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REVIEW ARTICLE

Profiling of Immunomodulatory Flavonois From Leafy Vegetables Available in West Bengal - An Overview of Their Mechanism of Action

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Abstract

In recent years, the increasing incidence of autoimmune diseases such as cancer and viral diseases including COVID-19 poses a serious problem in terms of prevention, diagnosis, prognosis and therapy. Various kinds of active immunomodulatory ingredients are one of the main components of the continuous breakthroughs of pharmaceuticals until recent research. Flavonoids are significant phytochemicals that are thought to be principally responsible for plants' immunomodulation function. These phytochemicals can also serve as a model for the creation of safe and effective immunomodulators as potential treatments for the prevention and treatment of various immune-related disorders. Leafy vegetables give stronger support for human health due to their overlapping nutritional and therapeutic advantages. The goal of the present review was to profile the immunomodulatory flavonoids from the leafy vegetables of West Bengal to support the prevalent immune-related disorders worldwide. The majority of the leafy vegetables with significant immunomodulatory action are outlined, along with their potential mechanisms and quantity of the response flavonoids. The present work will pick the interest of researchers and promote additional studies on these leafy vegetables-based immunomodulation agents as prospective therapies for the treatment the various immune-suppressed disorders in future days.

Keywords: Flavanoids, Immunomodulatory, Leafy Vegetables

1. Introduction

Public health is greatly impacted by nutrition and metabolism, highlighting the essential and undeniable role they play in overall wellbeing[1] immunity is our body's natural defense system[2,3]. It defends against diseases, infections and damaged cells in our body to maintain health and prolong life with all organs and processes contributing tools to build this immune system. The two broad categories of the immune system have been identified based on their distinct mechanisms and functions i.e. adaptive immune system (specific or acquired immune system) and innate immune system (non-specific immune system)[2]. Innate immunity consists of immune and non-immune components, while acquired immunity contains only an immune component. The adaptive immune system plays a vital role in immune cells, mainly ligamentous lymphocytes, which recognize pathogens based on their antigen receptors and react differently to them. B lymphocytes produce antibodies that block the activity of the pathogen, and T cells split into two subgroups: cytotoxic T cells (CD8 + T), which kill cancer cells directly, and T helper cells (CD4 + T), which secrete cytokines and mediators that other

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cells such as B lymphocytes and macrophages[4-6]. Th cells emit the cytokines that make up the types of antibodies produced by B cells, as well as activate and polarise monocytes and macrophages. As a result, Th cells perform a critical function in the immune system.

Many researches over the last few decades have demonstrated that natural substances may be widely utilized to treat a variety of ailments by controlling immunity. Flavonoids, for example, are widely dispersed in nature and have a wide range of biological actions. Flavonoid is a large group of polyphenolic compounds having a benzo-γ- pyrone found in plant kingdom[7,8]. As of 2021, over 6,000 different flavonoid species and their structures have been identified across the globe[3]. The common structure of this compound is phenylbenzopyrone (C_6-C_3-C_6). Flavonoids are categorized into various classes based on the level of saturation and the degree of openness of their central ring structure (Figure1)[1]. Flavonoids act as a unique UV filter and protect the plant from various biotic and abiotic loads. Flavonoids give different types of biological and antioxidant activity such as anti-cancer agents[9], anti-diabetic agents[10], anti-inflammatory, anti-viral[11] including immunomodulatory[12]. Large numbers of research have established the immunomodulatory potential of flavonoids.

The present review aims to provide a comprehensive overview of the immunomodulatory flavonoids present in leafy vegetables from West Bengal and their potential role in managing immune-related disorders globally.

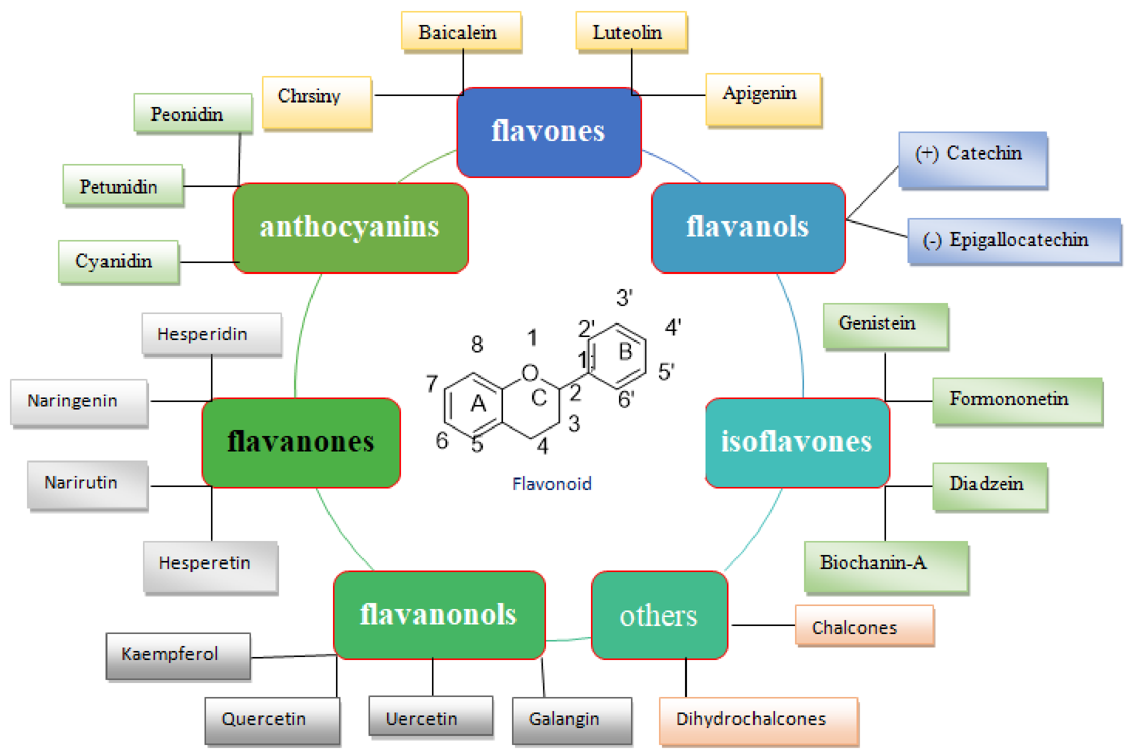

Figure 1. Classification of flavonoids, and there seven subgroups, according to their chemical and biological properties: flavanols, flavanones, flavones, flavonols, isoflavones, anthocyanins, and others.

2. Leafy Vegetables

Term vegetable was "food grown from plants, edible herbs, or roots". Based on the origin, the vegetables can be divided into different classes, such as bulb vegetables, fruit vegetables, inflorescence vegetables, leafy vegetables and root vegetables[13]. The plants whose leaves and sometimes its petiole, shoots are eaten called as leafy vegetables. Since ancient periods, these leafy vegetables are being used as a source of food[14]. The presence of vitamins, minerals, trace elements and fiber has a high nutritional value[13]. Leafy vegetables have a significant impact on the ecosystem and economy of rural communities in West Bengal. The rural communities preferred these vegetables for several reasons mainly because of easy availability in the area and, economy[15,16]. Many modern-day customers are not fully aware of the significance of leafy vegetables in their daily diets, which is reflected in the market demand.

3. Flavonoids from 40 Leafy Vegetables and their Biological Activity

In this study, a total of 40 leafy vegetables from 26 different families were examined. The Amaranthaceae family possesses maximum leafy vegetables, followed by Brassicaceae, Araceae, Fabaceae, Cucurbitaceae, Apiaceae, and others. Vegetables are a excellence source of high concentration of minerals, vitamins (vitamin C, vitamin A, vitamin K, vitamin B6, and others), provitamins, and carbohydrates, as well as a significant quantity of Zn, Mn, Cu, Se, Sr, Rb, and Br and fibre which allows one to preserve the digestive gadget healthy, and they're additionally low in fats and calories. It also includes several phytochemicals that aid in various forms of biological activity, such as antifungal, antioxidant, antibacterial, antiviral, and anti-carcinogenic action, as well as immunomodulatory function[13]. Different types of leafy vegetables, their scientific name, family and their different biological action are given in Table 1.

Table 1. List of 40 leafy vegetables available at West Bengal[13,17,18]

Sl. No.	Common Name	Scientific Name	Flavonoids	Biological Activity
Amaranthaceae family				
1	*Palong shak*	*Spinacia oleracia*	Quercetin, kaempferol, apigenin	Antioxidative, Antiproliferative, and Antiinflammatory properties
2	*Sada notey*	*Amaranthus tricolor*	Quercetin, kaempferol, Luteolin	Antimicrobial and Antioxidant activity.
3	*Lal notey*	*Amaranthus dubius*	Quercetin, kaempferol, Rutin	Antioxidant, Antimalarial, and Antiviral properties.
4	*Latamouri/ Gungutiya*	*Digera muricata*	Rutin and hyperoside flavonoids	Anthelmintic, Central nervous system depression (CNS) Depressant, Antiepileptic. Anti-inflammatory.
5	*Beet shak*	*Beta vulgaris*	Apigenin, Luteolin,	Anthelmintic, Central nervous system depression (CNS) Depressant, Antiepileptic. Anti-inflammatory.
6	*Kata note*	*Amaranthus viridis*	quercetin and lutein and rutin	Anticancer, Hypoglycemic, Aphrodisiac, Antimicrobial, Antioxidant activity.
7	*Sanchi*	*Alternanthera philoxeroides*	flavone glycoside, kaempferol	Antimicrobial, Antioxidant and cell proliferative properties, Antidiabetic
Fabaceae family				
8	*Methi shak*	*Trigonella foenumgraecum*	quercetin, kaempferol	Antidiabetic, Antinociceptive, Anticarcinogenic, Antioxidant, Anti-inflammatory, and Hypocholesterolemic
Meliaceae family				
9	Neem	*Azadirachta indica*	Quercetin	Antibacterial,Antifungal, Anti-inflammatory, Antipyretic,
Plantaginaceae family				
10	*Bramhi shak*	*Bacopa monnieri*	Apigenin, quercetin	Antibacterial, Anti-fungal, Anti-cancer, Anti-oxidant, Anti-inflammatory,

Table 1 continued...

Sl. No.	Common Name	Scientific Name	Flavonoids	Biological Activity
		Basellace family		
11	*Puishak*	*Basella albe*	4,7- dihydroxy kempferol	Androgenic activity, Antiulcer activity, Antioxidant, Cytotoxic and antibacterial activity,
		Apiaceae family		
12	*Thankuni*	*Hydrocotyle verticillata*	Quercetin, Rutin, kaempferol	Anti-inflammatory , Anti psoriatic, Antiulcer, Hepatoprotective, Anticonvulsant, Sedative, Immunostimulant .
		Tiliaceae family		
13	*Mitha pat*	*Corchorus olitorius*	Quercetin glycoside	Antioxidant, Anti-tumor, Hypoglycemic, Antimicrobial, Anti-inflammatory, Analgesic
		Umbelliferae family		
14	*Dhoney*	*Coriandrum sativum* L.	Apigenin, Quercetin, Kaempferol**.**	Diuretic, Antioxidant Activity, Anti-microbial Activity, Anti mutagenic, Anthelmintic activity.
		Asteraceae family		
15	*Hingcha*	*Enhydra fluctuans*	Baicalein-7-o-glucoside and Baicalein-7-o- diglucoside	Anti-microbial, Anti-inflammatory, CNS depressant, Anti-oxidant, Hepatoprotective, Analgesic, Anti-diarrheal, Thrombolytic, Anti-diabetic, Phagocytic, cytotoxic and Neuroprotective agents.
		Acanthaceae family		
16	*Kulekara*	*Hygrophila auriculata*	Apigenin 7-O- glucuronide, apigenin 7-O- glucoside, Apigenin, Luteolin.	Anticancer, Hypoglycemic, Aphrodisiac, Antimicrobial, Antioxidant, Lipid peroxidation, Hepatoprotective activity.
		Athyriaceae family		
17	*Dhekishak*	*Diplazium esculentum*	Quercetin,	Antioxidant, Antimicrobial, Antidiabetic, Immunomodulatory, CNS stimulant, and Antianaphylactic activities.
		Commelinaceae family		
18	*Kanchara*	*Commelina bengalensis*	Flavones C glycosides, flavonol O-glycoside, Quercetin	Laxative, Anti-inflammatory, Anti-microbial, Anti-cancer, Sedative, Analgesic, Hepatoprotective, Anti-depressant, Anti-viral, Antioxidant, Antidiarrheal, Demulcent, Emollient, Diuretic and Febrifuge.
		Cucurbitaceae family		
19	*Lau shak*	*Lagenaria siceraria*	Flavone C-glycosides	Analgesic, Anti-inflammatory, Anti hyperlipidemic, Diuretic, Hepatoprotective, Anthelmintic, and Antibacterial.
20	*Kumro shak*	*Cucurbita maxima Duchesne*	Flavonoids	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer and Neuroprotective agents.

Table 1 continued...

Sl. No.	Common Name	Scientific Name	Flavonoids	Biological Activity
21	*Telakuch*	*Coccinia granis*	Flavonoids	Antimicrobial, Antioxidant and cell proliferative properties, Antidiabetic activity.
Brassicaceae family				
22	*Fulkopi pata*	*Brassia oleracea*	Quercetin, kaempferol and isorhamnetin	Anticancer-activity against colon and lung cancers, Antioxidant activities, decrease of lipid Peroxidation under diabetic oxidative stress.
23	*Mulo shak*	*Raphanus sativus*	kaempferol-3	Antimicrobial and Antiviral activity.
24	*Sores shak*	*Brassica juncea*	Quercetin, kaempferol	Anticancer, Hypoglycemic, Aphrodisiac, Antimicrobial, Antioxidant
25	*Badhakopi pata/ Patakopi*	*Brassica oleracea*	Quercetin, kaempferol	Antimicrobial, Antioxidant and cell proliferative properties, Antidiabetic activity.
Araceae family				
26	*Kochu shak*	*Colocasia esculenta*	Apigenin, luteolin, anthocynin	Antihelminthic, Anti-diabetic and Anti-inflammatory
27	*Kharkolpata*	*Typhonium trilobatum*	Quercetin and catechin	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer and Neuroprotective agents.
28	*Mankachu*	*Alocasia macrorrhizs*	Flavonoids	Antidiuretic, Anticancer, Antioxidant, Antimicrobial, Antidiabetic, Antihyperglycemia
29	*Oal*	*Amorphophallus bulbifer*	Flavonoids	Antioxidant, Antimicrobial, Antidiabetic
Convolulaceae family				
30	*Kalmishak*	*Ipomoea aquatica*	Quercetin	Anthelmintic, central nervous system depression (CNS) depressant, Antiepileptic. Anti-inflammatory.
Molluginaceae family				
31	*Gima shak*	*Glinus oppositifolius*	Apigenin, kaempferol glycoside.	Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer and Neuroprotective agents.
Moringaceae family				
32	*Sojne pata*	*Moringa olelfera*	Flavanoids and flavanol glycoside	Anti-inflammatory, analgesic, Antipyretic, Antidiabetic, Antioxidant, Antiparasitic, Anticancer
Apiaceae family				
33	*Radhunipata*	*Trachyspermum roxburghianum*	Flavonois	Anti-inflammatory, Analgesic, Antipyretic, Neural and smooth muscle effects, Antimicrobial, Antidiabetic, Antioxidant, Antiparasitic,
Lamiaceae family				
34	*Pudina pata*	*Mentha aspera*	Kaempferol, fisatin	Anticancer, Hypoglycemic, Aphrodisiac, Antimicrobial, Antioxidant

Table 1 continued...

Sl. No.	Common Name	Scientific Name	Flavonoids	Biological Activity
Convolvulaceae family				
35	*Dhudla shak*	*Hewittia malabarica*	Flavonoids	Aphrodisiac, Antimicrobial,
Alliaceae family				
36	*Piyaz*	*Allium cepa*	Kaemferol, quercetin	Antioxidant activity
Malvaceae family				
37	*Lafa shak*	*Melva verticillata*	-	Anti-inflammatory, Analgesic, AntipyreticAntioxidant, Antiparasitic, Anticancer.
Menispermaceae family				
38	*Guloncha*	*Tinospora cordifolia*	Quarcetin	Anticancer, Hypoglycemic, Aphrodisiac, Antimicrobial, Antioxidant,Lipid peroxidation, Hepatoprotective and Hematopoietic activity
Acanthaceae family				
39	*Kalmegh*	*Andrographis paniculata*	5,2'- dihydroxy-7,8 dimethoxyflavone or skullcap flavone	Antimicrobial, Antioxidant and cell proliferative properties, Antidiabetic activity.
Verbenaceae family				
40	*Bhat*	*Clerodendrum inerme*	Flavonoid and flavonoid glycoside.	Anti-inflammatory, Analgesic, Antipyretic, Antidiabetic, Antioxidant, Antiparasitic.

4. Immunomodulatory Flavonoids

Immunomodulators are substances, either synthetic or biological, that aid in the modulation, suppression, or stimulation of both the adaptive and innate immune systems[12]. These agents can also be used to stimulate the immune response of certain people such as COVID-19, immunocompromised patients. Worldwide people start realizing the role of the immune system, because, dramatically increases life-threatening infections such as cancer patients, transplant recipients, AIDS patients and COVID-19. These immunomodulators can act the Both the innate and adaptive branches of the immune system can be modulated, suppressed, or stimulated by immunomodulators[10], it does not affect any immunological memory cells. Different types of herbal compounds are used as immunostimulants. This herbal compound rapidly increases the immunostimulant activity[15].

This review covers the flavonoids present in 40 leafy vegetables from 26 different plant families (Table 1). Several flavonoids with immunomodulatory activity were identified, including Quercetin, Kaempferol, Apigenin, Rutin, Luteolin and others (Table 2). Their immunomodulatory mechanism has also been explored in Table 2. The rest of the flavonoids from Table 1 has the hypothesized mechanism of action for immunomodulatory activity, such as Butein, Xanthohumol, Dihydroxanthohumol, Daidzein, and flavonoids like Urcetin, Galangin, Procyanidin. Narirutin, Arbutin, and don't exhibit immunomodulatory activity.

Table 2. Different types of flavonoids and their immunomodulatory mechanism

Sl No.	Name of the Flavonoids	Mechanism of Action	References
1	Quercetin	• Decrease the production of pro-inflammatory cytokines/ chemokines and the expression levels of MHC class II and co-stimulatory molecules.	19
2	Kaempferol	• Reduce iNOS and COX-2 activity through suppression of the signaling of STAT-1, NF-kappa B, and AP-1.	20
3	Apigenin	• Impairing DC phenotypic and functional maturation.	21
4	Naringenin	• Increasing NKG2D ligands (MIC-A/B, ULBP-1 and ULBP-2).	22
5	Baicalin	• Inhibiting mTOR activation.	23
6	Genistein	• Down regulating GATA-3 and STAT-6.	24
7	Tangeretin	• Down regulation of Notch and PI3K signalling pathway.	25
8	Daidzein	• Decreases TNF-α, IL-1β, MCP-1, NO, and iNOS expression at mRNA level.	26
9	Luteolin	• Decreased secretion of inflammatory mediators (INF-γ, IL-6) reduced COX-2, ICAM-1 expression.	27
10	EGCG	• Inhibiting mitogen-activated protein kinases and NF-κB;	28
11	Chrysin	• Reducing the expression of CD80, CD83, CD86 and HLA-DR.	29
12	Taxifolin	• Inhibiting T-bet, GATA-3 and RORγt.	30
13	Hesperetin	• Activating PI3K/ Akt/ IL-6 signalling pathway.	31

5. Five Most Potent Immunomodulatory Flavonoids

The leafy vegetables examined contained varying quantities of different flavonoids (Figure 1). Among those, five flavonoids apigenin, quercetin, kaempferol, luteolin, and rutin (Figure 2) were present in most of the leafy vegetables and are high in amount. Also, the immunomodulatory activities of these five flavonoids were found to be quite potent, according to the literature survey. The quantitative amounts of quercetin, apigenin, kaempferol, rutin and luteolin are recorded in the table below (Table 3).

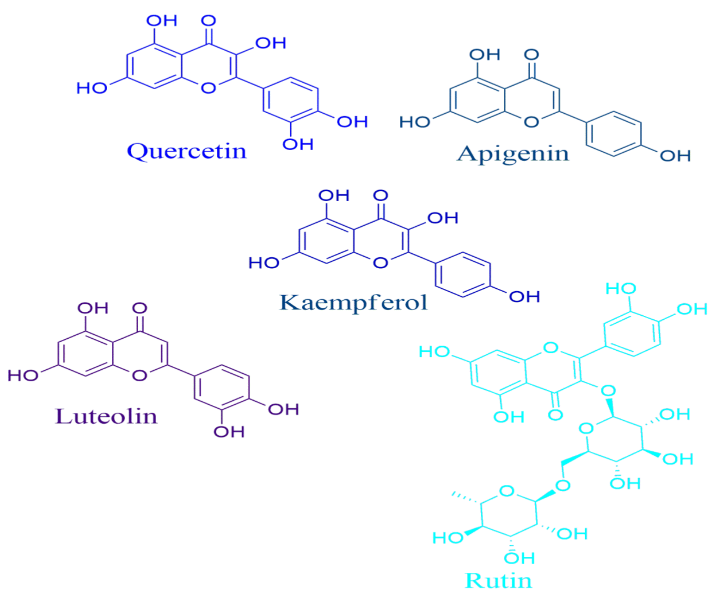

Figure 2. Chemical structure of five commonly available flavonoids.

Table 3. Quantity of five commonly available flavonoids in leafy vegetables and their total flavonoid content[27,32,33]

Sl No.	Common Name	Scientific Name	Quercetin (mg/100 g fw)	Apigenin (mg/100 g fw)	Kaempferol (mg/100 g fw)	Rutin (Mg/100gfw)	Luteolin (mg/100 g fw)	Tfc (mg qe/g)
1	Sada notey	*Amaranthus lividus*	4.4	1.8	0.0	0.005832	0.0	6.2
2	Lal notey	*Amaranthus cruentus*	0.0	0.0	0.0	24.5	0.0	0.0
3	Beet shak	*Beta vulgaris*	0.00228	0.00005	0.00104	1.2	0.0096	0.0
4	Badhakopi pata/ Patakopi	*Brassica oleracea*	0.0	0.0	0.0	3.11	0.0	0.0
5	Dhoney	*Apium graveolens*	0.0	2.5	0.0	0.598	1.0	3.4
6	Kharkolpata	*Momordica cochinchinensis*	78.0	0.0	32.0	0.0	0.0	110
7	Sojne pata	*Moringa oleifera*	89.8	0.0	36.3	23.39	0.0	129
8	Kumro shak	*Cucurbita maxima*	1.4	0.0	2.1	0.0	0.0	4.6
9	Telakuch	*Coccinia grandis*	6.19	0.0	111	45.63	0.0	118
10	Mitha pat	*Corchorus capsularis*	4.2	0.0	13.8	2.76	0.0	18.1
11	Kalmishak	*Ipomoea aquatica*	0.0	0.0	0.0	7.07	0.0	0.2
12	Puishak	*Basella alba*	0.0	1.4	1.4	0.0	0.0	2.8
13	Palong shak	*Spinacia oleracea*	5.0	17.0	3.0	0.0	0.0	25
14	Thankuni	*Hydrocotyle verticillata*	95.5	0.0	11.4	0.0	0.0	108
15	Kalmegh	*Polygonum odoratum*	118	0.0	25.5	3.77	0.0	144
16	Bramhishak	*Bacopa monnieri*	24.36	0.0	0.0	3.30000	0.0	24.36
17	Fulkopi pata	*Brassia oleracea*	21.9	0.0	0.0	0.000311	0.0	21.9
18	Mulo shak	*Raphanus sativus*	0.52	0.22	3.23	0.0052	1.95	5.92
19	Methi shak	*Trigonella foenumgraecum*	0.0	0.0	0.0	0.417	0.0	4.85
20	Piyaz	*Allium cepa*	149.75	0.0	83.2	0.157	39.1	272.05
21	Kata note	*Amaranthus viridis*	9.12	0.0	0.0	58.52	0.0	0.0
22	Sanchi	*Alternanthera philoxeroides*	1.886	0.0	0.9119	0.016	0.0	0.0

6. Mechanism of the Five Potent Immunomodulatory Flavonoid

6.1 Quercetin

It belongs to the flavonol group (Figure 1), the most common subclass of flavonoids (Figure 2). It is found in a wide range of foods and plants, including berries, apples, cabbage, grapes, capers, onions, shallots, tea, and tomatoes, as well as seeds, nuts, flowers, bark, and leaves[1]. These flavonoids are found in various forms in plants, often conjugated with sugars such as glucose, galactose, and rhamnose[34]. One unique characteristic of flavonoids is their potential to enhance both mental and physical performance while also reducing the risk of infections[10]. Quercetin has been proven to influence lipid and glucose metabolism by lowering oxidative stress.

Some experimental studies on different animal models (SNU-C4, SNU1, K562, NK-92 cell lines; C57BL/6J mice, EGFP mice and ApoE−/− mice), have indicated that this flavonoid efficiently reduces and regulate the production of pro-inflammatory cytokines and chemokines, as well as the expression levels of MHC class II and co-stimulatory molecules. Lipopolysaccharide (LPS) activated dendritic cells (DCs) under such conditions[1]. Quercetin also increased TNF- and IL-1 mRNA levels, This property of flavonoids may help to reduce apoptotic neuronal cell death resulting from microglial activation[10]. Quercetin considerably increases gene expression, as well as the generation of Flavonoids can downregulate interleukin 4

(IL-4), which is derived from Th-2 cells, and interferon (IFN) derived from Th-1 cells. The phenotypic expression of INF- cells was elevated whereas IL-4 positive cells were decreased[10].

Quercetin can also modulate the expression of Th2 cytokines, including IL-4 and IL-5. Activated mast cells regulate the secretion of chemical mediators (mMCPT-1, PGD2, Cys-L, and TSLP), and also suppressed the gastrointestinal cytoprotective activity[1,35]. Additionally, it has been found that flavonoids can inhibit the release of RcεRI mediators of pro-inflammatory cytokines, histamine and tryptase. The inhibition of TNF- by flavonoids can facilitate the translocation of NFB p65 to the nucleus[35] (Figure 3).

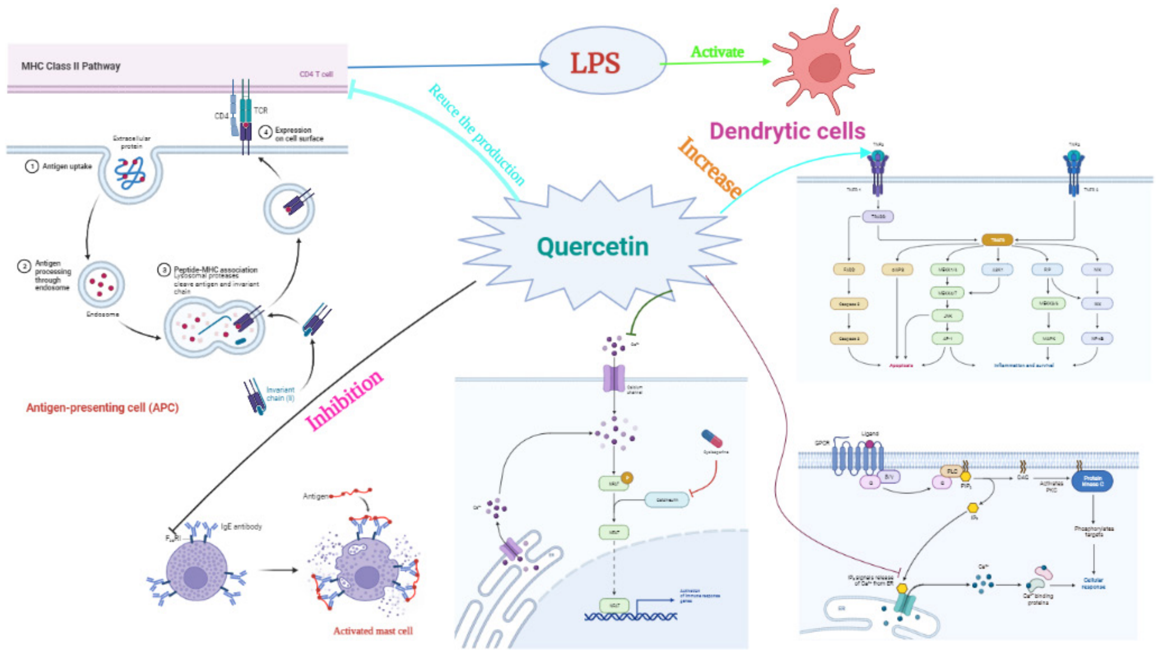

Figure 3. General mechanism of action of Quercetin.

6.2 Kaempferol

Kaempferol is classified as a flavonol (Figure 1), the most significant subtype of flavonoids. Its chemical name is 3,5,7trihydroxy2 (4hydroxyphenyl)4H1benzopyran4 one (Figure 2) . It is one of the most common aglycone flavonoids[9]. Kaempferol has biological effects that include Cardioprotective, neuroprotective, anti-inflammatory, antidiabetic, antioxidant, antibacterial, antitumor, and anticancer properties[36].

kaempferol reduced LPS-induced Tumour Necrosis Factor (TNF) and interleukin 1 (IL1) expression; inhibition of TNF-facilitates the translocation of NFB p65 to the nucleus. Inhibiting LPS-induced decreases in the levels of chondrogenic indicators, such as SOX9, Collagen II. The apoptotic signal-regulating kinase 1 (ASK1)/JNK1/JNK2/p38 signalling pathway was reduced by kaempferol. Kaempferol regulates the ASK1/MAPK signalling pathway and oxidative stress (Figure 4).

6.3 Apigenin

Apigenin is the subclass of flavones type of flavonoids (Figure 1), the chemical name of that compound is 4,5,7-trihydroxyflavone (Figure 2). And it is common type of dietary flavonoid, widely distributed in different types of leafy vegetables (*kachu shak*, *kalmi shak*), and herbs, such as orange, parsley, wheat sprouts, grapefruits, onion, celery, and chamomile tea[1]. Apigenins are generated in plants via the phenylpropanoid pathway, which transforms L-Phe in three phases. They give some different types of biological activity such as anti-proliferative, antioxidant, anti-cancer and anti-inflammatory activities. Apigenin is gaining popularity due to its ability to suppress the PI3K/Akt/mTOR signalling pathways (Figure 5).

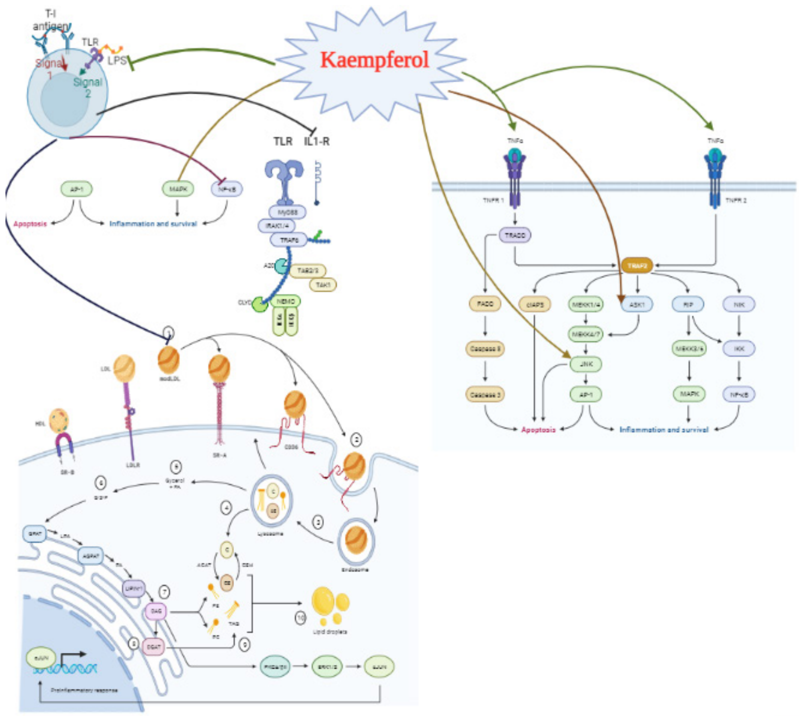

Figure 4. General mechanism of action of Kaempferol.

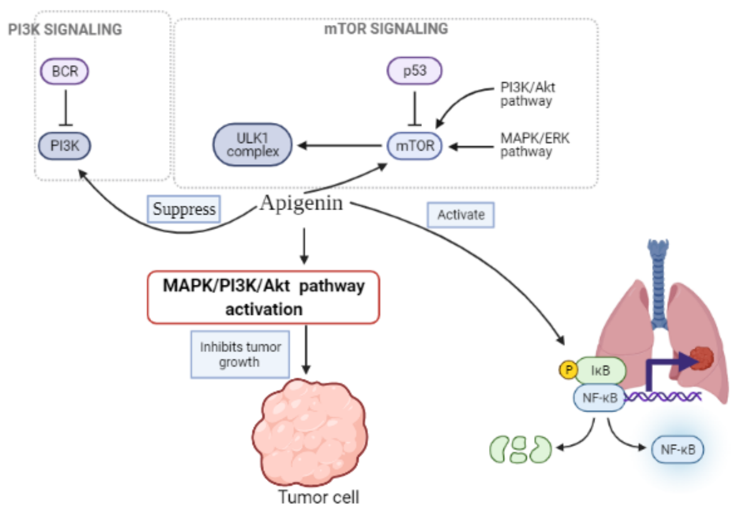

Figure 5. General mechanism of action of Apigenin.

Studies have shown that apigenin can inhibit tumour growth and induce apoptosis in HepG2 human hepatoma cells by activating the pentose phosphate system, which generates NADPH. This activation causes apoptosis via the PI3K/AKT and ERK1/2 MAPK pathways, lowers cancer cell viability, adhesion, and migration, and modulates angiogenesis and metastasis[37].

Apigenin has a significant effect on NF-B activation in the lungs. This discovery demonstrated Apigenin has been shown to have immune-regulatory functions in an organ-specific manner[1].

Apigenin, in combination with Quercetin and Luteolin, protects pancreatic beta-cells against cytokine-induced damage during inflammation. Recent research has demonstrated apigenin's inhibitory effect on mast cell secretion. Both apigenin and luteolin are potent inhibitors of T-cell responses in both mice and humans, particularly auto-reactive T cells. These findings suggest that apigenin plays an important regulatory role in the immune system[1].

6.4 Rutin

Chemical name of rutin is 3,3′,4′,5,7pentahydroxyflavone-3-rhamnoglucoside (Figure 2). Rutin is a powerful antioxidant that also has biochemical activities in cancer prevention and treatment. Around 130 derivatives have been synthesized from rutin[11]. It is present in plants such as buckwheat, green tea, and apples, as well as several types of leafy vegetables. Rutin reduces oxidative stress by inhibiting lipid peroxidation, making it anti-inflammatory, anticarcinogenic, neuroprotective, antiproliferative, antimetastatic, and antioxidative stress[38].

Reactive Oxygen Species (ROS) can damage DNA, cause chromosomal mutations, alter gene expression, interfere with cell division, and disrupt cell development. They may also decrease the activity of various proteins involved in antioxidant systems[39]. Rutin gives pharmacological benefits by inhibiting platelet aggregation and the downstream pathway of cyclooxygenase-1[40].

Rutin has been shown to have anti-inflammatory effects by reducing the expression of pro-inflammatory cytokines such as TNF-α, IL-6, IL1β, and COX2, and down-regulating inflammatory markers, as well as exhibiting antiproliferative effects. In both *in vivo* and *in vitro* studies, rutin's anti-inflammatory effect has been primarily attributed to the modulation of MAPKs and NF-κB signalling pathways[40] (Figure 6).

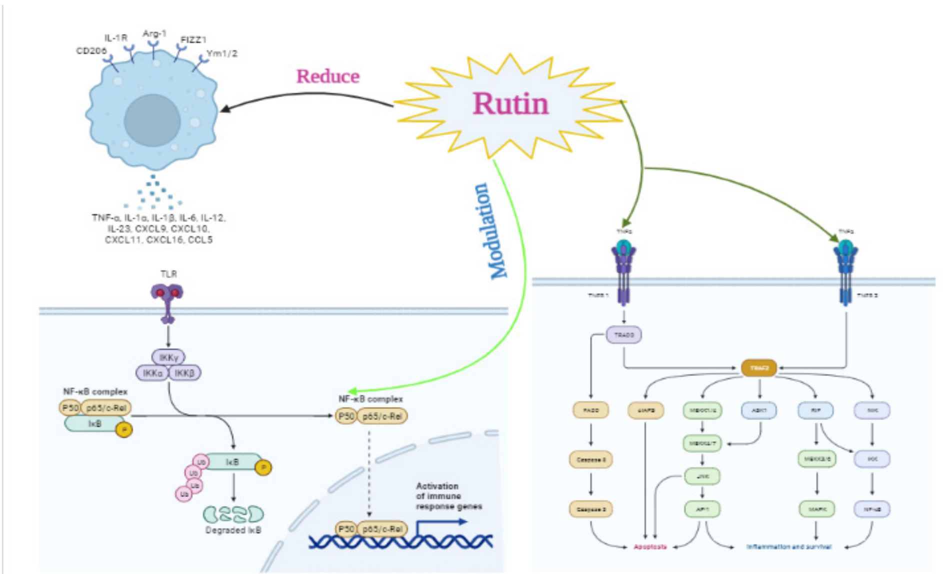

Figure 6. General mechanism of action of Rutin.

6.5 Luteolin

Luteolin is the common type of flavonoid in the subclass of flavones (Figure 1). The chemical name of that flavonoid is 3′,4′,5,7-tetrahydroxyflavone (Figure 2). It is widely distributed in different fruits medicinal plants and leafy vegetables (parsley, sweet peppers and celery)[1,41]. It also gives some immunomodulatory effects such as Multiple Sclerosis (MS), an T - cell-mediated autoimmune pathology[42]. Luteolin is commonly found in nature in glycosylated forms, but it has been observed that only the aglycone form of luteolin is absorbed in the body.

Luteolin therapy reduced lymphocyte infiltration and follicular loss in thyroid glands in an Experime ntal Autoimmune Thyroiditis (EAT) study. Luteolin has been shown to have anti-inflammatory properties by preventing the interferon-induced increase in cyclooxygenase 2 and the release of the pro-inflammatory cytokine tumour necrosis factor-alpha (TNF-α)[1]. It has been observed that luteolin therapy significantly inhibits T-cell proliferation and antigen-specific IFN-gamma production. Additionally, luteolin appears to be a potent inhibitor of mast cell histamine release (Figure 7).

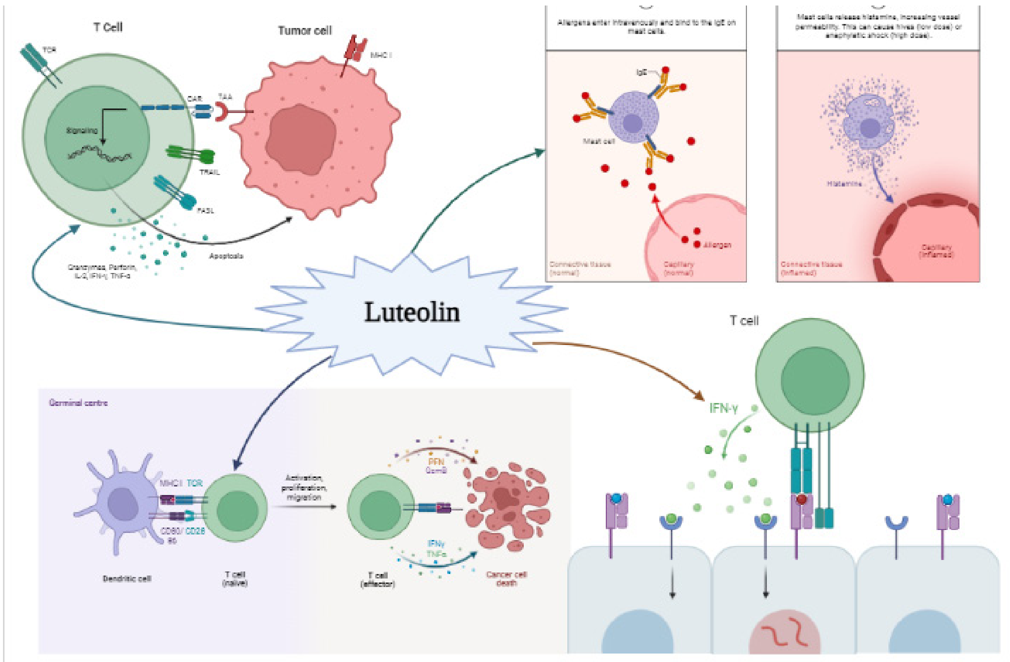

Figure 7. General mechanism of action of Luteonin.

7. Discussion

Over the last few decades, many research works have demonstrated that natural substances may be widely utilized to treat a variety of ailments by controlling immunity. Flavonoids, for example, are widely dispersed in nature and have a wide range of biological actions. These reviews indicate the various key role and typical pathways of the five most potent flavonoids for improving immunity, as well as explore their quantity among all leafy vegetables of West Bengal (Table 3)(Figure 2). Flavonoids have been shown to have a multifaceted effect on the immune system. They can increase the activity of Natural Killer (NK) cells by upregulating the associated activating receptors, and limit the maturation and differentiation of Dendritic Cells (DCs) by inhibiting co-stimulatory surface molecules, endocytosis, and chemotaxis, which contributes to T-cell activation. Flavonoids can also limit the development of neutrophil extracellular traps and the oxidative burst of neutrophils, reduce the activation of M1 type macrophages and promote M1 to M2 transition. Furthermore, flavonoids can promote Treg cell proliferation and increase CTL cell activity by influencing different transcription factors, inflammatory cytokines, and signal transduction pathways, while inhibiting Th cell activation or proliferation. Lastly, flavonoids can have inhibitory or activating effects on various B-lymphocytes.

Apigenin is gaining popularity due to its ability to suppress the PI3K/Akt/mTOR signaling pathways of Dendritic cells; PBMCs animal model/cell line. Apigenin has been shown to have a significant impact on NF-kB activity in the lungs, indicating its potential to exert immune-regulatory functions in an organ-specific manner. In a study, Apigenin administration (5 mg/kg or 10 mg/kg) prevented the OVA-induced increase in eosinophil count and Th17 cells. Apigenin, in conjunction with Quercetin and Luteolin, protects pancreatic beta-cells against cytokines during inflammation. Apigenin and Luteolin have been shown to have a potent inhibitory effect on T-cell responses in both mice and humans, especially auto-reactive T cells when used in combination. This suggests that apigenin and luteolin may have potential in the regulation of autoimmune diseases. The maximum amount of apigenin present in *Spinacia oleracea* (*Palong shak*) (17 mg/100g fw) and the minimum amount of apigenin present in *Beta vulgaris* 0.00005 mg/100gfw which is commonly known as *Beet shak*.

Quercetin is another immunomodulatory flavonoid that has been shown to effectively decrease the production of pro-inflammatory cytokines/chemokines and the expression levels of MHC class II and co-stimulatory molecules. Quercetin has been found to inhibit Lipopolysaccharide (LPS) -induced activation of Dendritic Cells (DCs). In that condition, Lipopolysaccharide (LPS) induced the activation of DCs. The amount of quercetin present in different leafy vegetables is shown in Table 3. and Maximum amount of quercetin present in *Allium cepa (*149.75 mg/100g fw) and the minimum amount of Quercetin present in *Beta vulgaris* (0.00228 mg/100g fw). The maximum amount of rutin present in *Amaranthus viridis* (58.52 mg/100g fw) and the minimum amount present in *Brassia oleracea* (0.000311 mg/100g fw). The maximum amount of kaempferol present in *Coccinia grandis* (111 mg/100g fw) and the minimum amount present in *Beta vulgaris* (0.00104 mg/100g fw). The maximum amount of Luteolin present in *Allium cepa* (39.1 mg/100g fw) which is commonly known as piyaz and the minimum amount present in *Beta vulgaris* (0.0096 mg/100g fw).

The processes by which these leafy vegetables and their bioactive compounds (flavonoids) modulate immunity are complicated, including several signal transduction pathways. The majority of these compounds have a wide range of pharmacological actions, and their targets and molecular processes are not well explored or understood till date. The pharmacological actions of these compounds are diverse, and many of their molecular targets and mechanisms are still not fully understood. While the documented biological activities of these leafy vegetables are based on cell-based *in vitro* and animal-based *in vivo* studies, more extensive clinical trials are needed to assess their efficacy and toxicity in humans.

8. Conclusion

Natural products and traditional remedies have long been a source of inspiration for the development of therapeutic agents. Over the years, several substances derived from leafy vegetables have been identified for their immunomodulatory properties. The identification and isolation of more selective immunomodulatory drugs derived from plants have the potential to mitigate the adverse effects and high costs associated with synthetic molecules. This review provides a comprehensive profiling of leafy vegetables in Bengal, including the potent immunomodulatory flavonoids found in these vegetables. As molecular-level investigations progress, it is expected that these vegetables and their derived compounds will continue to show significant advances and play a crucial role in treating various immune-related disorders in the future.

9. Abbreviations

Units and Symbols
Mg - Milligram
g - Gram
FW - Fresh Weight

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