Glycoside flavonoids of genus Morus: Phytochemistry, Biosynthesis and Pharmacological activities

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Abstract

Traditional natural products have been the focus of research to explore their medicinal properties. One such medicinally important plant is Morus alba, which is growing very quickly, especially in Asia, and has tremendous pharmacological activities due to the presence of phytochemicals in various parts of this plant. In this review, we provide a comprehensive summary of the phytochemistry, biosynthetic pathways, and pharmacological activities of glycoside flavonoids in the leaves of Morus alba has been studied. In the past centuries, mulberry has been considered a food source for silkworms, and its leaves are used to feed animals for livestock. However, in recent years, mulberry has attained a special place in research because of its phytochemical composition and benefits to human health, including antioxidant, anticancer, antidiabetic, and immunomodulatory effects. Phytochemicals are bioactive compounds present in plants that work with nutrients and dietary fiber to protect against diseases. Morus alba also contains many phytochemicals, such as flavonoids, phenols, and anthocyanins. The biosynthetic pathway is greener and more efficient for producing structurally diverse natural products. This pathway acts as an alternative to chemical synthesis and microbial transformation, which suffer from complex routes, difficult isomer separation, and high cost. The mechanism of the flavonoid biosynthesis pathway in mulberry leaves is not yet clear, and currently, this is a much-needed topic for scientists to explore.

Introduction

The presence of natural products in animals, plants, and minerals plays a vital role in the field of medicinal chemistry; therefore, researchers from antiquity are working on the extraction, isolation, identification, biological activities, and many properties of these natural organic products. Conventional medicines depend on phytochemical-rich plant extracts to cure many diseases because medicines obtained from natural sources are less toxic and have fewer side effects than synthetic medicines [1]. For the remedy of many health issues, to remove pain and discomfort, such as fragrance and flavor in food, everyone depends on the plant kingdom for their needs, and medicinal plants play a vital role in developing countries. Medicinal plants are the best source for the discovery of new compounds that lead to new drugs [2]. Medicinal plants of the Moraceae family are known for their versatile applications in many fields such as agriculture, cosmetics, food, and pharmaceuticals. One example of the family Moraceae is Morus alba (mulberry), which is the most commonly used medicinal plant in Asia, especially in China. Mulberry is highly produced in China and grows throughout many regions such as Asia, Africa, America, Europe, and India. The leaves of mulberry are only known for sericulture. But the leaves are not used in sericulture but can be used as medicines as well [3]. Mulberry is a rich source of many bioactive compounds such as flavonoids, amino acids, vitamins, polysaccharides, and steroids, which are used for the treatment of many infections and internal diseases.

Among these components, flavonoids have attracted more attention in recent studies because of their anti-inflammatory, anti-aging, and anti-hyperglycemic activities. [4,5]. Flavonoids belong to the polyphenolic family and incorporate at least 6000 molecules, primarily divided into phlobaphenes, aurones, isoflavonoids, flavones, flavonols, and anthocyanins [6]. Flavonoid compounds commonly found in mulberry are kuwanon (flavons), sangenon (flavanols), rutin (flavons), quercetin (flavonols), and catechins (flavanols).

Plants have an amazing tendency to identify changes in the environment, and then the rapid response to increases the opportunity and lowers the risk. The basic need to respond to these changes is the integration of growth, development, and metabolism, which leads to the evolution of many other mechanisms to regulate cellular functions. One of the mechanisms is glycosylation, which involves a large multigene family of glycosyltransferases (GTs). Glycosyltransferases can identify lipophilic small molecules, including hormones and secondary metabolites, as well as biotic and abiotic toxins in the environment [7,8]. Glycosylation of flavonoids increases their solubility and stability in plants. Flavonoids exist mainly in their glycosylated forms in plants. The final step in the biosynthesis of these glycosides is glycosylation, which plays a variety of roles in plant metabolism. Glycosyltransferases (GTFs) establish natural glycosidic linkages. They catalyze the transfer of saccharide moieties from an activated nucleotide sugar (also known as a glycosyl donor) to a nucleophilic glycosyl acceptor molecule, the nucleophile of which can be oxygen-, carbon-, nitrogen-, or sulfur-based. GTFs play an important role in glycosylation, and it is important to understand the chemistry of these enzymes in mulberry leaves.

1. Phytochemistry of Mulberry leaves

1.1 Nutritional values Mulberry leaves

The main components of mulberry leaves with pharmacological properties are phenols, polysaccharides, steroids, amino acids, lignins, and volatile components. The leaves of mulberry are also a good source of ascorbic acid. It contains carotene, vitamin B1, folic acid, folinic acid, and vitamin D. The nutritional composition of mulberry leaves in dry condition is 15.31-30.91% protein, 2.09-7.92% fat, 9.9-13.85% crude fiber, 27.60-43.6% neutral dietary fiber (NDF) and 11.3-17.24% ash [9]. Evidence suggests that mulberry leaves also contain some additional therapeutic agents such as Moran 20k and 1-deoxynojirimycin (DNJ) as antidiabetic agents, and isoprene-substituted flavanones such as Kuwanon C and kuwanon as antimicrobial agents [10,11]. The nutritional values are shown in Figure 1.

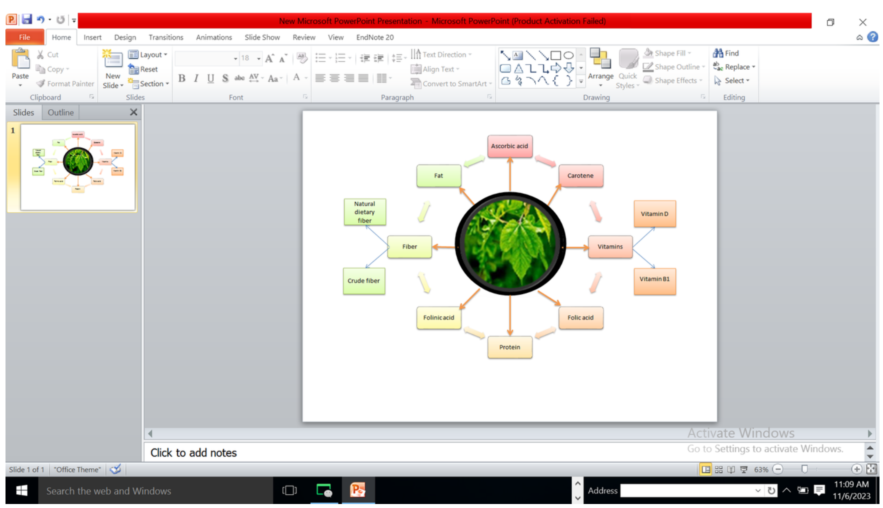

Fig.1. Nutrition's present in Mulberry leaves

Following are the most common bioactive components isolated or extracted from the mulberry leaves:

1.2 Flavonoids

The *Morus* plants are known to be abundant in flavonoids and are shown in figure 2. The flavonoids in the mulberry leaves are mainly in the forms of quercitin and kaempferol [12]. Kuwanons, moracinflavans, moragols andmorkkotins are some of the main flavonoids found in mulberry leaves. The total contents of isoquercetin, rutin, kaempferol and astragalin are ranging from 4.34 mg/g to 0.53 mg/g [13].The major antioxidant flavonoids are flavonol, glycosides, rutin, quercitin-3-(6-malonylglucoside) and isoquercitin have been extracted from the leaves of mulberry [4]. The flavonoids possess many medicinal properties like anti-inflammatory, antioxidant, antiallergic, antithrombotic, hepatoprotective, antiviral and carcinogenic in human being.

Flavonol glycosides	mg/100g (Dried weight)
Quercetin-3-(6-malonylglucoside)	754-1046
Rutin	487-659
Isoquercitrin	168-220
Astragalin	26-36

Table 1: Flavonol glycosides in mulberry leaves

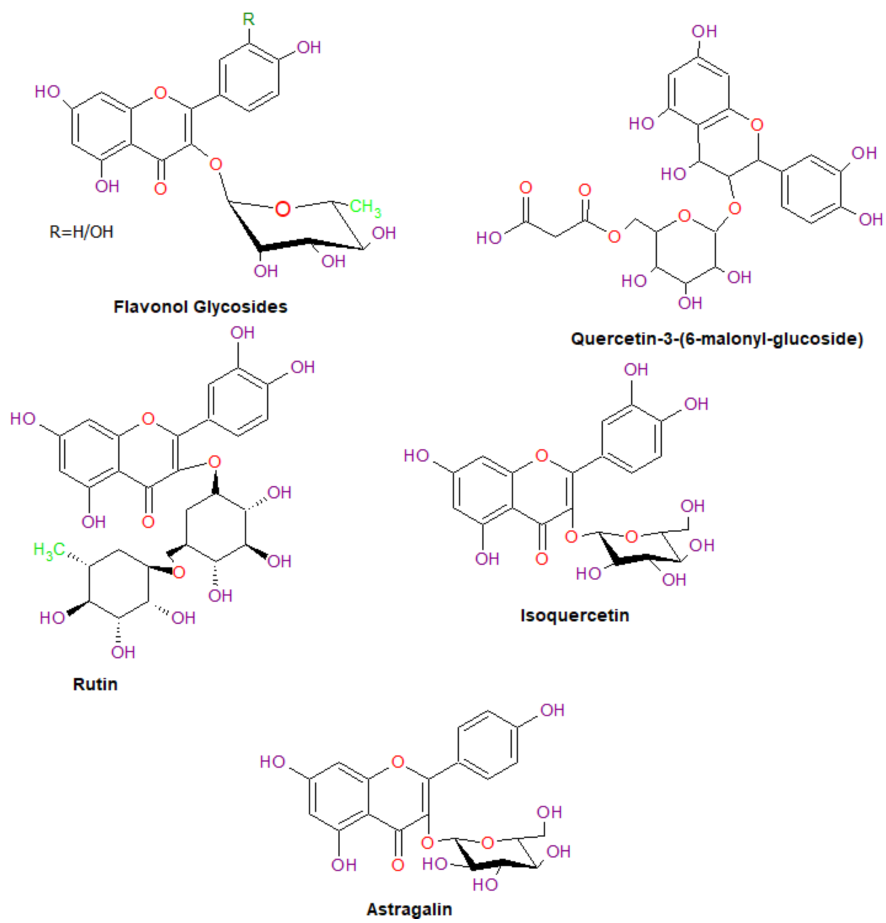

Fig. 2 . The structure of flavonoids present in mulberry leaves.

1.3 Alkaloids

The alkaloids present in large concentration in mulberry leaves are aurantiamide acetate, 1-deoxynojirimycin (1-DNJ) which is most potent glycosidase inhibitor that decrease the blood sugar level, D-fagomine (FAG), 2-O- αDgalactopyranosyl-deoxynojirimycin (GAL-DNJ), 4-O-β-D-dlucopyranosyl-fagomine (Glu-FAG), isofagomine, cis-5-hydroxypipecolic acid, trans-5-hydroxypipecolic acid, methylpyrrolidine carboxylic acid and pipecolic acid.The main active constituent in Mulberry leaves is 1-Deoxynojirimycin (DNJ) and has attracted attention of many researchers because of its effective and specific inhibition of different enzymes involved in wide range of important biological processes such as maturation of the sugar chains in glycoproteins, intestinal digestion [14]. The DNJ acts as an antihyperglycemic agent which slow down the rate of carbohydrate degradation to monosaccharides and it can also interrupt glucose absorption and then reduce post prandial blood glucose levels [15]. The structure of DNJ is same as the sugars is shown in figure 3.

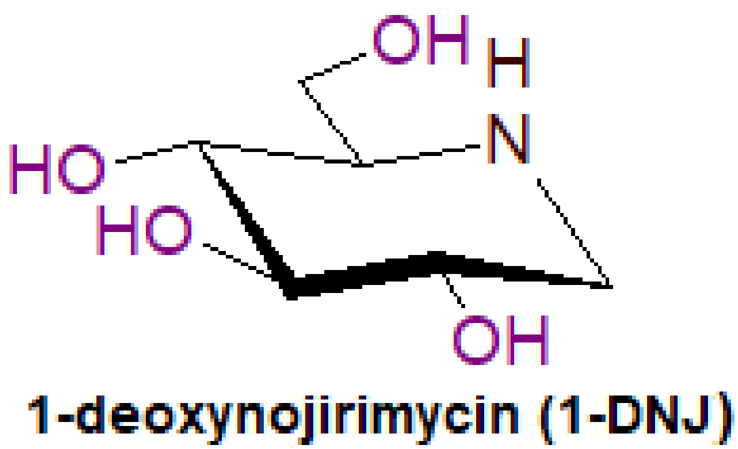

Fig. 3 .The chemical structure of alkaloid

1.4 Anthocyanins

A natural phenolic compound anthocyanins are responsible for the colors of fruits, flowers and leaves. Anthocyanins are the best source for the health benefits as it act as antioxidant, anti-inflammatory etc. Anthocyanins has very high inhibitory ability on lipid oxidation and has antimetastatis activity to inhibit migration of B16-F1 cells [16,17].

1.5 Polysaccharides

Polysaccharides are the main active bioactive components of mulberry leaves. Polysaccharides are long-chain macromolecular polymers that are generally used as thickeners and gelling agents in the food industry because of their ability to retain water and form hydrogels. These mulberry leaf polysaccharides have received increasing attention because of their multiple biological activities. These polysaccharides promote insulin expression and regulate liver glucose metabolism [18]. In mulberry leaves, polysaccharides are mainly present in the inner epidermal cells [19]. Glucose, galactose, arabinose, fructose, xylose, rhamnose, glucuronic acid, galacturonic acid, mannose, and sorbose are monosaccharides that combine with each other and form many types of polysaccharides in mulberry leaves [20].

1.6 Amino acids

Amino acids are the building blocks for the synthesis of proteins which includes antioxidant enzymes. Some of the amino acids can directly scavenge oxygen free radicals. Out of 20 essential amino acids present in mulberry leaves, four amino acids, Asparagine, Alanine, Proline and GABA were observed and their structures are shown in figure 4. Mulberry leaves contains high amino acid contents which are nutritively superior and also promote growth and development of silkworm [21,22].

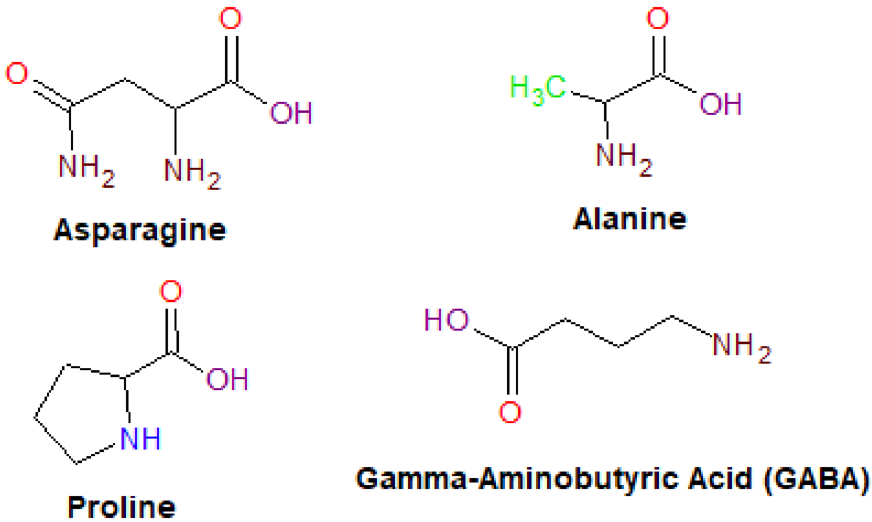

Fig.4. Chemical structure of amino acid present in mulberry leaves

2. Biosynthesis

The biosynthetic pathway is greener and more efficient for producing structurally diverse natural products. This pathway acts as an alternative to chemical synthesis and microbial transformation, which suffer from complex routes, difficult isomer separation, and high cost. Biosynthesis, also known as anabolism, is a simple compound that is joined together and forms a macromolecule using enzymes. Extreme reaction conditions and many toxic chemicals are required for the chemical synthesis of flavonoids [23]. One of the advantages of biosynthesis is the production of rare and expensive natural products because of the development of molecular biological tools and availability of genome information from a variety of organisms. Biosynthesis can be used in simple and complex transformations without disturbing the blocking and deblocking steps, which are common in organic synthesis [24]. The mechanism of the flavonoid biosynthesis pathway in mulberry leaves is not yet clear, and currently, this is a much-needed topic for scientists to explore. Flavonoids are synthesized through the shikimate pathway followed by the phenylpropanoid metabolic pathway and possess approximately 15 carbon atoms arranged in three aromatic rings linked as C6-C3-C6 [25]. Several enzymes are involved in the shikimate pathway, which is a six-step reaction in the biosynthesis of shikimic acid. The reaction as shown in figure 5 begins with a simple aldol condensation reaction of phosphoenolpyruvic acid and D-erythrose 4-phosphate [Dias MC, Pinto DCGA, Silva AMS. (2021) Plant flavnoids: Chemical Characteristics and Biological Activity. Molecules, 26:5377.]. The end product of chorismic acid is converted into the amino acid phenylalanine by the action of prephenate-aminotransferase (PhAT) and aromate-dehydrate (ADT) enzymes [Tariq H, Asif S, Andleeb A, Hano C, Abbasi BH. (2023) Flavonoid Production: Current trends in plant metabolic engineering and de novo microbial production. Metabolites, 13:124.].

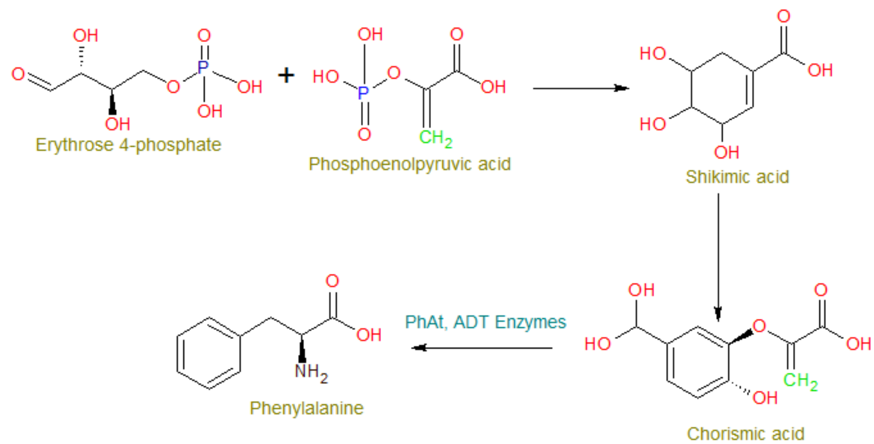

Fig. 5. Shikimate pathway

The biosynthesis of flavonoids starts with the condensation of one p-coumaroyl-CoA molecule (shikimate-derived, B ring) with three molecules of malonyl-CoA (polyketid origin, A ring) to give chalcone (2'4'6'4-tetrahydroxychalcone) catalyzed by chalcone synthase (CHS) enzymes. Chalcones are subsequently isomerized to flavanone by chalcone flavanone isomerase (CHI). The pathway diverges from these central intermediates into several side branches, each providing a different class of flavonoids.

The initial steps in this pathway are referred to as the general phenylpropanoid pathway [26]. In phenylpropanoid pathway, an aromatic amino acid, phenylalanine first converted to p-coumaroyl-CoA through the activity of phenylalanine ammonia lyase (PAL), cinnamic acid 4-hydroxylase (C4H), and 4-coumarate-CoA ligase (4CL). PAL catalyzed the deamination of phenylalanine to trans-cinnamic acid in the first committed step called as deamination of phenylalanine to trans-cinnamic acid [27]. In the phenylpropanoid pathway, the second step involves the activity of C4H, a cytochrome P450 monooxygenase, in plants. This enzyme catalyzes the hydroxylation of trans-cinnamic acid and generates p-coumaric acid. This was the first oxidation reaction [28]. The third step in the general phenylpropanoid pathway involves the activation of p-coumaroyl-CoA by the action of 4-courmarate: CoA ligase. In plants, the activity of 4CL is positively correlated with anthocyanin and flavonol content in response to stress [29], whereas PAL, C4H, and 4CL are generally coordinately expressed [30]. Chalcone synthase (CHS) catalyzes the stepwise condensation of three acetate units to yield naringenin chalcones. In vitro, naringenin chalcone is transformed into naringenin by chalcone isomerase (CHI) [31]. Naringenin is a very important flavonoid frame catalyzed by FNS I and FNS II (flavone synthases I and II) and IFS (isoflavone synthase) to form flavones and isoflavones respectively [32]. Naringen can also be catalyzed by flavanone-3-hydroxylase (F3H), flavonol-3'-hydroxylase (F3'H) and flavonol-3'5'-hydroxylase (F3'5'H) to synthesized dihydro-myricetin, dihydro-kaempferol and dihydro-quercetin, respectively [33]. The flavonol synthase (FLS) converted dihydro flavonols into flavonols (like kaempferol, quercetin and myricetin), which further was catalyzed by dihydroflavonol 4-reductase (DFR) to generate leucoanthocyanidins [34], further catalyzed by leucoanthocyanidin dioxygenase (LDOX) to produce anthocyanidins [35]. anthocyanidins and leucoanthcyanidins were again converted to proanthocyanidins catalyzed by leucoanthocyanidin reductase (LAR) and anthocyanidine reductase (ANR), repectively [36]. variations in anthocyanins is then responsible for the stabilization of vacuolar anthocyanins which includes glycosylation, methylation and acylation [37].

In general phenylpropanoid pathway shown in figure 6 is common to all the downstream metabolites like flavonoids and lignin. The metabolic pathway continues through a series of enzymatic modifications to yield flavonols, dihydroflavonols and anthocyanins.

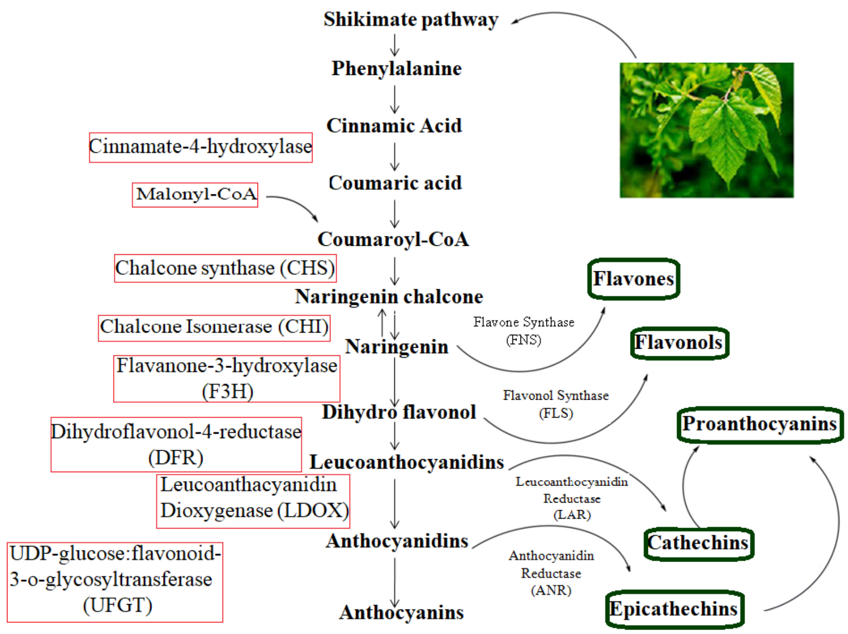

Fig.6. Phenylpropanoid pathway for flavonoids

There are some factors which influence the expression of the genes involved in the biosynthesis pathways for flavonoids and leading to the changes in their availability. Those factors can be the environmental conditions (like light, water availability and temperature), the hormones (like jasmonic acid) and also some physical injuries [38].

3. Pharmacological activities

The presence of general phytochemicals and biological activities of mulberry leaves are shown in figure 7. Mulberry leaves used widely as a medicine in Asian countries especially in China. The Mulberry leaves are rich in flavonoids and many other bioactive compounds which functions to lowering the blood sugars, reducing blood lipids, resisting viruses, aging and also helps in enhancing the immunity. The major bioactive ingredients in plants are flavonoids. The flavonoids act as effective antioxidants and metals chelators because of the phenolic compounds. The flavonoids can also be a potent anticancer promoters and cancer chemopreventive agents.

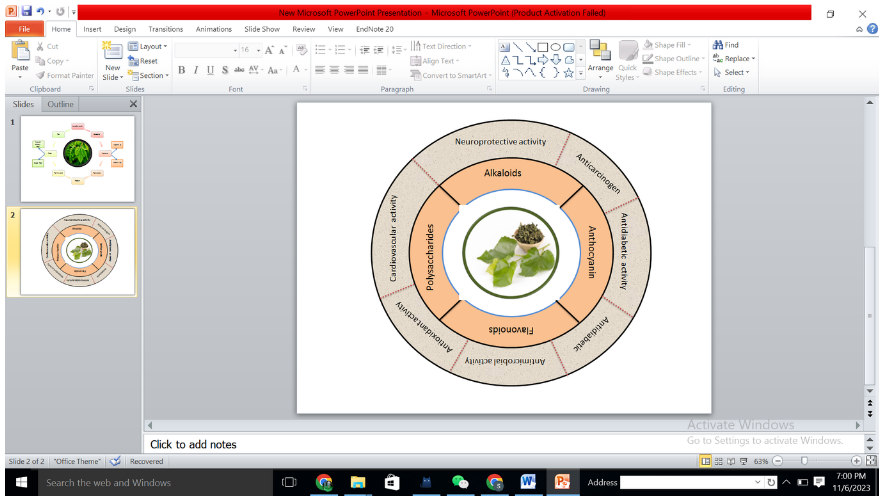

Fig.7. Pharmacological applications of mulberry leaves

3.1 Antioxidant

The working of anti-oxidants is shown in figure 8. The substances which hold back the deterioration by oxidation like of fats, oils, foods etc., some components like Vitamin C, Vitamin E etc. responds to the damaging effects of oxidation in a living organism. The free radicals possessing an unpaired electron which are considered as fragments of molecules that are generally very reactive. They are continuously produced in the cells as by-products of metabolism [39]. Plants naturally have tendency to develop some specific anti-oxidative defense enzymes like catalase, peroxidase, polyphenol oxidase, ascorbate peroxidase and glutathione reductase etc. to control the free radicals of oxygen under various environmental stress conditions [40]. To avoid threats related to the oxidative stress, antioxidant is required in the form of food supplement [41].

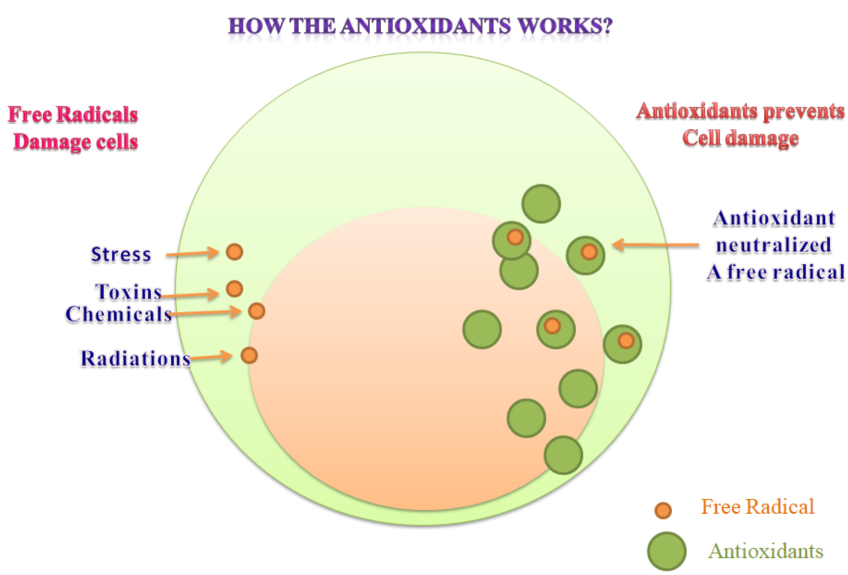

Fig.8. Working of anti-oxidants

Wide range of flavonoids present in the mulberry leaves which make it a very good antioxidant agent. The strong antioxidative properties of mulberry leaves are because of the presence of higher values of phenolic and flavonoid compounds [42,43]. Flavonoids with high antioxidant activity can be used in many ways, like (i) it can inhibit reactive oxygen species inhibition, (ii) leukocyte immobilization, (iii) inhibit nitric oxide and (iv) xanthine oxidase inhibition [44]. As the flavonoids have physiological functions in plants, it's an important part of the human diet [45].The higher amount of quercetin in mulberry leaves maybe responsible for the reduction of oxidation process [5,46]. Three flavonoids quercetin, rutin and isoquercetin are considered as main antioxidant components in mulberry leaves ethanol extracts [4].

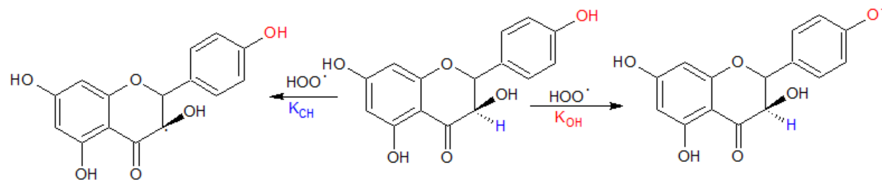

Fig.9. Flavonoids as Antioxidants

3.2 Anticarcinogenesis

Flavonoids have been considered as high impact on the cancer prevention and some selected isoflavones have tendency to inhibit cancer in a number of animal models. Some studies revealed that the flavonoids inhibit carcinogenesis *in vitro* and significant results also indicates that they also do it *in vivo* [47,48]. Flavonoids are the dominant class of phenolic compounds in mulberry leaves.

The mulberry leaves shows effective cytotoxic behavior against the cancer cells and the mechanism is shown in figure 10. Many anticancer compounds found in mulberry leaves like Kaempferol, Kuwanon S, Morusin, with strong activity towards cytotoxic cell lines Hela, MCF-7 and Hep3B. The galactose binded lectin isolated from the mulberry leaves has cytotoxic activity on the human breast cancer with IC50-8.5 µg and also on colon cancer cell with IC50-16 µg. [49]. Many phenolic compounds are present in mulberry leaves which induces anticancer activity in hepatoma cells by cell cycle captured at G2-M phase and inhibition of topo-isomerase II activity. The anthocyanins are also a phenolic compound present in mulberry leaves and has beneficial effects in decreasing the risk of cancer because of its high chemopreventive properties [50]. Another flavonoid present in mulberry leaves is Quercetin which is capable of reducing the progress of human β leukemia [51].Many other clinical trials revealed the therapeutic potential of mulberry leaves against cytotoxicity as a cheap and easily available source for the treatment of cancer and reduces the invasiveness of cancer cells. The flavonoids and quercetins present also exhibited selective cytotoxicity against human ovarian cancer and gastric cancer [52].

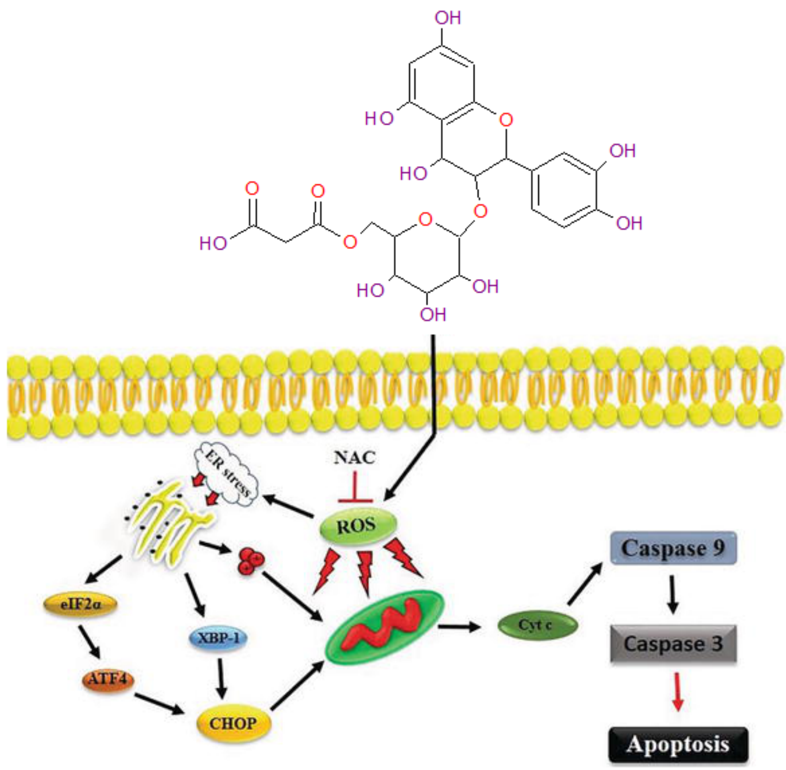

Fig.10. Mechanism of mulberry leaves as anti-cancer agents

3.3 Antidiabetic Activity

Recent studies revealed that the total flavonoid in mulberry leaves exhibit excellent hypoglycemia properties. Mulberry leaves contains several iminosugars which includes 1-deoxynojirimycin and to inhibit α-amilase and galactosidase[53-55].The most effective are nojirimycin derivatives among all the iminosugars. The oral antidiabetic drug used in Europe is a synthetic derivative of 1-Deoxynojirimycin.

The flavonoids and their related constitution present in mulberry leaves are also have anit-diabetic related effects and the mechanism of anti-diabetic activity is shown in figure 11. The mulberry leaves of the South-American mulberry species were found to contain some benzofurane derivatives and are active on STZ induced diabetic rats [56]. Some of the purified flavone fractions of mulberry was found to activate α-glucosidase enzymes which in turn increase the blood glucose level [57].The polysaccharides present in leaves of mulberry have strong potential for the inhibition of α-glucosidase.

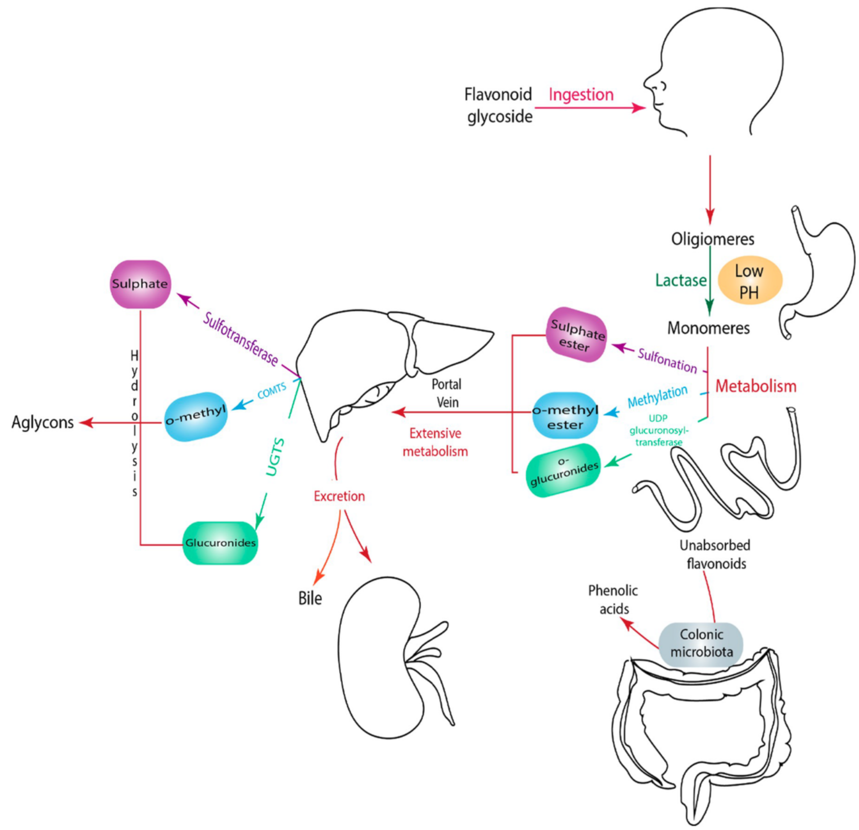

Fig.11. Mechanism of anti-diabetic activity of mulberry leaves

3.4 Antimicrobial activity

Fresh fruits and vegetables are living tissues which continue their metabolic activities thereafter harvested. Antimicrobials helps in protection against the microbial organisms which in turn used for the production antibiotics. Mulberry leaves contained Kuwanon C, Mulberrofuran G and albanol B with strong antibacterial activity with MIC's ranging from 5-30 mg/ml [58].

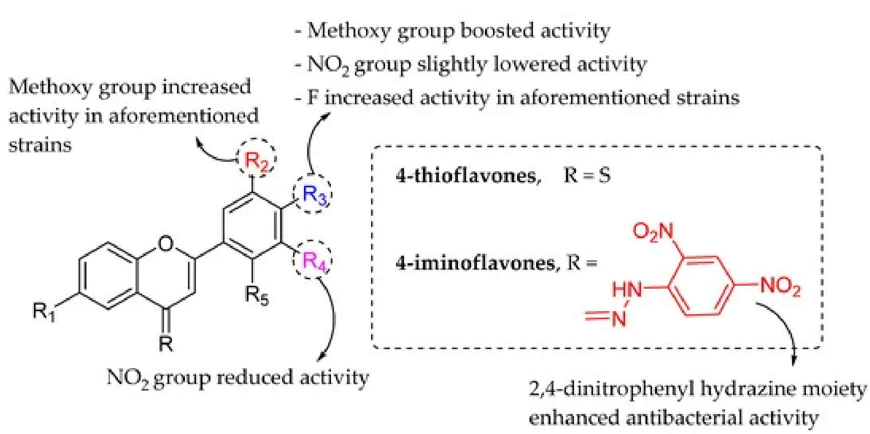

Fig.12. Antimicrobial activity of flavonoids

The mulberry leaves extract in ethanol shows moderate antibacterial activity whereas in aqueous it was little [59]. Some microbes like*E. Coli, Salmonella typhimurium, Staphylococcus epidermis, S. aureus, Candida albicans* and *Saccharomyces cerevisiae* can be inhibited by the flavonoids present in mulberry leaves [60].

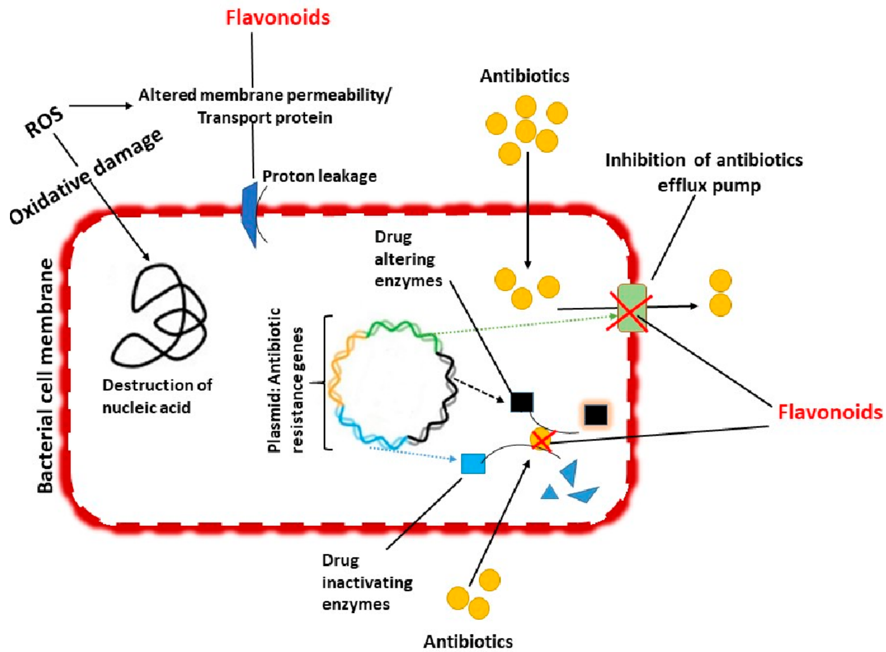

Fig.13. Mechanism of antimicrobial activity

3.5 Cardiovascular activity

In many eastern countries used mulberry leaves to treat cardiovascular diseases. In china people use mulberry for decreasing blood pressure which is the main reason for the cardiovascular disorder. The mulberry leaves also helps to reduce the serum cholesterol, hypertension and to prevent artherosclerosis [61]. The cardiovascular diseases basically related to heart or blood vessels. High concentration of iron present in mulberry leaves which helps in better distribution of oxygen by boosting the production of RBCs. Reveratrol is a flavonoid present in the mulberry leaves helps in increasing the formation of nitric oxide (NO) which acts as a vasodilator. This will relaxes blood vessels and reduces the chances of blood clot formation and subsequent heart diseases like heart strokes. The resveratrol helps to remove the restrictions in blood vessels which in turn reduces the chance of heart failure [62].

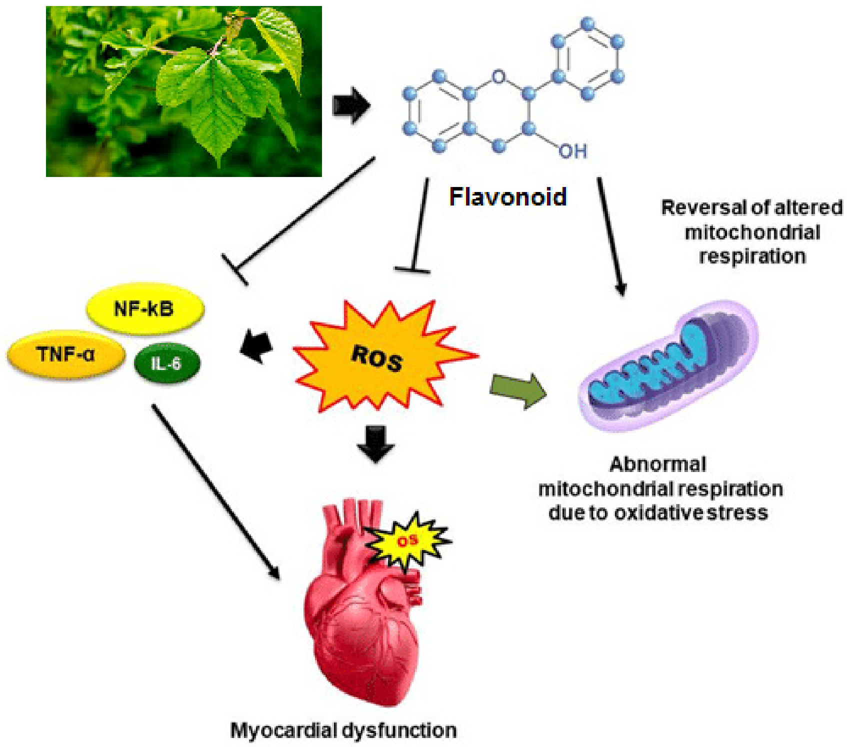

Fig. 14. Cardiovascular mechanism of flavonoids

3.6 Neuroprotective activity

The neuroprotection activity is related to prevent the disease progression and other injuries due to the loss of neurons and halting. The cognitive disorders and many other neuronal dysfunctions are treated with the use of medicinal plants and mulberry is most prominent among all. Recent studies reports that the phenolic compounds, anthocyanins and flavonoids possess neuroprotective effects [63,64]. The polyphenols and alkaloids present in mulberry leaves can improve the cognitive function and also suspend the neural-degeneration [65].The isolated compounds from mulberry leaves can be used as neuroprotective agents for neurodegenerative diseases treatment [66]. One of the most common diseases with neurodegenerative disorder is Alzheimer's diseases. The extract of mulberry leaves reduces the risk of Alzheimer's disease. The leaf extract inhibit the amyloid β-peptide (1-42) fibril formation and attenuation of amyloid β-peptide (1-42) induced neurotoxicity [67]. The mechanism of neuroprotective activity is shown in figure 15.

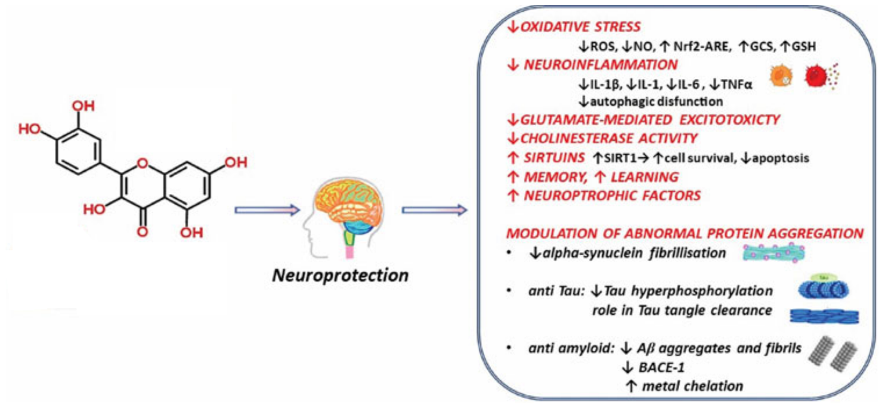

Fig. 15. Mechanism of Neuroprotective activity

Future perspectives and challenges

Glycosylation is one of the most important physiological and biochemical reactions in nature given its crucial role in a multitude of essential processes. This intrinsic importance has attracted long-standing and wide research attention on the characteristics of GTs to facilitate their application in glycosylation reactions for the metabolic engineering of natural product biosynthesis. Although several GTs have been found to be suitable for altering glycosylation patterns, low catalytic activity and stringent substrate specificity remain limiting factors in the diversification of PNPs for industrial fermentation systems.

Many enzymes and genes are involved in flavonoid pathways and have been characterized. However, many aspects of flavonoid biology remain unidentified. The expression patterns and activity of some transcription factors that regulate this branched pathway have not yet been identified. Very little evidence exists for protein-protein interactions that form metabolic channels and increase the efficiency of this pathway. These problems can be solved by achieving efficient engineering of flavonoid pathways in plants. The great biodiversity of plants that arose during evolution has generated a concomitant variety of flavonoid structures known to date, many of which are yet to be discovered. Further analysis of different plant species will allow the discovery of novel structures and possibly new metabolic pathways. Future studies will also contribute to the improvement of floricultural, food, pharmaceutical, and chemical industries. Moreover, evidence of beneficial functions of flavonoids in human health and the use of natural compounds for the prevention and treatment of different pathologies are continuously increasing worldwide, and interest will continue to grow among researchers in the coming years.

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