

Keywords: Flavonoids, sources, extraction methods, purification, structural analysis.

1. Introduction

Flavonoids signify a notable and substantial class of secondary metabolites that are naturally produced by plants. They are regarded as indicators of the quality of fruits and medicinal plants, rendering them a compelling component for assessing quality in agricultural practices and product development. In the human body, flavonoids have a vast array of biological and pharmacological properties that include preventing, protecting, treating, and lessening the symptoms of many chronic illnesses [1]. The bioavailability of these compounds, which make up the majority of the human diet daily, is dependent on their concentration in the sources and the pace at which they are absorbed into the site of action [2]. Therefore, to regulate the quality and effectiveness of these goods, it is imperative to ascertain the amount of flavonoids in their matrices [3].

They depend on organization for their functions. The structural classification of flavonoids, the extent of hydroxylation, diverse substitutions and conjugations, along with the degree of polymerization, all play a significant role in determining their chemical composition [4]. The potential health benefits associated with the antioxidant characteristics of these polyphenolic compounds have generated considerable scholarly interest in these substances. Through the mechanisms of free radical scavenging and/or metal ion chelation, the functional hydroxyl groups present in flavonoids influence their antioxidant activities [5, 6]. Metal chelation may be essential for preventing the production of radicals that harm certain biomolecules [7, 8].

In order to stabilize free radicals and facilitate antioxidant defense, the functional –OH groups present in flavonoids possess the capability to donate electrons through resonance mechanisms [9]. Flavonoids may be divided into six main types based on their structural makeup: flavones, flavonols, flavan-3-ols, flavanones, isoflavones, and anthocyanins [9]. Flavonoids are utilized in the culinary, cosmetic, and pharmaceutical sectors because of their exceptional antioxidant properties [10]. Nevertheless, the utilization of these antioxidants within industrial contexts demands extraction methodologies that are both of superior purity and quality. Consequently, numerous techniques for flavonoid extraction have been explored, and recently, there has been a notable advancement in environmentally sustainable extraction methods and strategies that yield higher outputs [11].

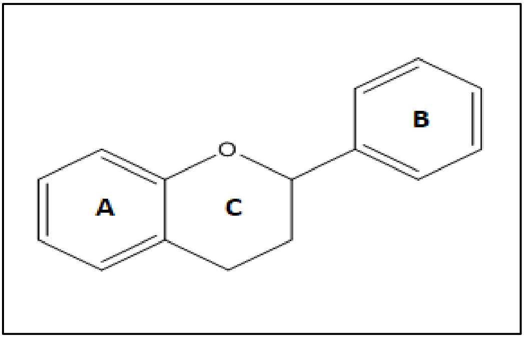

Fig 1: Basic nucleus of Flavonoid

2. Flavonoid Structure and Classification

Flavonoids are extensively distributed in nature and are often found in phenolic compounds that have C6-C3-C6 (phenyl-benzopyran) as their nucleus. In general, flavonoids can be classified as flavonoids (2-phenyl-benzopyran), iso-flavonoids (3-phenyl-benzopyran), and neo-flavonoids (4-phenyl-benzopyran) based on the various connecting locations of the aromatic ring (B ring) and the benzopyran group (C ring) [12]. Furthermore, because of their comparable C6-C3-C6 skeletons and recalled minor flavonoids, chalcone, and aurone are also considered to be flavonoids [13]. The flavonoid aromatic ring (B ring) is joined to the second position of the benzopyran group (C ring) in a limited sense. Flavone, flavonone, flavonol, dihydroflavonol, flavon, flavonol, and anthocyanidin are the general divisions of flavonoids, which depend on whether the C ring contains a double bond at the second and third locations and if the third position is attached to a hydroxyl group. Further modifications, including hydroxylation, acylation, methylation, prenylation, and glycosylation, take place at various places based on these fundamental structures to produce flavonoids with a variety of structures and functions [14]. Additionally, certain flavonoids undergo polymerization to create macromolecules like proanthocyanidins. Interestingly, the diverse flavonoid structures also influence the distinct ways in which they interact with other biological molecule-level [15].

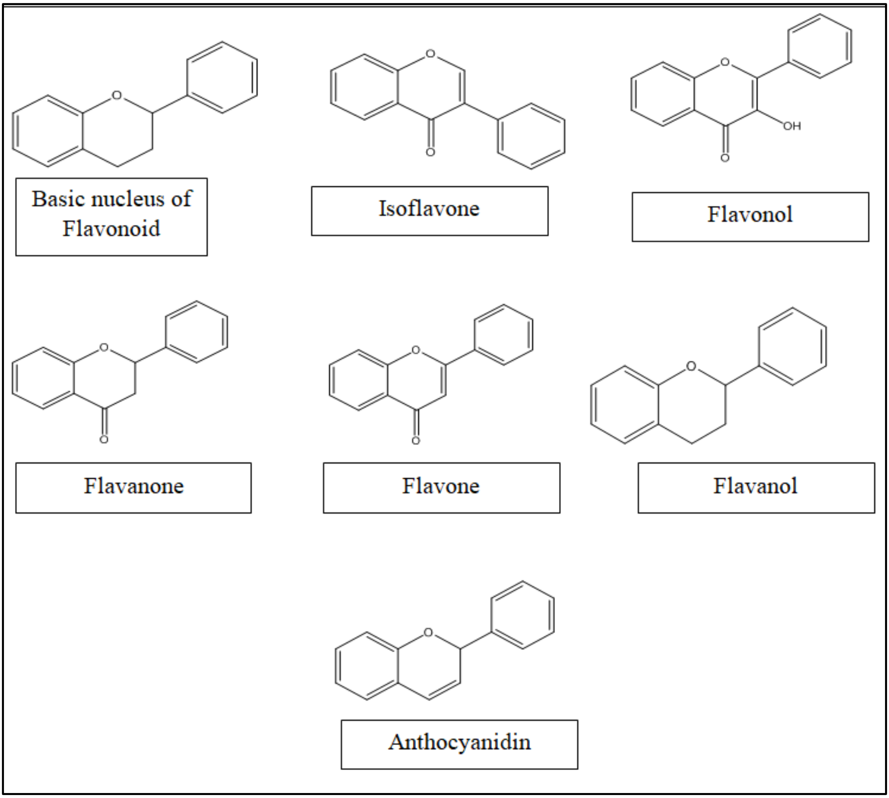

Fig 2: Subclass/categories of flavonoids

3. Natural Sources of Flavonoid

The most prevalent phenolic chemicals in plants, particularly those that can perform photosynthesis, are flavonoids. They are in charge of flavor, color, vitamin and enzyme preservation, and the inhibition of fat oxidation. The variability in environmental conditions and the level of light exposure represent two among many determinants influencing the spatial distribution of flavonoids within plant systems. For instance, an increase in light intensity promotes the biosynthesis of flavonoids in their more oxidized forms.

Although flavonoids are also present in humans and animals, they are supplied by the body's plant-based food rather than being produced on-site [16, 17]. Flavonones, isoflavonoids, flavans (flavanols), flavonols, flavones, and anthocyanins are among the ten chemical groups that make up plant-derived flavonoids. The predominant flavonoids encountered in dietary sources are flavonols; however, flavones and flavanones are present in various plant species and citrus fruits, respectively. Isoflavones are particularly plentiful in soy-derived products, while anthocyanins are notably concentrated in strawberries and other berry varieties [18]. Fruits, vegetables, and tea all contain anthocyanins and catechins [17].

Table 1: Different sources and subgroups of flavonoid

Sub-groups	Characteristic flavonoid	Sources
Flavanols	Epicatechin, epigallocatechin gallate, gallocatechin, procyanidin, catechins,	Flowers and Fruits, apples, tea, hops wine, beer, fruit juice.
Flavones	Chrysin, luteolin, apigenin, luteolin, diosmetin	Herbs, fruits, cereals, parsley, vegetables, thyme, flowers
Flavanones	Neohesperidin, naringenin, Hesperidin	Cumin, Citrus fruits, oranges, peppermint, grapefruits
Flavonols	Kaempferol, myricetin, Isorhamnetin, quercetin, rutin	Cherries, Onions, apples, kale, broccoli, tomatoes, tea, red wine, berries, Tartary buckwheat
Anthocyanin	Cyanidin, pelargonidin, malvidin delphinidin	Tea, fruits, honey, cereals, vegetables, olive oil, nuts, cocoa
Isoflavonoids	Genistein, formononetin, daidzein, glycitein,	Legumes (soybeans)

3.1 Flavonoid Occurrence in Food

3.1.1 Flavonols (3-hydroxy flavones)

Flavonols are one of the most studied subgroups of flavonoids due to their antioxidant qualities. The primary sources of these phytochemicals are fruits, vegetables, and plant-based drinks including red wine, black tea, and green tea. The main foods that contain flavonols include red lettuce, broccoli, tomatoes, onions, apples, and grape berries [19]. Quercetin is probably the most prevalent dietary flavonol found in food among the several types. Although it may be found in many fruits and vegetables, onions have the highest quantities of it. Depending on the food's availability, different countries have different sources of quercetin. For instance, quercetin is mostly found in tea in Japan and the Netherlands, whereas it is mostly found in wine in Italy. In Finland, Greece, and the United States, apples and onions are the chief foods that contain quercetin. There are several glycosidic forms of quercetin found in plants, but the most prevalent one is quercetin-3-rutinoside, sometimes referred to as rutin or quercetin-3-rhamnoglucoside. Onions contain quercetin, which is referred to as quercetin-3V-glucoside or quercetin-4V-glucoside, depending on whether it is attached to one or two glucose molecules. Quercetin arabinosides, which are found in berries, and quercetin galactosides, which are found in apples, are further recognized dietary quercetin glycosides. The diet also contains other flavonols such as isorhamnetin (onion), myricetin (berries), and kaempferol (broccoli) [20].

3.1.2 Flavanones

Numerous citrus fruits contain flavanones. Juices are known to have several hundred milligrams of flavanones per liter, even though their concentration is greater in solid tissues. The two main flavanones present in citrus fruits are narirutin (naringenin-7-rutinoside) from mandarins and hesperidin (hesperetin-7-rutinoside) from oranges. Naringin and Narirutin (20%) are flavanones that are present in grapefruits. Naringenin is another flavanone that is known to be present in tomatoes and tomato-based products. Naringenin chalcone, which is present in tomato skin, particularly when the tomatoes are fresh, is transformed into naringenin when tomato ketchup is made [20].

3.1.3 Flavones

In structure, flavones are identical to flavonol molecules, except they include an additional –OH group substituted at the carbon 3 position. Lutein and apigenin are the two main dietary flavones. Broccoli, peppers, onion leaves, celery, chrysanthemum flowers, parsley, cabbages, carrots, and apple skins contain the latter, whereas wheat sprouts, onions, oranges, parsley, chamomile, and tea have the former [21].

3.1.4 Anthocyanidins

The natural pigments known as anthocyanidins are what give things their color. They give fruits and vegetables their blue, purple, red, and orange hues. Over 500 distinct anthocyanidins have been identified in the literature to date. Anthocyanidins are mostly found in fruits, vegetables, nuts, tea, honey, olive oil, chocolate, and berries including blueberries and blackcurrants. Malvidin, cyanidin, delphinidin, and pelargonidin are a few other dietary anthocyanidins [19].

3.1.5 Isoflavones

A particularly unique subclass of flavonoid chemicals, iso-flavones are made up of a 3-phenyl chromen frame that is chemically formed from a 2-phenyl chromen skeleton by an aryl relocation process. Legumes, mainly soy, frequently contain them. Additionally, they have been found in sunflower seeds, clover sprouts, black beans, lima beans, chickpeas, and green split peas. The two main iso-flavones found in the human diet are genistein and daidzein [22].

3.1.6 Flavanols

Flavanols are a multifaceted class of polyphenols that include polymeric procyanidins known as condensed tannins and the monomeric flavan-3-ols (catechin, epicatechin, etc.). Fruits and goods made from them contain them. Additionally, they can be found in cereals, apples, kiwi, tea, red wine, and chocolate. Except for broad beans and lentils, they are effectively missing from vegetables and legumes. Flavanols have also been found in fruit and vegetable peels and seeds [19].

3.2 Medicinal plants rich in flavonoids

The therapeutic value of medicinal plants has recently attracted more attention, which may be because of their phenolic components, particularly flavonoids [50, 51]. Flavonoids have been consumed by humans seeing as the dawn of life for humans on earth, around 4 million years ago. They exhibit a diverse range of biological characteristics that promote human well-being and diminish the probability of illness.

It is believed that oxidative alteration of LDL cholesterol is a major factor in atherosclerosis. By scavenging free radicals, the isoflavone glabridin, a noteworthy polyphenolic component present in *Glycyrrhiza glabra* (fabaceae), prevents LDL oxidation [52]. By serving as flavorings, colorants, and antioxidants, flavonoids are commonly referred to affect the stability and quality of food [53, 54]. The flavonoids found in berries may assist older adult’s cognition and may also have a beneficial influence on

Parkinson's disease. In hypertensive rats, the total flavonoid portion of *Astragalus complanatus* has been shown to have an anti-hypertensive effect [55]. The risk of dementia incidence has been negatively correlated with antioxidant flavonoid intake [56].

The solubility of flavonoids may be a key factor in their medicinal effectiveness. With the possible exception of an occasional allergic reaction, humans are not able to experience immediate harmful consequences from consuming flavonoids due to their poor solubility in water, short intestinal half-life, and decreased absorption. The flavonoid's poor water solubility frequently poses an issue for their potential medical uses. Therefore, the creation of semisynthetic, water-soluble flavonoids, such as inositol-2-phosphate quercetin and hydroxyl-ethyl-rutosides, has been linked to the management of micro bleeding and hypertension [57].

Table 2: Plants used as medicine that are high in flavonoids

Plant	Flavonoids	References
Aloe vera (Asphodelaceae)	Luteolin	[23]
Acalypha indica (Euphorbiaceae)	Kaempferol glycosides	[23]
Azadirachta indica (Meliaceae)	Quercetin	[24]
Andrographis paniculata (Acanthaceae)	5-hydroxy-7,8-dimethoxyflavone	[25]
Bacopa moneirra (Scrophulariaceae)	Luteolin	[23]
Betula pendula (Betulaceae)	Quercetrin	[25]
Butea monospermea (Fabaceae)	Genistein	[26]
Bauhinia monandra (Fabaceae)	Quercetin-3-o-rutinoside	[26]
Brysonima crassa (Malphigaceae)	(+)-Catechin	[27]
Calendula officinalis (Compositae)	Isorhamnetin	[25]
Cannabis sativa (Compositae)	Quercetin	[25]

Citrus medica (Rutaceae)	Hesperidin	[23]
Clerodendrum phlomidis (Verbenaceae)	Pectolinarigenin	[24]
Clitoria ternatea (Fabaceae)	Kaempferol-3-neohesperidoside	[28]
Glycyrrhiza glabra (Leguminosae)	Liquiritin	[25]
Mimosa pudica (Mimosoideae)	Isoquercetin	[29]
Limnophila indica (Scrophulariaceae)	3,4-methlenedioxyflavone	[29]
Mentha longifolia (Lamiaceae)	Luteolin-7-o-glycoside	[30]
Momordica charantia (Curcurbitaceae)	Luteolin	[31]
Oroxylum indicum (Bignoniaceaea)	Chrysin	[29]
Passiflora incarnate (Passifloraceae)	Vitexin	[25]
Pongamia pinnata (Fabaceae)	Pongaflavonol	[32]
Tephrosia purpurea (Fabaceae)	Purpurin	[29]
Tilia cordata (Tiliaceae)	Hyperoside	[25]

4. Novel Extraction Methods of Flavonoids

4.1 UAE

The separation of bioactive compounds from natural goods is a common usage for ultrasound, an intensification technology that has applications in the provisions and pharmaceutical sectors [33]. The intensification procedure is based on the incident known as acoustic cavitation, which is the result of cycles of compression and expansion brought on by ultrasonic waves that pass through the liquid [34]. The rupture that follows, which releases bioactive compounds, is dependent on the circumstances of extraction [35, 36]. The intermolecular connections between the target chemicals and the sample matrix are disrupted, and mechanical consequences on the extraction solvent and sample matrix composition are produced when bubbles finally collapse and release the energy they have accumulated. Cell destruction and size of particle reduction are two mechanical outcomes that allow for greater substantial solvent-sample interaction and, consequently, better mass transfer, resulting in higher yields in less time [36]. A further implication of cavitation is the enhanced solubilization of the amalgamation produced during the extraction process, due to the ultrasonic waves promoting the liquid's movement beyond the shear forces and turbulence generated by the rupture of bubbles [37, 38].

UAE may generally be investigated in more environmentally friendly procedures because of its great efficiency, which permits less energy and solvent usage. The UAE provides higher-purity end products, simpler manipulation and processing, faster extractions with excellent reproducibility, and a quicker return on investment than traditional extraction methods [39, 40]. A range of matrices that include a variety of bioactive components from different groupings, like fruits, seeds, vegetables, teas, or flowers, are covered by UAE [41, 42]. However, the circumstances and permutations of the process's variables need to be carefully specified based on the kind of sample and substance to be extracted. These factors encompass the frequency, typically ranging from 20 kHz-100 MHz, the ultrasonic energy output, the time span of exposure, the thermal conditions, the quantity and formulation of the sample, as well as the selection, volume, and concentration of the solvent employed [43, 44].

4.1.1 Factors Affecting UAE Methods

Natural product extraction is a complicated process in which any component, either alone or in combination, can have an impact on the outcome. Assessing every element of the process, including the solvent, power, sample, frequency, intensity, duration, temperature, and apparatus type, as well as how they interact, is crucial.

4.1.2 Solvent for extraction

The most important factor in any extraction process is unquestionably the solvent selection. When choosing the extraction solvent, the main factors to be taken into account should be the ability to dissolve and the effectiveness of its connections with the matrix. The parameters of the solvent that must be watched include polarity, vapor pressure, viscosity, pH, surface tension, melting and boiling temperatures, density, specific gravity, and the effect on the activity and purity of the extracted substance [45]. These elements should be carefully considered primarily because they lower the cavitation threshold, which makes it more difficult to remove the compounds from the matrix [45].

The targeted intermediate and outcomes, the solvent's ability to interact with the target compounds under extraction conditions, and the process of extraction characteristics and their suitability for the solvent should all be considered. The solvent's physicochemical and biological characteristics are crucial as they not only interact with the extracted substances and treated material but also determine the medium's nature. Potential modifications in the solvents that may occur during the extraction process may have a substantial influence on the long-term viability of the flavonoids and the efficacy of the therapies [46]. At the proper temperature, a solvent with a low vapor pressure promotes cavitation, enhancing the process's ultrasonic effects. On the other hand, viscous fluids, such as oils, allow the waves to amplify more, which prevents ultrasonic waves from propagating and the cavitation's mechanical impacts on the sample [47, 48].

Utilizing organic solvents such as acetone, acetonitrile, ethanol, methanol, and petroleum ether, along with water and their combinations, flavonoids are commonly extracted from various plant matrices, including herbs, industrial byproducts, stems, or seeds. Through the application of organic solvents like methanol, acetone, ethanol, and isopropanol in conjunction with diverse concentrations of water, flavonoids have been effectively extracted from botanical materials via UAE. There are extraction methods where either water or an organic solvent is utilized exclusively. Some studies indicate that the oxidative degradation of sensitive flavonoids can be mitigated by acidifying the extraction solvents [49]. Hydrogen cations (H+), generated by acidic compounds, play a pivotal role in the stabilization of free radicals potentially produced during the process of ultrasonication. [46]. A multitude of investigations have illustrated that organic solvents, such as methanol, exhibit enhanced efficacy in the extraction of flavonoids due to their polar characteristics. Contemporary research emphasizes the utilization of non-harmful and biodegradable alternatives, such as ethanol, in extraction techniques to alleviate the environmental repercussions associated with organic solvents, while achieving similar or possibly enhanced effectiveness [50]. Novel substitutes for hazardous solvents include ionic or eutectic solvents that integrate citric and lactic acids, in addition to multiphasic systems like cloud point extraction [51-54].

The solvent pH can also influence the extraction efficacy by varying the ionic strength, specifically affecting the chemical's solubility and relations to the sample matrix. The ideal pH for removing flavonoids from plant matrices has been assessed in several researches. A recent study [55] investigated how the pH of the solvent affects the quantity of flavonoids recovered from *Euonymus alatus* and discovered that recoveries decreased in higher pH ranges and increased in lower pH ranges (2.5–3.5). Another study that evaluated polyphenols in this case discovered that the solvent's pH affects how well they are extracted from pomegranate peel, having the best consequences taking place in an acidic medium [56]. Lower extraction yields were observed at pH values higher than 7.0.

For the UAE of bioactive chemicals from the bark of *Citrus reticulata*, faintly acidic electrolyzed water (pH 6.20) was the most effective method in favor of extracting total phenolic components. However, it has been found that acid-electrolyzed water with a pH of 3.24 produced greater flavonoid yields [57]. In the UAE, an investigation into the polyphenolic content in the leaves of *Satsuma mandarin* revealed that a superior yield of total flavonoids was observed at a pH level of 2 in aqueous solutions, thus exemplifying the significant impact of pH on phytochemical extraction. The maximum concentrations of total flavonoids and total phenolic compounds were detected in acidic environments [58].

Higher flavonoid yields are often generated in acidic media, according to findings found in the literature. The breakdown of phenolics attached to proteins and carbohydrate polymers is supported by an acidic pH, which explains this tendency for polyphenols [59]. According to El-Abbassi et al. (2014), phenols that have been protonated at low pH levels acquire a hydrophobic character, facilitating their easier passage through the micelles and stronger interaction with the hydrophobic micellar surfactant. Phenols deprotonate and become more ionic at higher pH values. As a result, hydrophobic phenolic compounds become less soluble in micelles because of the increased proton activity. Accordingly, when the pH decreases, more phenols are removed [60, 61].

4.1.3 Sample

The amount of chemicals recovered from the sample matrix will depend on the material's structure, moisture, resilience, and composition. The sample may be fresh or dried, depending on the target compounds (plants, oleaginous, seeds, yeast, algae, etc.). Therefore, it is crucial to prepare the sample matrix before extraction, particularly since certain chemicals are sensitive to preparation procedures including sifting, drying, and homogenization. The sample preparation guarantees extraction effectiveness