6. Medicinal plants as antioxidant agents: Understanding their mechanism of action and therapeutic efficacy

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Abstract. The present review focuses on the antioxidant activities of the various medicinal plants, their bio-availability, mechanism of action and therapeutic efficacy. Antioxidant of medicinal plant origin may exert their effects on biological systems by different mechanisms. Efforts have been made to explore the structure and functional groups that involve removing the oxidants that most often occurs in the biological systems. Some of the mechanisms have been dealt deeply in this review. Many oxidants have been implicated in a number of disease, removal or minimization of oxidants exposure and at the same time increasing the antioxidant ability of the biological system may reduce the damage.

Medicinal plants produce significant amounts of antioxidants such as flavonoids, phenolics and polyphenolics compounds to prevent the body from oxidative stress that could be caused by reactive oxygen and nitrogen species. Therefore, this review assessed the role of important antioxidants to combat these reactive species.

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1. Introduction to medicinal plants

The use of plants as medicines goes back to early man. Certainly the great civilizations of the ancient Chinese, Indians, and North Africans provided written evidence of man's ingenuity in utilizing plants for the treatment of a wide variety of diseases. In ancient Greece, for example, scholars classified plants and gave descriptions of them thus aiding the identification process. Theophrastus has been described by some as the father of botany but little, if anything, has been recorded on his distant relative J.B. Theophrastus who extolled the virtues of medicinal plants and forecast the possibility of discovering flavonoids. As Europe entered the dark ages much of this information would have been lost had it not been for the monasteries that acted as centers for the production of medicinal plants which were used to heal the suffering of mankind.

It was not until the 19th century that man began to isolate the active principles of medicinal plants and one particular landmark was the discovery of quinine from Cinchona bark by the French scientists Caventou and Pelletier. Much less is known about the isolation of quinine by J.B. Caventou and J.B. Pelletier. Such discoveries led to an interest in plants from the New World and expeditions scoured the almost impenetrable jungles and forests in the quest for new medicines [1].

Like other Plants, medicinal plants synthesize a vast range of organic compounds that are traditionally classified as primary and secondary metabolites although the precise boundaries between the two groups can in some instances be somewhat blurred. Primary metabolites are compounds that have essential roles associated with photosynthesis, respiration, and growth and development. These include phytosterols, acyl lipids, nucleotides, amino acids and organic acids. Other phytochemicals, many of which accumulate in surprisingly high concentrations in some species, are referred to as secondary metabolites. These are structurally diverse and many are distributed among a very limited number of species within the plant kingdom. Although ignored for long, their function in plants is now attracting attention as some appear to have a key role in protecting plants from herbivores and microbial infection, as attractants for pollinators and seed-dispersing animals, as allelopathic agents, ultra violet (UV) protectants and signal molecules in the formation of nitrogen-fixing root nodules in legumes. Secondary metabolites are also of interest because of their use as dyes, fibers, glues, oils, waxes, flavoring agents, drugs and perfumes, and they are viewed as potential sources of new natural drugs, antibiotics, insecticides and herbicides.

In recent years the role of some secondary metabolites as protective dietary constituents has become an increasingly important area of human
nutrition research. Unlike the traditional vitamins they are not essential for short-term well-being, but there is increasing evidence that modest long-term intakes can have favorable impacts on the incidence of cancers and many chronic diseases, including cardiovascular disease and Type II diabetes [2].

Medicinal plants antioxidant activity is mainly due to the presence of secondary metabolites [3]. Based on their biosynthetic origins, plant secondary metabolites can be divided into three major groups: flavonoids and allied phenolic and polyphenolic compounds, terpenoids and nitrogen-containing alkaloids and sulphur-containing compounds [2].

1.1. Phenolic compounds (flavonoids and phenolic acids)

Phenolic compounds possess one or more aromatic rings and one or more hydroxyl groups. They are the products of secondary metabolism in plants, providing essential functions in the reproduction and the growth of the plants; acting as defense mechanisms against pathogens, parasites, and predators, as well as contributing to the color of plants. In addition to their roles in plants, phenolic compounds in diet provide health benefits. They can be grouped into two major categories:

Cranberry, apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pea grape fruit, broccoli, spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, cucumber and others, shown on figure 1.1, are some of the best food sources of phenolic compounds [4].

![Figure 1.1. Best dietary sources of phenolic compounds.](image-url)
1.1.1. Phenolic acids

The simplest Phenolic compounds commonly found in plants. Generally, they can be classified in two broad categories based on their chemical nature: Benzoic acid derivatives and cinnamic acid derivatives as elucidated in figure 1.2.

1.1.2. Flavonoids

Flavonoids are a group of polyphenolic compounds, which are widely distributed throughout the plant kingdom. Many have low toxicity in mammals. Flavonoids exhibit several biological effects such as anti-inflammatory, anti-hepatotoxic and anti-ulcer actions. They also inhibit enzymes such as aldose reductase and xanthine oxidase. They are potent antioxidants and have free radical scavenging abilities. Many have antiviral actions and some of them provide protection against cardiovascular mortality. They have been shown to inhibit the growth of various cancer cell lines in vitro, and reduce tumour development in experimental animals.

Flavonoids occur as aglycones, glycosides and methylated derivatives. The flavonoid aglycone consists of a benzene ring (A) condensed with a six member ring (C), which in the 2-position carries a phenyl ring (B) as a substituent (Figure 1.3). Six-member ring condensed with the benzene ring is either a flavonols and flavonones or its dihydroderivative (flavanols and flavanones). The position of the benzenoid substituent divides the flavonoid

Figure 1.2. Two major classes of Phenolic acids with examples.
class into flavonoids (2-position) and isoflavonoids (3-position). Flavonols differ from flavonones by hydroxyl group the 3-position and C2-C3 double bonds. Flavonoids are often hydroxylated in position methyl ethers and acetyl esters of the alcohol group are known to occur in nature. When glycosides are formed, the glycosidic linkage is normally located in positions 3 or 7 and the carbohydrate can be L-rhamnose, D-glucose, gluco-rhamnose, galactose or arabinose [5].

1.2. Terpenoids

The term terpene refers to a hydrocarbon molecule, while terpenoid refers to a terpene that has been modified, for example by the addition of
oxygen [6]. Terpenes or isoprenoids, are one of the most diverse classes of secondary metabolites which play variety functional roles in plants as hormones (gibberellins, abscisic acid), photosynthetic pigments (phytol, carotenoids), electron carriers (ubiquinone, plastoquinone), mediators of polysaccharide assembly (polyprenyl phosphates), and structural components of membranes (phytosterols). More than 55,000 different terpenoids have been isolated, and this number has almost doubled each decade, many of which are of plant origin. Terpenoids are essential for plant growth, development, and general metabolism. Terpenoids are found in almost all plant species [7, 8, 9].

In plants, terpenoid biosynthesis occurs by two different pathways to synthesize the main building block Inositol pyrophosphate (IPP), (a) the Mevalonic acid pathway or HMG-CoA reductase pathway that occurs in cytosol and produces IPP for sesquiterpenoids, (b) methylerythritol phosphate/1-deoxy-D-xylulose (MEP/DOX) pathway forms IPP in the chloroplast for mono and diterpenoids [10].

Generally based on the number of building blocks, i.e., the isoprenoid units, terpenoids are classified into several classes, such as Hemeterpene, monoterpenes (e.g., carvone, geraniol, d-limonene, and perillyl alcohol), diterpenes (e.g. retinol and trans-retinoic acid), triterpenes [e.g., Betulinic Acid (BA), lupeol, oleanic acid, and Ursolic Acid (UA)], and tetraterpenes (e.g., α-carotene, β-carotene, lutein, and lycopene) [11]. Different terpenoids molecules have antioxidant, antiviral, antibacterial, antimalarial, antiinflammatory, inhibition of cholesterol synthesis, antiallergenic, anti hyperglycemic, immunomodulatory and anticancer activities [12, 13].

1.3. Alkaloids

Alkaloids are a diverse group of low molecular weight, nitrogen-containing compounds mostly derived from amino acids. Alkaloids are thought to play a defensive role in the plant against herbivores and pathogens. Plant-derived alkaloids currently in clinical use include analgesics, antineoplastic agent, gout suppressant, muscle relaxants, antiviral, cytotoxic, antinociceptive, anticholinergic, antiinflammatory and DNA-binding activities and some of them have also been used in the treatment of Alzheimer’s disease, myasthenia gravis and myopathy [14,15].

Alkaloids can be classified into families, on the basis of structural similarities and the amino acids that are used for their biosynthesis. Some alkaloids are also produced using building blocks derived from other secondary metabolic pathways, such as terpenoids, polyketides and peptides [16]. Some of the important classes of alkaloid are shown below: Terpenoid
Indole Alkaloids (TIAs) comprise a family of greater than 3000 compounds that includes the antineoplastic agent’s vinblastine and camptothecin, the antimalarial drug quinine, and the rat poison strychnine. Some TIAs have been proposed to play a role in the defense of plants against pests and pathogens. TIAs consist of an indole moiety provided by tryptamine and a terpenoid component derived from the iridoid glucoside secologanin [17].

The benzylisoquinoline alkaloids are a very large and diverse class of alkaloids with > 2500 defined structure. This family contains such varied physiologically active members as emetine (an antiamoebic), colchicines (a microtubule disrupter and gout suppressant), berberine (an antimicrobial against eye and intestinal infections), morphine (a narcotic analgesic), codeine (a narcotic analgesic and antitussive), and sanguinarine (an antimicrobial used in oral hygiene) [18].

Tropane alkaloids (TPAs) occur mainly in the Solanaceae. There principal characteristics pyrollic ring derived from the ornithine and arginine aminoacids by a chemical reaction catalysed by ornithine decaboxylase and Arginine decarboxylase respectively. This class of alkaloid includes the anticholinergic drugs atropine, hyoscyamine, and scopolamine, and the narcotic tropical anesthetic cocaine. Although nicotine is not a member of the tropane class, the N-methyl-\(\Delta^1\)-pyrorrolinium cation involved in TPA biosynthesis is also an intermediate in the nicotine pathway [17, 19].

Purine alkaloids such as caffeine, theobromine, and theacrine are widely distributed in the plant kingdom. Caffeine, a nonselective adenosine \(A_1\) and \(A_{2A}\) receptor antagonist, is the most widely used psychoactive substance in the world. Evidence demonstrates that caffeine and selective adenosine \(A_{2A}\) antagonists interact with the neuronal systems involved in drug reinforcement, locomotors sensitization, and therapeutic effect in Parkinson's disease (PD) [20].

2. Metabolism of phenolic compounds

2.1. Intakes

Flavonoids intake seem to vary greatly between countries; the lowest intakes (2.6 mg/d) are in Finland and the highest intakes (68.2 mg/d) are in Japan. Quercetin is the most important contributor to the estimated intake of flavonoids, mainly from the consumption of apples and onions. A major problem in cohort studies of flavonoids intake is that only a limited number of flavonoids can be measured in biological samples, and more importantly, only a relatively small number of fruit and vegetables are used to make an accurate estimation [21].
2.2. Intestinal absorption

Much about the intestinal mechanisms of the gastrointestinal absorption of polyphenols remains unknown. Most polyphenols are probably too hydrophilic to penetrate the gut wall by passive diffusion, but the membrane carriers that could be involved in polyphenol absorption have not been identified. The unique active transport mechanism that has been described is a Na\(^+\)-dependent saturable transport mechanism involved in cinnamic and ferulic acid absorption in the rat jejunum [22].

In foods, all flavonoids except flavanols are found in glycosylated forms, and glycosylation influences absorption. The fate of glycosides in the stomach is not clear. Experiments using surgically treated rats in which absorption was restricted to the stomach showed that absorption at the gastric level is possible for some flavonoids, such as quercetin and daidzein, but not for their glycosides. Most of the glycosides probably resist acid hydrolysis in the stomach and thus arrive intact in the duodenum. Only aglycones and some glucosides can be absorbed in the small intestine, whereas polyphenols linked to a rhamnose moiety must reach the colon and be hydrolyzed by rhamnosidases of the microflora before absorption. The same probably applies to polyphenols linked to arabinose or xylose, although question has not been specifically studied. Because absorption occurs less readily in the colon than in the small intestine because of a smaller exchange area and a lower density of transport systems, as a general rule, glycosides with rhamnose are absorbed less rapidly and less efficiently than are aglycones and glucosides. This has been clearly shown in humans for quercetin glycosides: maximum absorption occurs 0.5–0.7 hour after ingestion of quercetin 4'-glucoside and 6–9 hours after ingestion of the same quantity of rutin (quercetin-3-rutinoside). The bioavailability of rutin is only 15–20% of that of quercetin 4'-glucoside, its structure shown in figure 2.1. Similarly, absorption of quercetin is more rapid and efficient after ingestion of onions, which are rich in glucosides, than after ingestion of apples containing both

![Figure 2.1. Structure of quercetin 4'-glucoside.](image-url)
glucosides and various other glycosides. In the case of quercetin glucosides, absorption occurs in the small intestine, and the efficiency of absorption is higher than that for the aglycone itself. The underlying mechanism by which glucosylation facilitates quercetin absorption has been partly elucidated. Glucosides could be transported into enterocytes by the sodium-dependent glucose transporter (SGLT1). They could then be hydrolyzed inside the cells by a cytosolic-glucosidase [23].

Another pathway involves the lactase phloridzin hydrolase, a glucosidase of the brush border membrane of the small intestine that catalyzes extracellular hydrolysis of some glucosides, which is followed by diffusion of the aglycone across the brush border. Both enzymes are probably involved, but their relative contribution for the various glucosides remains to be clarified. Quercetin 3-glucoside, which is not a substrate for cytosolic-glucosidase, is certainly absorbed after hydrolysis by lactase phloridzin hydrolase, at least in rats, whereas hydrolysis of quercetin 4'-glucoside seems to involve both pathways. In humans, whatever the mechanism of deglucosylation, the kinetics of plasma concentrations is similar after ingestion of quercetin 3-glucoside or quercetin 4'-glucoside. Isoflavone glycosides present in soya products can also be deglycosylated by glucosidases from the human small intestine. However, the effect of glucosylation on absorption is less clear for isoflavones than for quercetin [24].

2.3. Metabolism

2.3.1. Flavonoids conjugation

A. Glucuronidation of flavonoids in the intestine

Although flavonoids aglycons were supposed to be rapidly absorbed after oral ingestion, their plasma concentrations are found to be very low whereas the phase II metabolites such as glucuronides, sulfates, and methylated conjugates seem to be predominant in blood circulation. Therefore, liver and intestine are thought to be responsible for the extensive first-pass metabolism of flavonoids, and glucuronidation mediated by various UDP glucuronosyltransferases (UGTs) is suggested to be one of the most important metabolic pathways of flavonoids in both liver and intestine. Quite a number of studies in human have demonstrated the contribution of UGTs to the first-pass glucuronidation of flavonoids. For instance, after intake of kaempferol in human, 3-O-glucuronide conjugate of kaempferol was found to be the predominant form in plasma. Epicatechin glucuronide was detected as the main metabolite in human plasma after ingestion of flavonoid procyanidins and flavan-3-ols enriched cocoa milk drinks. The conjugate
metabolites, namely epicatechin-3'-O-glucuronide, 4'-O-methyl- epicatechin-3'-O-glucuronide, and 4'-O-methyl-epicatechin-5 or 7-O-glucuronide, were identified in human after intake of epicatechin. It was also found that quercetin-3-O-glucuronide together with quercetin-3'-O-sulfate andisorhamnetin-3-O-glucuronide was dominant in human plasma after oral administration of quercetin [25].

B. Glucuronidation of flavonoids in the liver

Comparing with the investigation of intestinal first-pass metabolism of flavonoids, studies on the hepatic first-pass glucuronidation of flavonoids were limited. By comparing the concentrations of Quercetin after its intravenous and intra portal administration to rats, the hepatic extraction ratio was determined to be about 52.6%, and extensive hepatic glucuronidation of Quercetin was suggested due to the finding of glucuronides as the major metabolites of quercetin. Although limited studies were designed to specifically evaluate the contribution of glucuronidation of flavonoid in the liver, its importance should be aware of due to high content of UGTs present in the liver [26].

C. Enzymes mediating glucuronidation of flavonoids

Glucuronidation is a process of metabolism catalyzed by UDP-glucuronosyltransferases (UGTs). To date, more than 20 UGT isoforms have been identified from the endoplasmic reticulum of different tissues responding for catalyzing the biotransformation of hydrophobic substrates to hydrophilic glucuronides. Liver was found to contain most of UGT isoforms and UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 are thought to be the most important for the drug glucuronidation in the liver. Studies on the extrahepatic distribution discovered that intestine also contains a large number of UGTs. For example, UGT 1A1, 1A3, 1A4, 1A6, 2B15, and 2B4 were also revealed in the intestine, whereas UGT 1A7, 1A8, and 1A10 were found only expressed in the intestine but not in the liver. By using recombinant human UGTs, specific isoforms of UGT have been identified for the glucuronidation of various flavonoids. UGT 1A3 was reported to mediate the glucuronidation of flavonoids including naringenin, apigenin, galangin, fisetin, 7-hydroxyflavone, genestein and quercetin. Kaempferol and quercetin were demonstrated to be the substrate of UGT 1A9. UGT 1A9, 1A1, and 2B15 were reported to catalyze the glucuronidation of galangin. UGT 1A1, 1A8, and 1A9 were involved in the glucuronidation of luteolin and quercetin. Moreover, UGT 1A7 displayed differential activities toward flavonoids such
as chrysin, apigenin, galangin, fisetin kaempferol, morin, quercetin, etc. UGT 1A10 mainly found in gastrointestinal tract catalyzed the glucuronidation of a number of flavonoids, including apigenin, chrysin, luteilin morin, daidzine genistein naringenin. Recent study on baicalein was found that it is the substrate of various UGT isozymes including UGT 1A1, 1A3, 1A8, 1A7, 1A9, and 2B15 [27].

D. Tissue uptake of flavonoids

When single doses of radiolabeled polyphenols (quercetin, epigallocatechin gallate, quercetin 4'-glucoside, resveratrol) are given to rats or mice killed 1–6 hour later, radioactivity is mainly recovered in blood and in tissues of the digestive system, such as the stomach, intestine, and liver. However, polyphenols have been detected by high performance liquid chromatography (HPLC) analysis in a wide range of tissues in mice and rats, including brain, endothelial cells, heart, kidney, spleen, pancreas, prostate, uterus, ovary, mammary gland, testes, bladder, bone, and skin. The concentrations obtained in these tissues ranged from 30 to 3000 ng aglycone Equivalents/g tissue depending on the dose administered and the tissue considered. It is still difficult to say whether some polyphenols accumulate in specific target organs. A few studies seem to indicate that some cells may readily incorporate polyphenols by specific mechanisms. The endothelium is likely to be one of the primary sites of flavonoid action. Energy-dependent transport system is active in aortic endothelial cells for the uptake of morin. This system may also transport other hydroxylated phenolic compounds. Microautoradiography of mice tissues after administration of radiolabeled epigallocatechin gallate or resveratrol indicated that radioactivity is unequally incorporated into the cells of organs. Regional selectivity has also been observed in the prostate and the brain [24].

E. Elimination of flavonoids

Metabolites of polyphenols may follow two pathways of excretion, i.e., via the biliary or the urinary route. Large, extensively conjugated metabolites are more likely to be eliminated in the bile, whereas small conjugates such as monosulfates are preferentially excreted in urine. In laboratory animals, the relative magnitude of urinary and biliary excretion varies from one polyphenols to another. Biliary excretion seems to be a major pathway for the elimination of genistein, epigallocatechin gallate, and eriodictyol. Biliary excretion of polyphenols in humans may differ greatly from that in rats because of the existence of the gall bladder in humans; however, this has
never been examined. Intestinal bacteria possess-glucuronidases that are able to release free aglycones from conjugated metabolites secreted in bile. Aglycones can be reabsorbed, which results in enterohepatic cycling [24]. The general metabolism process, oral ingestion, absorption, Glucuronidation, tissue uptake and elimination of flavonoids are elucidated in figure 2.2.

F. Toxicity of flavonoids

There is much controversy regarding the purported toxic or even mutagenic properties of quercetin. At a conference of the Federation of American Societies for Experimental Biology in 1984 on mutagenic food flavonoids, carcinogenicity was reported in just 1 of 17 feeding studies conducted in laboratory animals. Furthermore, at early time researchers also reported that high doses of quercetin over several years might result in the formation of tumors in mice. However, in other long-term studies, no carcinogenicity was found. In contrast with the potential mutagenic effects of flavonoids in earlier studies, several more recent reports indicate that flavonoids, including quercetin, seem to be antimutagenic in vivo. A large clinical study, in which 9959 men and women were followed for 24 years, showed an inverse relation between the intake of flavonoids (for example quercetin) and lung cancer. One possible explanation for these conflicting data is that flavonoids are toxic to cancer cells or to immortalized cells, but are not toxic or are less toxic to normal cells. If this is true, flavonoids might play a role in the prevention of cancer [21].

Figure 2.2. Possible routs of dietary flavonoids after oral injection [28].
3. Free radicals and oxidative stress

3.1. Free radicals

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the terms collectively describing free radicals and other non-radical reactive derivatives also called oxidants. Radicals are less stable than non-radical species, although their reactivity is generally stronger. A molecule with one or more unpaired electron in its outer shell is called a free radical. They include hydroxyl (OH•), superoxide (O2•−), nitric oxide (NO•), nitrogen dioxide (NO2•), peroxyl (ROO•) and lipid peroxyl (LOO•). Also, hydrogen peroxide (H2O2), ozone (O3), singlet oxygen (1O2), hypochlorous acid (HOCl), nitrous acid (HNO2), peroxynitrite (ONOO•), dinitrogen trioxide (N2O3), lipid peroxide (LOOH), are not free radicals and generally called oxidants, but can easily lead to free radical reactions in living organisms [29]. Biological free radicals are thus highly unstable, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells, cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing, and inflammatory diseases [30].

Reactive oxygen species (ROS) mostly originate from three sources: the mitochondrial electron transport chain, NADPH oxidase and myeloperoxidase of neutrophils and xanthine oxidase of endothelial cells (figure 3.1). However,
Primary sources of ROS are the mitochondrial respiratory chain and xanthine oxidase. There is also a delayed and amplified generation of ROS due to the inflammatory response initiated by cytokines released from the damaged cells [31].

To deal with reactive species (RS), the body is equipped with an effective defense system, which includes: enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GP), and glutathione reductase (GR); high molecular weight antioxidants such as albumin, ceruloplasmin, and ferritin; and an array of low-molecular-weight antioxidants such as ascorbic acid, α-tocopherol, β-carotene, glutathione (GSH), and uric acid [32].

In health, balance between production of ROS/RNS and antioxidant defenses lies slightly in favour of ROS/RNS production. Oxidative stress occurs when there is an imbalance between free radical reactions and the scavenging capacity of antioxidative defense mechanism of the organism (figure 3.1).

Oxidative stress is a severe disruption of balance in favour of ROS/RNS. In principle, oxidative stress can result from increased production of ROS/RNS, excessive activation of phagocytic cells in chronic inflammatory diseases, diminished antioxidants. For example, mutations affecting antioxidant defense systems and depletions of dietary antioxidants and micronutrients [33].

### 3.2. Antioxidant activities of phenolic compounds

Antioxidants may intervene at different levels in the oxidative process (for example, by scavenging for free radicals and lipid peroxyl radicals,

![Figure 3.2. Oxidative stress: occurs when there is an imbalance between the production of ROS and /or RNS at one side and cellular defenses in the other.](image-url)
removing oxidatively damaged bio-molecules, and having other types of action). Antioxidants can be grouped into two: synthetic and natural antioxidants [35]. Many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) have been used to retard the oxidation process [36].

In recent years, there has been a growing interest in the search for natural antioxidants, especially secondary metabolites, for three principal reasons:

A. Numerous clinical and epidemiological studies have demonstrated that consumption of fruits and vegetables is associated with reduced risks of developing chronic diseases such as cancer, cardio-vascular disorders and diabetes;
B. Safety consideration regarding the potential harmful effects of the chronic consumption of synthetic antioxidants in foods and beverages; and
C. The public’s perception that natural and dietary antioxidant are safer than synthetic analogues. The result has been an increased interest in spices, aromatic and medicinal plants as sources of natural antioxidants [37].

3.2.1. Phenolic acids

The antioxidant activity of Phenolic acids is related to the acid moiety and the number and the relative positions of hydroxyl groups on the aromatic ring structure. Hydroxycinnamic acids are more effective antioxidant than hydroxybenzoic acids due to the increased possibilities for delocalization of phenoxy radical (figure 3.3). Benzoic and cinnamic acid, neither of which possesses free hydroxyl groups, have no free radical scavenging activities. Di- and tri-hydroxylation increase the activity over a single hydroxyl group with the position of the hydroxyl groups being the most important factor.

![Figure 3.3](image_url)

**Figure 3.3.** (a) polyhydroxybenzoic acid and (b) polyhydroxycinnamic acid.
Hydroxylation in the 2- and 4- positions or in the 3-, 4-, 5-positions confer the greatest antioxidant activity. As substituents increase the electron density on the hydroxyl group cause a decrease in the dissociation energy of the O-H bond. Therefore electron-donating substituents will increase the antioxidant activity, as in case of vanillic acid relative to the p-hydroxybenzoic acid.

Hydroxycinnamic acid esters, such as caffeoyltaric acid, p-coumaroyltartaric acid and chorogenic acid, exhibit greater antioxidant activity than the parent hydroxycinnamic acids, possibly due to increased possibilities for electron delocalization [38].

### 3.2.2. Flavonoids

Flavonoids have long been acknowledged for their unique antioxidant properties. They may prevent production of oxidants (e.g. by inhibition of xanthine oxidase and chelation of transition metals), inhibit oxidants from attacking cellular targets (e.g. by electron donation and scavenging activities), block propagation of oxidative reactions (by chain-breaking antioxidant activity), and reinforce cellular antioxidant capacity (through sparing effects on other antioxidants and inducing expression of endogenous antioxidants). Flavonoids also possess anti-inflammatory and anti-platelet aggregation effects through inhibiting relevant enzymes and signaling pathways, resulting ultimately in lower oxidant production. Finally, flavonoids used as vasodilator and have a lot medicinal values [30].

#### 3.2.2.1. Free radical scavenging activity of flavonoids

Flavonoid antioxidants function as scavengers of free radicals by rapid donation of a hydrogen atom to radicals. In general, the radical-scavenging activity of flavonoids depends on the molecular structure and the substitution pattern of hydroxyl groups, that is, on the availability of phenolic hydrogens and on the possibility of stabilization of the resulting phenoxyl radicals via hydrogen bonding or by expanded electron delocalization (figure 3.4). Previous structure-activity relationship (SAR) studies of flavonoids have pointed to the importance of the number and location of the phenolic OH groups present for the antiradical efficacy. The structural requirement considered to be essential for effective radical scavenging by flavonoids is the presence of a 3’-, 4’-dihydroxy, i.e., a O-dihydroxy group (catechol structure) in the B ring, possessing electron donating properties and being a radical target.
Also, the 3-OH moiety of the C ring is also beneficial for the antioxidant activity of flavonoids. The C2-C3 double bond conjugated with a 4-keto group, which is responsible for electron delocalization from the B ring, enhances further the radical-scavenging capacity, and saturation of the 2, 3-double bond is believed to cause a loss of activity potential. Also, the presence of both 3-OH and 5-OH groups in combination with a 4-carbonyl function and C2-C3 double bond increases the radical scavenging activity. In the absence of the O-dihydroxy structure in the B ring, hydroxyl substituent in a catechol structure on the A-ring was able to compensate and become a larger determinant of flavonoid antiradical activity. Figure 3.5, summarizes the structural criteria that modulate the free radical scavenging activity of flavonoids [39].

In summary, these structural features contribute to the increase of the phenoxy radical stability, that is, the radical scavenging activity of the parent
flavonoid. DPPH• is a free radical compound and it has been widely used to test the free radical scavenging ability of flavonoids. The scavenging of DPPH• by flavonoid (free radical scavenger) can be represented as depicted in figure 3.6 and 3.7 [40].

3.2.2.2. Chelation of transition metals by flavonoids

The antioxidant activity of flavonoids can be explained through their chelating action (figure 3.8). They bind with transition metal particularly iron and copper and thus inhibit of transition metal-catalysed free radical formation. The two most likely points of attachment of transition metal ions to the flavonoids are the o-diphenolic groups at the 3’, 4’,-dihydroxy positions

![Figure 3.5. Structure of representative flavonoid.](image)

![Figure 3.6. Structure of flavonols and their oxidized form.](image)

![Figure 3.7. In vitro free radical scavenging activity of flavonoids.](image)
in the B ring and the ketol structures, 4-keto, 3-hydroxy or 4-keto, 5-hydroxy in the C ring. Chelated in this way, transition metals would be unavailable to interact with other compounds and initiate biologically damaging reactions. Flavonoids inhibit lipid peroxidation, oxidation of linoleic acid and Fe$^{2+}$ catalyzed oxidation of glutamine synthase, through free radical scavenging and removal of metal ions from catalytic sites via chelation[41]. Due to their reducing power these phytochemicals act as both antioxidant and pro-oxidant depending upon the exposed environment. They act as prooxidant in the absence of free radicals. The classical antioxidants, α-tocopherol and vitamin C, are also reported to behave in a similar fashion. Catechins, abundant in green tea, also possess the antioxidative and pro-oxidative characteristics of Cu$^{+2}$ induced low density lipoprotein (LDL) oxidation[42].

3.2.2.3. Inhibition of xanthine oxidase activity by flavonoids

The xanthine oxidase pathway has been implicated as an important route in the oxidative injury to tissues, especially after ischemia-reperfusion. Both
Xanthine dehydrogenase and xanthine oxidase are involved in the metabolism of xanthine to uric acid in the last two steps of purin metabolism. Xanthine dehydrogenase is the form of the enzyme present under physiologic conditions, but its configuration is changed to xanthine oxidase during ischemic conditions. Xanthine oxidase is a source of oxygen free radicals. In the reperfusion phase (i.e. reoxygenation), xanthine oxidase reacts with molecular oxygen, thereby releasing superoxide free radicals (figure 3.9 and 3.10) [21].

Flavonoids such as luteolin, silibinin, and quercetin inhibit xanthine oxidase, and suggested that these compounds, or derivatives of them, may be useful leads in the development of clinically useful inhibitors of XO.

Quercetin and luteolin have stronger inhibition effect relative to silibinin. It seems likely that luteolin, silibinin, and quercetin position in the active site of XO with the dihydroxybenzene functionality of their benzopyran moiety directed toward the molybdenum cofactor, their benzopyran intercalated between Phe914 and Phe1009, their C1 carbonyl groups directed toward Arg880 [43].

**Figure 3.9.** Metabolism of hypoxanthine xanthine to uric acid under normal physiological condition.

**Figure 3.10.** Reperfusion phase of ischemic condition and xanthine oxidase inhibition by quercetin.
3.2.2.4. Inhibition of NADPH oxidases activity by flavonoids

Initially, NADPH oxidases were thought to be enzymes present only in phagocytes of the innate immune response, where they are responsible for generating large amounts of $O_2^\cdot$ to kill invading pathogens (“oxidative burst”). Upon activation, $O_2$ is reduced to $O_2^\cdot$ by the transfer of one electron from the reducing equivalent NADH or NADPH (figure 3.11). However, these enzymes that exist only to produce free radicals appeared unexpectedly in nonphagocytic cells, and it became evident that NADPH oxidases exist in various cell types [44]. Recently, a novel flavonoid derivative S17834 \[6,8\]-diallyl 5, 7-dihydroxy 2-(2-allyl 3-hydroxy 4-methoxyphenyl) 1-H benzo(b)pyran-4-one\] has been reported to directly inhibit vascular NADPH oxidase in vitro [45]. Although not yet investigated for this mechanism in ischemia–reperfusion, flavonoids have shown ability to suppress enzyme activity and/or expression of NADPH oxidases [30].

![Figure 3.11. Generation of superoxide by NADPH oxidases.](image)

3.2.2.5. Reinforcement of cellular antioxidants by flavonoids

Human studies have shown depletion of non-enzymatic antioxidants such as glutathione, ascorbic acid, and vitamin E following myocardial ischemia–reperfusion. Hydrophilic antioxidants, such as ascorbate and glutathione, have shown to work at the front line of defense against oxidative stress, protecting lipophilic antioxidants such as ubiquinol and vitamin E from oxidation. Ascorbic acid also helps to regenerate vitamin E from its oxidized form, and is in turn recycled by glutathione, although vitamin C is also needed for the recovery of glutathione from its oxidized form. In such a network, flavonoids are proposed to act as intermediate antioxidants, protecting lipophilic antioxidants and being protected by hydrophilic antioxidants [30].
3.2.2.6. Induction of phase two enzymes activities of flavonoids

The antioxidant effect of flavonoids and other phytochemicals may be exerted indirectly through induction of phase two enzymes. Phase two enzymes are proteins whose expression is coordinately regulated by an antioxidant response element (ARE) located in the promoter region of the corresponding genes. Since phase two enzymes are committed to neutralization and detoxification of xenobiotics and electrophiles, inducers of such genes may deliver protection against oxidative stress. One of the phase two enzymes, heme oxygenase-1, has been recognized as an important mediator of the delayed phase of ischemia preconditioning, and its over expression has led to reduced ventricular remodeling and hypertrophy and better myocardial recovery and contractile function.

Over the last decade, a large number of investigations have indicated the ability of flavonoids to induce phase two enzymes in animals and human cell cultures. This ability of epigallocatechin gallate has recently been reviewed. However, whether flavonoids can induce phase two enzymes in heart and thereby provide advance protection against ischemia–reperfusion injury is not yet investigated [46].

3.3. Antioxidant activity of terpenoids: Carotinoids

Carotenoids are natural pigments synthesized by plants and microorganisms, but not by animals. Carotenoids are classified as follows: 1) Carotenoid hydrocarbons are known as carotenes and contain specific end groups. Lycopenes have two acyclic end groups. β-Carotene has two cyclohexene type end groups. 2) Oxygenated carotenoids are known as xanthophyls. Examples of these compounds are a zeaxanthin and lutein (hydroxy), b) spirilloxanthin (methoxy), c) echinenone (oxo), and d) antheraxanthin (epoxy) [47].

Carotenoids exert many important functions, among which are the outstanding antioxidant effects in lipid phases by free radical scavenging or singlet oxygen quenching. With regard to antioxidants activity in biological systems, carotenoids appear to be involved protection against both singlet and triple oxygen (as radical chain- breaking antioxidants). The best documented antioxidant action of carotenoids is their ability to quench singlet oxygen (which is known to be capable of damaging lipids, DNA and of being mutagenic). This results in an excited carotenoid, which has the ability to dissipate newly acquired energy through a series of rotational and vibrational interactions with the solvent, thus regenerating the original unexcited carotenoid, which can be reused for further cycles of singlet oxygen.
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quenching: $^1\text{O}_2 + Q^3\text{O}_2 + ^3Q$ where $^1\text{O}_2$ represents singlet oxygen, $Q$ denotes quencher molecules, $^3\text{O}_2$ and $^3Q$ denotes triple oxygen and quencher respectively. The quenching activity of a carotenoid mainly depends on the number of conjugated double bonds of the molecule and is influenced to a lesser extent by carotenoid end groups (cyclic or acyclic) or the nature of substituents in carotenoids containing cyclic end groups. Lycopene (eleven conjugated and two non conjugated double bonds) is among the most efficient singlet oxygen quenchers of the natural carotenoids. The prevention of lipid peroxidation by carotenoids has been suggested to be mainly via singlet oxygen quenching [48, 49].

β -Carotene is also scavenger of peroxyl radicals, especially at low oxygen tension. This activity may be also exhibited by others carotenoids. The interactions of carotenoids with peroxyl radicals may precede via an unstable β-carotene radical adduct. Carotenoid adduct radicals have been shown to be highly resonance stabilized and are predicted to be relatively unreactive. They may further undergo decay to generate non radical products and may terminate radical reactions by binding to the attacking free radicals. Carotenoids act as antioxidants by reacting more rapidly with peroxyl radicals than do unsaturated acyl chains. In this process, carotenoids are destroyed [50].

3.4. Antioxidant activities of alkaloids: Berberine

The antioxidant activity of berberine has been widely demonstrated. First, it was reported that berberine (fig.3.12) can scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) in similar fashion with flavonoids. For instance, among the RNS, peroxynitrites (ONOO−) generated through the reaction between nitric oxide (NO·) and superoxide anion radical. Secondly, berberine can inhibit lipid peroxidation and show protective effects against low-density lipoprotein (LDL) oxidation. Thirdly, it has inhibitory effects on lipoxygenase and xanthine oxidase, two important ROS-derived

![Molecular structure of berberine](image)

Figure 3.12. Molecular structure of berberine [53].
sources. Berberine also significantly increased superoxide dismutase activity and decreased superoxide anion and malondialdehyde (MDA) formation. In addition, it was found that berberine can also bind catalyzing metal ions (transition metals like iron and copper ions), which can reduce the concentration of metal ions in lipid peroxidation [51,52].

4. Other medicinal efficacy of secondary metabolites of medicinal plants

4.1. Flavonoids

4.1.1. Flavonoids anti-inflammatory activity

Flavonoids have shown the capacity to inhibit enzymes involved in eicosanoid pathways, including phospholipase A2, cyclooxygenases and lipoxygenases, thereby limiting production of inflammatory mediators such as prostaglandins and leukotrienes. Flavonoids can also inhibit production of pro-inflammatory cytokines, such as tumor necrosis factor -α (TNF-α), interleukin (IL)-1β, IL-6, and interferon-γ, as well as chemotactic agents (figure 18) [30].

Cyclooxygenase and lipoxygenase play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Neutrophils containing lipoxygenase create chemotactic compounds from arachidonic acid. They also provoke the release of cytokines. Selected phenolic compounds were shown to inhibit both the cyclooxygenase and 5-lipoxygenase pathways. This inhibition reduces the release of arachidonic acid. The exact mechanism by which flavonoids inhibit these enzymes is not clear. Quercetin, in particular, inhibits both cyclooxygenase and lipoxygenase activities, thus diminishing the formation of these inflammatory metabolites.

Flavonoids also inhibit both cytosolic and membrane tyrosine kinase. Integral membrane proteins, such as tyrosine 3-monooxyxygenase kinase, are involved in a variety of functions, such as enzyme catalysis, transport across membranes, and transduction of signals that function as receptors of hormones and growth factors, and energy transfer in ATP synthesis. Inhibition of these proteins results in inhibition of uncontrolled cell growth and proliferation. Tyrosine kinase substrates seem to play key roles in the signal transduction pathway that regulates cell proliferation. Another anti-inflammatory property of flavonoids is their suggested ability to inhibit neutrophil degranulation. This is a direct way to diminish the release of arachidonic acid by neutrophils and other immune cells [21].
4.1.2. Antiplatelet aggregation activities of flavonoids

Thromboxane A2 (TxA2) is a powerful unstable inducer of platelet activation and aggregation produced by sequential arachidonic acid metabolism by cyclooxygenase and thromboxane synthase, upon activation of platelets with agonists such as adenosine diphosphate, thrombin or collagen. Once generated, TxA2 acts in an autocrine and paracrine manner, increasing activation and recruitment of the surrounding platelets to the site of vascular damage.

TxA2 effects on platelets, and on other target cells, are mediated via interaction with specific seven- transmembrane G-protein-coupled receptors (GPCR). The TxA2 receptor (TP) is encoded by a single gene that is alternatively spliced in the carboxyl terminus resulting in two variants, TPa (343 residues) and TPb (407 residues), that share the first 328 amino acids. Several studies have shown that flavonoids impair agonist-induced TxA2 formation through the inhibition of arachidonic acid liberation and metabolism by cyclooxygenase and TxA2 synthase activities[55]. Moreover, studies using TxA2 analogues indicate that certain flavonoids, apigenin and genistein, may behave as TP antagonists (figure 4.1) [56].

Figure 4.1. Structural features explaining the TP antagonistic activity of flavonoids: (A) flavonoid active core believed to interact with the thromboxane A2 receptor; (B) specular relationship between certain elements within the structure of thromboxane A2 (the heterocyclic ring conjugated with a double bond and the adjacent hydroxyl group) and apigenin (γ-pyrone side of the A and C conjugated rings) [57].

Figure 4.1. The proposed action of flavonoids. Flavonoids ‘F’ “=” and “↓” denote enzyme inhibition and down regulation of expression, respectively [54].
4.1.3. Flavonoids against cardiovascular disease

The potential of photochemical constituents of plant material for the maintenance of health and protection from coronary heart disease is also raising interest among scientists and food manufactures as consumers move towards functional foods with specific health effects. Cardiovascular diseases (CVD) is the name for the group of disorders of the heart and blood vessels and include hypertension (high blood pressure), coronary heart disease (heart attack), cerebrovascular disease (stroke), heart failure, peripheral vascular disease [21].

4.1.4. Flavonoids activities against hypertension

Flavonoids in plants available as flavones (containing the flavonoid apigenin found in chamomile); flavanones (hesperidin - citrus fruits; silybin- milk thistle flavonols (tea: quercetin, kaempferol and rutin grapefruit; rutin buckwheat; ginkgo flavonglycosides - ginkgo), play a major role in curing the cardiovascular diseases. Flavonoids block the angiotensin-converting enzyme (ACE) that raises blood pressure (figure 4.2). Flavonoids also protect the vascular system and strengthen the tiny capillaries that carry oxygen and essential nutrients to all cells [21].

![Figure 4.2. Flavonoids inhibit Angiotensin Converting Enzyme that raises blood pressure.](image)

4.1.5. Flavonoids activities against coronary heart disease and vascular disorder

Increased low density lipoprotein (LDL) and especially oxidized LDL are recognized as risk factors in coronary artery disease (CAD). Certain
flavonoids were potent inhibitors of the modification of LDL. Flavonoids also inhibited the cell-free oxidation of LDL mediated by CuSO4. The flavonoids appeared to act by protecting LDL against oxidation caused by the macrophages, as they inhibited the generation of lipid hydroperoxides and protected α-tocopherol, a major lipophilic antioxidant carried in lipoproteins, from being consumed by oxidation in the LDL. Thus the flavonoids protected α-tocopherol (and possibly other endogenous antioxidants) in LDL from oxidation, maintained their levels for longer periods of time, and delayed the onset of lipid peroxidation. While the mechanisms by which flavonoids inhibit LDL oxidation are not certain, the following possibilities have been advanced. First, they may reduce the generation or release of free radicals in the macrophages or may protect the α-tocopherol in LDL from oxidation by being oxidized by free radicals themselves. Second, flavonoids could regenerate active α-tocopherol by donating a hydrogen atom to the α-tocopheryl radical; the latter is formed when it transfers its own hydroxyl hydrogen atom to a lipid peroxyl radical to terminate the chain reaction of lipid peroxidation. Third, flavonoids may sequester metal ions, such as iron and copper, thereby diminishing the engendered free radicals in the medium. Preliminary evidence indicated that the isoflavone genistein inhibits Cu-mediated LDL oxidation in a time- and concentration-dependent fashion [25].

4.1.6. Antitumor activities of flavonoids

Cancer has emerged as a major public health problem in developing countries, matching the industrialized nations. A healthy lifestyle and diet can help in preventing cancer. Chronic inflammation is associated with a high cancer risk. At the molecular level, free radicals and aldehydes, produced during chronic inflammation, can induce deleterious gene mutation and posttranslational modifications of key cancer-related proteins. Chronic inflammation is also associated with immune suppression, which is a risk factor for cancer.

Flavonoids of medicinal plants are proposed as an antitumor agent in various researches. There mechanism of actions could be: by inhibition of protein Kinase activities, antiprolifration activities, induction of apoptosis, and inhibition of metastasis, migration and angiogenesis of the tumor cell (figure 4.3) [58].

4.1.6.1. Inhibition of protein kinase activity

Protein kinase C (PKC), the ubiquitous, Ca^{2+} and phospholipid-dependent, multifunctional serine- and threonine- phosphorylating enzyme,
Antiproliferation

Anti-tumor activities of flavonoids

Inhibition of protein kinase

Induction of Apoptosis

Inhibition of metastasis

**Figure 4.3.** Antitumor activities of Flavonoids.

plays a role in a gamut of cellular activities, including tumor promotion, mitogenesis, secretary processes, inflammatory cell function and T lymphocyte function. Certain dietary flavonoids turned out to be potent inhibitors of PKC in vitro. Out of different flavonoids examined, quercetin was the most efficient inhibitor of PKC by competitively blocking the ATP binding site on the catalytic unit of PKC. Flavonoids, impairing the activities of other ATP-utilizing enzymes, cause inhibition by competitively binding to the ATP binding site [59, 60].

### 4.1.6.2. Induction of apoptosis

Cell death in multicellular organisms occurs by two distinct mechanisms, apoptosis and necrosis. Apoptosis, also called programmed cell death, plays a cardinal role in embryonic development, metamorphosis, hormone-dependent atrophy, as well as in the maintenance of tissue homeostasis. Apoptosis is the result of complex signal transduction pathways, bringing about gene mediated cell death. Being a process regulated by specific gene activity, apoptosis is sensitive to mutations.

Apoptosis is one of the important pathways through which anticancer agents inhibit the growth of tumor cells. Resistance of tumor cells to cytostatic agents is a major problem in the treatment of advanced cancers. Understanding the signaling pathways that control cytostatic agent induced apoptosis in tumor cells is critical to ultimately improving anticancer therapy. At present, only a few potential anticancer agents such as the flavonoids seem
to cause apoptosis. Quercetin flavonoids induced apoptosis, characterized by typical morphological changes, in certain tumor cell lines. Quercetin also inhibited the synthesis of heat shock protein (HSP) 70 in these cell lines. There was an association between this effect and the induction of Quercetin induced apoptosis. Furthermore, quercetin and luteolin induced apoptosis in a wide range of tumor cells such as A431, MiaPaCa-2, Hep G2 and MCF 7. The citrus flavone, tangeretin (5,6,7,8,4’-pentamethoxyflavone), induced apoptosis in HL-60 cells, at concentrations greater than 2.7 µM, the flavone had little effect on the mitogen stimulated blastogenic response of human peripheral blood mononuclear cells [61].

4.1.6.3. Inhibition of metastasis, migration and angiogenesis

The spread of cancer through metastasis represents one of the gravest dangers of the disease. In human cancers of the breast, liver, colon, lung and ovary, the production of certain matrix metalloproteinases (MMPs) correlates with cancer invasion/metastasis. At least 20 genes encoded different MMPs, which one can categorize into four subclasses based on structural organization and substrate specificity: collagenases, gelatinases, stromelysins and membrane-type MMPs. MMPs belong to a rapid growing family of zinc-dependent endopeptidases that are capable of degrading a variety of extracellular matrix (ECMs). Collectively, MMPs degrade most components of the extracellular matrix. Tumor cells probably need more than one MMP, as well as more general degradative enzymes to cross the tissue barriers they encounter. There are multiple levels in the regulation of the activities of MMPs, including the expression and secretion of MMPs, and the activation processes of MMPs. Endogenous inhibitors such as 2-macroglobulin, and the tissue inhibitors of metalloproteinases (TIMP) cause inhibition of MMPs in vivo, once activated. Four structure-related tissue inhibitors, TIMP-1 to TIMP-4, regulate MMP activity. The secretion of MMPs is necessary for tumor invasion, as indicated by the observations that treatment with antibodies or inhibitors against MMPs abolished the invasive behavior of certain tumor cells. Therefore, one would expect to limit the metastatic potential of cancer cells by the suppression of the secretion and of the action of the activated MMPs in cancers. Certain studies report that flavonoids likeluteolin and quercetin influence the level of MMPs [62].

4.1.6.4. Antiproliferative activities

Dysregulated proliferation appears to be a hallmark of susceptibility to neoplasia. Cancer prevention is generally associated with inhibition, reversion
or retardation of cellular hyperproliferation. The molecular mechanism of antiproliferation may involve the inhibition of the prooxidant process that causes tumor promotion. It is generally believed that the formation of growth promoting oxidants (reactive oxygen species, ROS) is a major “catalyst” of the tumor promotion and progression stages, which follow the initiation stage (carcinogen metabolic activation to mutagens). The prooxidant enzymes induced or activated by various tumor promoters, for example, phorbol esters, include the arachidonate metabolizing enzymes, cyclooxygenases (COX), and lipoxygenases (LOX). In addition, inhibition of polyamine biosynthesis could be a contributing mechanism to the antiproliferative activities of flavonoids. Ornithine decarboxylase is a rate-limiting enzyme in polyamine biosynthesis, which has been correlated with the rate of DNA synthesis and cell proliferation in several tissues. Several experiments show that flavonoids can inhibit ornithine decarboxylase induced by tumor promoters, and thus cause a subsequent decrease in polyamine and inhibition of DNA/protein synthesis. Furthermore, flavonoids are also effective at inhibiting signal transduction enzymes, for example, protein tyrosine kinase (PTK), protein kinase C (PKC), and phosphoinositide3-kinases (PIP3), which are involved in the regulation of cell proliferation [63].

4.1.7. Flavonoids protection against myocardial ischemia–reperfusion injury

Besides antioxidant effects, flavonoids possess other properties that alleviate ischemia–reperfusion injury; for instance they help to better re-establish blood flow in post-ischemic hearts. A variety of flavonoids and polyphenols have shown the capacity to dilate blood vessels. Their mechanism of action is various and may be exerted in endothelium-dependent and/or -independent manners.

Some polyphenols, such as quercetin and resveratrol, can induce vasorelaxation by both mechanisms, although in the absence of endothelium much higher concentrations of polyphenols are probably required. The endothelium-dependent relaxation effect of polyphenols is mediated by nitric oxide. Nitric oxide (NO˙) is an important signaling molecule with vasodilatory, anti-inflammatory, and anti-platelet activities. The up-regulatory effect of polyphenols on NO˙ levels occurs through either activation of endothelium nitric oxide synthase (eNOS) or by removing O2˙− and thereby inhibiting consumption of NO˙. Other than increasing eNOS activity, flavonoids may additionally induce eNOS expression. It has been reported that in ischemic-reperfused hearts a part of beneficial effect of epigallocatechin gallate is mediated through induction of eNOS.
Intraperitoneal injection 1 mg/kg resveratrol one hour before coronary ligation in rats induced expression of eNOS and nNOS (neuronal NOS) while blocking expression of inducible nitrogen oxide synthase (iNOS) which contrary to eNOS produces excessive amounts of NO\(^{-}\) associated with formation of peroxynitrite and oxidative stress [64,65].

As eNOS is a calcium-dependent enzyme, elevation of intracellular Ca\(^{2+}\) has been suggested as the mechanism of the endothelium dependent NO\(^{-}\)-mediated vasorelaxation by polyphenols (figure 4.4). Polyphenols likely increase intracellular Ca\(^{2+}\) by stimulating both Ca\(^{2+}\) entry from extracellular milieu and Ca\(^{2+}\) release from intracellular Ca\(^{2+}\) stores. Surprisingly, the rise of Ca\(^{2+}\) by polyphenols occurs as a result of increased production of O2\(^{-}\)\^- as application of superoxide dismutase plus catalase attenuated the Ca\(^{2+}\) elevation. These results suggest that the effect of polyphenols on NO\(^{-}\) levels can occur both through stimulating O2\(^{-}\)\^- production inside endothelial cells (stimulating eNOS activity), and through scavenging O2\(^{-}\)\^- in the interstitial fluid (preserving NO\(^{-}\)).

**Figure 4.4.** Effect of flavonoids on endothelium-dependent vasorelaxation (31).
NO' is generally produced by eNOS attached to the endothelium plasma membrane and delivered to smooth muscle cells where it manifests its biological functions. In smooth muscle cells, NOU activates guanylate cyclase which synthesizes cyclic GMP (cGMP), an important mediator of vasodilation. cGMP acts by activating protein kinase G which affects a number of target proteins including those involved in Ca\(^{2+}\) channels, decreasing cytosolic Ca\(^{2+}\) through activating endoplasmic reticulum Ca\(^{2+}\) uptake and inhibiting extracellular Ca\(^{2+}\) entry. The eventual low intracellular Ca\(^{2+}\) in smooth muscle cells mitigates cellular contractility and yields relaxation. In contrast to the aforementioned polyphenol-induced vasorelaxation, inhibition of NO'-cGMP-mediated vasorelaxation has also been observed with some flavonoids [66].

The mechanism of endothelium-independent relaxation by polyphenols is yet uncertain, but signaling pathways downstream of cGMP might be activated in smooth muscle cells independently of NO'. Among downstream mechanisms are inhibition of protein kinase C and phosphodiesterases (a family of enzymes responsible for the breakdown of the vasorelaxants cyclic AMP (cAMP) and cGMP), inhibition of Ca\(^{2+}\) influx from extracellular and intracellular resources and activation of voltage-gated K\(^{+}\) channels [67].

The blockage of extracellular Ca\(^{2+}\) influx and endoplasmic reticulum Ca\(^{2+}\) release by polyphenols is appealing as it could be one of the possible mechanisms of polyphenol protection of hearts from Ca\(^{2+}\) overload in states of ischemia–reperfusion. Flavonoids may also promote vasorelaxation by stimulating production of prostacyclins by endothelial cells. It is found that 3 weeks oral administration of grape seed proanthocyanidins increased production of prostacyclins in ischemic and ischemic-reperfused hearts. Proanthocyanidins can also cause vasodilation through suppressing the rennin–angiotensin system by acting as angiotensin receptor antagonist as well as inhibiting angiotensin converting enzyme. Furthermore, vasodilatory effects of flavonoids may partly be exerted by scavenging peroxynitrite and therefore preserving tetrahydrobiopterin from oxidation. Alternatively, resveratrol has shown to elevate tetrahydrobiopterin levels by increasing activity of the rate limiting enzyme in tetrahydrobiopterin synthesis [31].

4.1.8. Inhibition of metalloproteinases by flavonoids

Matrix metalloproteinases (MMP) are a family of proteases that play a major role in protein degradation and tissue remodeling. Elevation of plasma levels of MMP has been documented after ischemia–reperfusion-related morbidities such as myocardial infarction, restenosis, and heart failure. Since increased activity of MMP is associated with ventricular dilation and cardiac
remodeling, inhibitors of MMP may play as effective strategies to prevent chronic consequences of the injury [68].

Polyphenolic compounds in red wine and green tea have shown ability to inhibit activation of metalloproteinase-2. In green tea, the inhibitory effect seemed to correlate with the gallic acid moiety of the catechins as the inhibitory activity of epigallocatechin gallate and epicatechin gallate was more than that of epigallocatechin while catechin and epicatechin showed the least effect. Epigallocatechin gallate dose-dependently decreased activation of metalloproteinase-2 in human umbilical endothelial cells. Similarly, quercetin dose-dependently decreased expression of metalloproteinase-9 in human aortic smooth muscle cells. The flavonoids inhibition of metalloproteinases has also been demonstrated in ischemic-reperfused hearts. The inhibition of metalloproteinases by phenolic compounds has been speculated to occur transcriptionally through suppression of DNA binding activity of NF-κB and AP-1. Moreover, quercetin has shown to stimulate expression of metalloproteinase-1 tissue inhibitor in human vascular endothelial cells treated with oxidized LDL. It has been suggested that high doses of polyphenols inhibit activation of metalloproteinases and prevent angiogenesis, while low doses of polyphenols show angiogenic effects without altering activity of metalloproteinases [21].

4.2. Terpenoids

4.2.1. Anti-inflammatory effect of terpenoids

4.2.1.1. Inflammation

Inflammation is caused by a variety of stimuli including physical damage, ultra violet irradiation, microbial invasion, and immune reactions. The classical key features of inflammation are redness, warmth, swelling, and pain. Inflammation cascades can lead to the development of diseases such as chronic asthma, rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and psoriasis [69].

Transcription factors, such as nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1), or signal transducer and activator of transcription (STAT), are involved in the induced expression of a variety of proteins, and especially cytokines controlling the inflammatory response. NF-κB is present in the cytosol of many cell types, usually as a heterodimer composed of p50 and p65 subunits, held in the inactive state by IκB inhibitory subunit. During physical damage, microbial invasion, stress and immune reaction, IκB inducibly phosphorylated, and subsequent ubiquitinylated. These post-translational modifications tag the molecule for the subsequent proteolytical
degradation by the ubiquitin-26 S proteasome pathway. This induced degradation of IκB proteins unmasks the nuclear localization sequences of the DNA-binding subunits of the NF-kB dimer and allows NF-kB (heterodimer p50/p65) to enter the nucleus, to bind to its DNA sequence, and to induce transcription. The target genes whose transcription is mainly regulated by NF-kB include many cytokines, cell adhesion molecules, such as intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule 1, as well as acute-phase proteins and immunoreceptors (described in figure 4.5). AP-1 refers to a family of protein dimers, usually composed of c-jun/c-fos subunits, which after stress-induced phosphorylation enter the nucleus and due to binding to consensus sequences enhance the expression of the appropriate genes. Activation of NF-kB and/or AP-1 is often induced by the proinflammatory cytokines, such as IL-1 or TNF-α. On the other hand, STAT3 belongs to the family of transcription factors activated by such cytokines as IL-6 and is involved in the up-regulation of acute phase protein synthesis in liver cells [70, 71].

![Figure 4.5. Schematic overview of NF-kB signaling pathways [16].](image)

### 4.2.1.2. Terpenoids as modulators of NF-kB signaling pathways

The molecular cascade of signaling events involved in NF-kB activation, provides several steps for specific inhibition of NF-kB activity. Inhibition of NF-kB activation can occur by different mechanisms including (a) inhibiting the activation of IKK complex, (b) targeting the proteasomal degradation of
or (c) interfering the translocation of NF-κB to the nucleus, or the binding of NF-κB to DNA. Several agents including natural products such as sesquiterpene lactones helenalin, 11α, 13-dihydrohelenalin, and chamissonoloid, sesterterpenes (cyclolinteinone) and ent-kaurane diterpenes (oridonin and poncicin) (figure 4.6) have been shown to inhibit either nuclear translocation of p65 or binding of NF-κB to DNA. However, the molecular target through which these natural compounds exert their effect not clearly understood. Nevertheless, the most effective and selective approach for the inhibition of NF-κB activation is provided by inhibitors of the IKK activity by sesquiterpene lactones such as parthenolide, kaurane diterpenes such as kamebakaurin; and labdane diterpenes such as hispanolone derivatives are examples of the specifically targeting of IKK kinase activity [72,73]. Consequently, downstream events, such as release of cytokines IL-1β, IL-6 or TNF-α production and lymphocyte proliferation are also inhibited [74].

![Figure 4.6. Structures of the investigated sesquiterpene lactones [73].](image)

**4.2.2. Terpenoids as anticancer agents**

Many studies have shown that several of the dietary monoterpens are effective in the prevention and treatment of cancer. Among these, Perillyl alcohol (POH) (p-metha, 1, 7-diene-6-ol or 4-isopropenyl-Cyclohexenecarbinol) found in the essential oils of several plants (lavendin, mints, cherries, etc.) and synthesized by the mevalonate pathway. It has well established chemopreventive activity in rodent mammary, skin, liver, lung, colon, and fore stomach cancers and also chemotherapeutic activity in pancreatic, mammary, and prostatic animal tumor models, leading to regression of existing malignant tumors. Several mechanisms have been
It has been shown that POH affects the expression of several regulators of cell cycle and apoptosis. There is also evidence that POH inhibits the post-translational isoprenylation of the Ras small GTPase superfamily of proteins, which are known to play a key role in many signal transduction pathways, including those that stimulate tumor-associated angiogenesis [75].

Limonene ((1-methyl-4-(1-methylethenyl) cyclohexane), monoterpen, has well established chemopreventive activity against many cancer types. Limonene (figure 4.7) has been shown to inhibit the development of spontaneous neoplasms in mice; dietary limonene also reduces the incidence of spontaneous lymphomas in p53 mice. Furthermore, when administered either in pure form or as orange peel oil (95% d-limonene), limonene inhibits the development of chemically induced rodent mammary, skin, liver, lung and fore stomach cancers [76].

D-limonene induces phase I and phase II carcinogen-metabolizing enzymes (cytochrome p450), which metabolize carcinogens to less toxic forms and prevent the interaction of chemical carcinogens with DNA. It also inhibits tumor cell proliferation, acceleration of the rate of tumor cell death and/or induction of tumor cell differentiation. Furthermore, as Preclinical data indicates d-limonene and its metabolites modulate Ras prenylation via farnesyl transferase inhibition. Many prenylated proteins regulate cell growth and/or transformation. Impairment of prenylation of one or more of these proteins might account for the antitumor activity of d-limonene. It was found that d-limonene attenuates gastric cancer through increasing apoptosis, while decreasing DNA synthesis and ornithine decarboxylase activity of cancer cells. D-limonene inhibits hepatocarcinogenesis via inhibition of cell proliferation, enhancement of apoptosis, and blockage of oncogene expression [77, 78].

Terpenes, such as farnesol a sesquiterpene and geraniol a monoterpen, have also been shown to have chemotherapeutic activities towards cancer cells. Although cell culture studies utilizing diverse cancer cell lines demonstrated the pronounced effects of farnesol (FOH) (figure 4.8 A) and

![Figure 4.7. Structure of Limonene.](image)
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Geraniol (GOH) (Figure 4.8 B) on the inhibition of cell proliferation, there is scarce information on the in vivo cancer chemopreventive potential of these acyclic isoprenoids. In rats FOH inhibited chemically induced colon and pancreatic carcinogenesis and GOH inhibited mammary carcinogenesis. FOH and GOH cell proliferation inhibition is based on their ability to posttranscriptionally inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activities, thus reducing synthesis of cholesterol and intermediaries of the mevalonate pathway, such as farnesyl and geranylgeranyl pyrophosphates. These mevalonate derivatives are important for protein farnesylation and geranylgeranylation of certain proto-oncogenes. Differently from normal cells, preneoplastic and neoplastic liver lesions present a loss in the transcriptional down regulation mechanism of HMG-CoA reductase by sterols and of cholesterogenesis. However, in these lesions this enzyme retains sensitivity to isoprenoid-mediated inhibition. Thus isoprenoids could represent potential chemopreventive agents against hepatocarcinogenesis. It was proposed that FOH could exert anticarcinogenic actions through farnesoid X activated receptor (FXR) - mediated gene expression. This nuclear receptor was initially shown to be activated by FOH but not by GOH. Subsequently bile acids were identified as the endogenous ligands for FXR, which plays a central role in bile acids and cholesterol metabolism. Activators of FXR such as FOH are potent cell proliferation inhibitors and apoptosis inducers [13, 79].

Moreover, monoterpenes such as carveol, uroterpenol, and sobrerol have shown activity against mammary carcinomas. Carvone has been analyzed as an agent reducing pulmonary adenoma and fore-stomach tumor formation. Several plant triterpenes exhibited in Vitro antitumor activity. Betulinic acid has been shown to induce apoptosis of several human tumor cells; including melanoma and glioma, and ursolic acid and oleanolic acid reduced leukemia cell growth and inhibited the proliferation of several transplantable tumors in animals [13].

Plant derived diterpenoids are the most effective anticancer agents approved by the Food and drug administration (FDA). Taxol, registered as a

![Figure 4.8](image)

**Figure 4.8.** Chemical structure of fernesol (A) a sesquiterpene and geraniol (B) a monoterpene.
trademark Taxol® by Bristol-Myers Squibb, and known generically as paclitaxel, is a secondary compound with a very complex chemical structure and one of the most effective anti-cancer drugs ever developed (Figure 4.9).

Taxol extracted and identified in 1971 from the inner bark of Taxus brevifolia (the pacific yew tree), is a potent antimitotic agent with excellent activity against breast and ovarian cancers. Taxol exhibits a unique mode of action. It acts as microtubulin stabilizing agent while the other anticancer agents destabilize this process. Actually, tubulin polymerizes to microtubulin and again microtubulin converts into tubulin. In a normal case, this process is in equilibrium later on, fixed-size 24-nm microtubulin bundles are formed and the cell multiplication process takes place, whereas taxol makes stabler bundles of microtubulins of size 22 nm (Figure 4.10). Due to this, a defective polymerization process occurs and thus, these cells have unnatural ‘bundles’ of microtubules and no mitotic spindle. The cancerous cells lack a check point to detect the absence of a spindle and attempt to continue the cell cycle, which leads to cell death. Because of this reason, taxol is sometimes also referred as a ‘spindle poison’ [80, 81].

4.2.3. Antiparasitic and antibacterial activity of terpenoids

Increased resistance in many pathogens towards currently used medicines, creating a serious threat to the treatment of infectious diseases.
Drug resistance is one of the most serious global threats to the treatment of infectious diseases. In addition to resulting in significant increases in costs and toxicity of newer drugs, antibiotic resistance is eroding our therapeutic armamentarium. Resistant strains of bacteria are continuing to increase, both in number and in variety, but not significantly different newer antibiotics are yet available. Treatment of infections caused by these resistant bacteria has become very difficult. Since they are resistant to many antibiotics, therapeutic options have become limited. Therefore, alternative methods of treatment are sought after. For over several years medicinal plants have served as the models for many clinically proven drugs, and are now being reassessed as antimicrobial agents [82].

As a result of monoterpenes lipophilic character, they preferentially partition from an aqueous phase into membrane structures. This results in membrane expansion, increased membrane fluidity and permeability, disturbance of membrane-embedded proteins, inhibition of respiration, and alteration of ion transport processes in gram-negative bacteria, evidencing that monoterpenes uptake is also determined by the permeability of the outer envelope of the target microorganism. However, specific mechanisms involved in the antimicrobial action of monoterpenes remain poorly characterized [83].
4.3. Alkaloids

4.3.1. Antitumor activities of alkaloids

The antitumor properties of the alkaloids extracted from Amaryllidaceae are well known. The first described compound with a cytostatic effect was lycorine (figure 4.11), which has been shown to exhibit antitumor activities, e.g. it can suppress leukaemia cell growth, reduce cell survival by arresting the cell cycle and inducing the apoptosis of tumor cells and it can also induce apoptosis and cell cycle arrest in a pre-B lymphoid cell line (KM3). Other types of Amaryllidaceae alkaloids likewise exhibit cytostatic effects, e.g. those of tazettine or crinine type. However, the most promising candidates are the narciclasin type pancratistatin and narciclasine [80]. Berberine is the major constituent of Coptis Chinese, a yellow benzylisoquinoline alkaloid, in vitro, induces apoptosis in both HL-60 and WEHI-3 cell lines in association with caspase-3 activity and MMP depolarization. In both berberine-treated cell lines, berberine increased accumulation of Bax and Bad, but decreased the expression of Bcl-2 and led to the depolarization of mitochondrial membrane potential (MMP), thus further enhancing the release of cytochrome c and increasing the activation of caspase-3, finally leading to apoptosis (figure 4.12). This suggests the potential application of berberine in the treatment of leukemia cells [85].

Cryptolepine, which is the main indoloquinoline alkaloid found in the roots of the climbing shrub Cryptolepis sanguinolenta (Periplocaceae), has cytotoxic properties in several cancer cell lines (for example B16 melanoma cells), and the mechanisms involved may include intercalation into DNA and inhibit its synthesis. In addition, it stabilizes topoisomerase II-DNA covalent complexes and stimulates the cutting of DNA at a subset of preexisting topoisomerase II cleavage sites. The mode of cryptolepine intercalation into DNA is peculiar in that it occurs at non alternating CG rich sequences [86, 87].

![Figure 4.11. Structure of lycorine [84].](image)
Figure 4.12. A proposed model for the berberine mechanism of apoptosis in leukemia cells [85].

4.3.2. Gout suppressant activity of alkaloids

Acute gouty arthritis (GA) is characterized by intense inflammation induced by monosodium urate (MSU) crystal deposition in articular joints and periarticular tissues. Interactions of MSU crystals with synovial lining cells, macrophages and other synovial cells attract a massive neutrophils influx into the joints via interleukin 8 (IL-8) chemotaxis, which drives episodes of acute gouty inflammation. Ingestion of MSU crystals by phagocytes after CD14 and Toll-like receptor (TLR) 4 engagements also elicits production of proinflammatory mediators that propagate and sustain intense acute GA [88].

Leukocyte chemotaxis in general has been shown to be receptor mediated, and the activated receptor in stimulated cells is believed to deliver a signal to the motility elements of the cells (such as microfilaments and microtubules), resulting in their orientation and subsequent directional migration towards the chemoattractant. The evidence for a functional role of microtubules in leukocyte chemotaxis has been somewhat controversial. However, work from several laboratories has suggested that microtubules play an important role in leukocyte chemotaxis. It was shown that exposure of leukocytes to attractants induces microtubule assembly before locomotion. This assembly was blocked by colchicine, which also prevented their proper orientation toward chemoattractants (figure. 4.13) [89].
Colchicine and vinca alkaloids each have a specific binding site on the tubulin dimer. Both compounds cause metaphase arrest, and either can be used to treat acute gouty arthritis. Structural studies show unfolding of a small region in the carboxy terminal of beta tubulin induced by colchicines. Such unfolding is thought to prevent the necessary contacts for microtubule polymerization through GTP hydrolysis. Colchicine and other tubulin disruptive molecules inhibit a characteristic and specific protein phosphorylation pattern that occurs in neutrophils exposed to monosodium urate [90].

Colchicine may also alter the distribution of adhesion molecules on the surface of both neutrophils and endothelial cells, leading to a significant inhibition of interaction between white blood cells (WBC) and endothelial cells interfering with their transmigration [91].

![Diagram](image_url)

**Figure 4.13.** A model of the mechanism of action of colchicines [92].
4.3.3. Antimalarial activity of alkaloids

Malaria, a tropical blood-borne protozoan disease caused by parasites of the genus Plasmodium, is one of the most important infectious diseases in the World. Nowadays, new antimalarial drugs have become an urgent need because of the declining efficiency of classical medication, and the rapid extension of chloroquine resistant strains of Plasmodium falciparum. Drug resistance is responsible for the spread of malaria to new areas, the recurrence of malaria in areas where the disease had been eradicated and plays an important role in the occurrence and severity of epidemics in some parts of the World [93, 94].

Cryptolepine, which has cytotoxic effect for some cancer cell lines, also has antimalarial activity for drug resistant strains of Plasmodium falciparum. Investigations into its molecular mechanisms of action have revealed that the antiplasmodial mode of action may be different from the cytotoxic mode of action. Cryptolepine shares with chloroquine, and related quinoline antimalarial drugs, the property of binding to heme and prevents its conversion to hemazoin. The drug-heme complex is considered to be toxic to the parasite [95].

4.3.4. Alkaloids for Alzheimer’s disease treatment

Alzheimer’s disease (AD) is the most frequent form of dementia characterized by memory loss and abnormal mental and physical behavioral changes. It is a progressive disorder leading β-amyloid plaque formation, neurofibrillary tangle formation, oxidative and inflammatory processes and deficiency in the neurotransmitter called acetylcholine in the brain [96].

A consistent neuropathological occurrence associated with memory loss is a cholinergic deficit, which has been correlated with the severity of AD. Therefore attempts to restore cholinergic function have been a rational target for drugs used to treat the symptoms of AD. Approaches to enhance cholinergic function in AD have included stimulation of cholinergic receptors (e.g. the stimulation of nicotinic receptors by nicotine), or by prolonging the availability of acetylcholine (ACh) released into the neuronal synaptic cleft. This may occur by inhibiting ACh hydrolysis by acetylcholinesterase (AChE), through the use of AChE inhibitors [97]. Molecules including phystostigmine, galanthamine, and huperzine A are the alkaloid-type of compounds isolated from the plants which possess remarkable anticholinesterase (inhibition of acetylcholinesterase responsible for hydrolysis of acetylcholine) effects to treat AD [83, 98].
5. References


