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Potential mechanisms for the antioxidant effects of Jasonia montana

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Abstract: The use of medicinal plants as a dietary supplement is increasing in parallel with the research on it's therapeutically roles on human health. A literature survey indicated that polyphenols from *Jasonia montana* represent a promising antioxidant activity. Quercetin is the most common Jasonia montana polyphenols. Also, Jasonia montana quercetin derivatives act as antioxidants in vitro by scavenging reactive oxygen and nitrogen species and chelating redox-active transition metal ions. This review will discuss the mechanism of antioxidant and chelating activity of quercetin in an attempt to understand its mechanism of action, which may pave the way for possible therapeutic applications.

Key Words: Jasonia montana, antioxidants, chelating activity, quercetin and polyphenols

Introduction

In recent years, polyphenols have been reported to possess various pharmacological actions, including anti-obesity[1, 2], antidiabetic [3, 4] and anti-cancer [5]. Many plant extracts and plant products have been shown to have significant antiobesity and anti-diabetic activities, [6,7] which may be an important property of medicinal plants associated with the treatment of several ill fated diseases including obesity, diabetes and atherosclerosis. Plant-derived polyphenols minimize obesity induced diabetes [8]. Among these herbal resources, the plant *Jasonia montana* occurs in the Mediterranean and adjacent areas, [9] including the Sinai Peninsula [10]. The herb has a strong aromatic odor and is used in traditional medicine for diarrhea, stomachache, and chest diseases [10]. *Jasonia montana* is one of the most common medicinal plants. The *Jasonia montana* owes its therapeutical activity to different groups of effective substances, which make up the complex effect of the drug.

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A literature survey indicated that some mono- and sesquiterpenes, [11-14] flavonoids, [15] Essential oils are of greatest importance among all effective substances [16]. Poly-phenols exist in many plants and are especially abundant in *Jasonia montana* [17], whose dried leaves are used as antioxidant. *Jasonia montana* and polyphenol-enriched plant extracts have no known toxicity. Thus polyphenols from *Jasonia montana* and possibly other plant sources represent a promising potential species [18]. These polyphenols are more potent antioxidants than vitamins C and E [19]. Polyphenol rich extracts from *Jasonia montana* inhibit lipid peroxidation in experimental animals [18-20]. Not surprisingly, plants such as *Jasonia montana* contain high levels of polyphenols, [19] which are excellent scavengers of reactive and represent a promising antiobesity effect. The different extracts of the plant were also tested for hypoglycemic, antidiabetic and anticholestatic activities [18, 20]. Recently, Hussein and Farghaly [21] studied the protective activity of *Jasonia* ethanolic extract against liver and kidney damage induced by iron-overloaded in adult rats and suggest that the aerial parts of *J. montana* extract may effectively normalize the impaired antioxidant

status in iron-overloaded rats model experiment. *In vivo* tests have been conducted with *Jasonia montana* to determine, for example, its, hypoglycemic [18], antioxidant, anticholestatic [20] and antihaemostatic [21] activities.

a- Free radicals

The term "free radicals" designates a family of compounds characterized by great reactivity due to the impaired electron in the outer orbital. To this group belong reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical and hydrogen peroxide, as well as reactive nitrogen species (RNS) which include nitric oxide and peroxynitrite. Although structurally different, free radicals share similar mechanisms to harm body's cells and tissues through damage on proteins, DNA and lipids [22]. The alterations of membrane functions occurring as a consequence of phospholipid modifications represent a relevant, radical species-dependent injury, either when considering the organism as a whole, or a specific integrated function, such as the immune response [23]. The potential therapeutic applications of antioxidants in free radical-related diseases led to the hypothesis of their use to slow down or reverse, for example, symptoms associated with neurodegenerative disorders, such as Alzheimer's disease (AD) and Parkinson's disease (PD). Such effect could occur through a block of proinflammatory cytokines action and the resulting oxidative damage [23-28]. However, several clinical studies demonstrated that not only malnutrition, but also the excess of certain nutrients (e.g. iron, alphatocopherol, beta-carotene, ascorbic acid) may set into motion oxidation phenomena and, therefore, cell injury [29, 30].

b- Oxidative Stress

The body is normally under a dynamic equilibrium between free radical generation and quenching. The physiological defense systems to counteract free radicals encompass endogenous enzyme systems, such as catalase, glutathione reductase and superoxide dismutase, as well as glutathione, urate and coenzyme Q, or exogenous factors (β-carotene, vitamin C, vitamin E and selenium) [31]. All these molecules have an antioxidant effect due to their ability to transform ROS into stable and harmless compounds or by scavenging both ROS and RNS with a redox based mechanism [31]. Very recently, a main role in the fight against oxidative stress has been assumed by enzymes such as heme oxygenase (HO) and biliverdin reductase (BVR). Heme oxygenase is a microsomal enzyme which metabolizes heme into ferrous iron, carbon monoxide and biliverdin (BV); the latter is then reduced by BVR into bilirubin (BR), a molecule endowed with strong antioxidant and antinitrosative activities [32-35]. Interestingly, all these protective factors act in a concerted way, enhancing the antioxidant defense system of the cell. When the balance between ROS/RNS and antioxidants turns in favor of the former, oxidative/nitrosative stress occurs. Although oxidative stress is associated with most diseases, routine assay methods are not nowadays available in the clinical practice. A strategy widely used to determine oxidative stress is measurement of malonyldialdehyde, F2-isoprostanes, or 8-hydroxydesoxyguanosine. Actually, these molecules are regarded as the most reliable markers available [36]. A classic example of an oxidation product apparently leading to disease is oxidized cholesterol in low-density lipoprotein (LDL), which displays a higher atherogenic potential than native LDL, and mainly involved in the pathogenesis of atherosclerosis and coronary heart disease (CHD) [37].

At the cellular level, a large body of data clearly demonstrated that ROS, when produced in low amounts and in a controlled manner, are physiological components of the signalling generated by cytokines, growth factors and neurotrophic peptides [38-43], although they may also activate apoptotic cell death [44]. Extracellularly generated ROS can diffuse through anion channels into the cytoplasm; the resulting variation in the cell redox state leads to modulation of an array of transcription factors (eg. NFkB, AP-1), protein kinases (e.g. AKT, JNK, p38), and receptor activated MAP kinases involved in apoptosis [38,45-47]. Moreover, the proapoptotic molecules Fas and Fas ligand (FasL) undergo positive transcriptional regulation after exposure to oxidants [48]. Interestingly, Krammer and Colleagues demonstrated that in vitro administration of vitamin E suppresses FasL mRNA expression and protects T cells of HIV-1 infected individuals from Fas mediated apoptosis [49]. Moreover, it was demonstrated that administration of combinations of vitamin E and C to cultures of human umbilical vein endothelial cells (HUVEC) treated with lipopolysaccharide could prevent apoptosis by upregulation of *Bcl-2* [50].

c- Phytochemical studies:

High content of flavonoids and phenolic compounds was found in *Jasonia montana* such as polyphenols[11-14], Essential oils and flavonoids[15]; 3,6,7,3`,4`-pentamethoxy quercetin (artemitin), 3,6,7,3`-tetramethoxy quercetin (chrysosplenetin), 3,6,3`,4`-tetramethoxy quercetin, 3,6,7- trimethoxy kaempferol, 3,6,3`-trimethoxy quercetin (jaceidin), 3,6,4`-trimethoxy quercetin (centaureidin), 3,3`,4`-trimethoxy quercetin, 3,6-dimethoxy quercetin, 3,3`-dimethoxy quercetin, 7,4`-dimethoxy quercetin, quercetin, quercetin-3-O-α-D-4C1-glucopyranoside, 3,5-dicaffeoyl-quinic acid, caffeic acid, quercetin-3-O-L- 1C4-rhamnopyranoside (Quercitrin) and quercetin-3- O-α-D-4C1 glucuronopyranoside [51] which may be responsible for free radical activity. There are eighteen phenolic quercetin derivatives were isolated from the chloroform, ethyl acetate and n butanol fractions of *Jasonia montana* [51].

Most studies assessing the antioxidant properties of quercetin utilize the aglycone form; however, analysis of plasma after quercetin consumption indicates that quercetin metabolites, like glucuronide (quercetin-3-*O*-β-D-glucuronide), are the primary compounds circulating in the blood [52]. The metabolites are also what are primarily found in plants [53]. The aglycone is used in studies because there are few quercetin metabolites commercially available. The chemical structure of the metabolites, however, is possible (Figure 1)

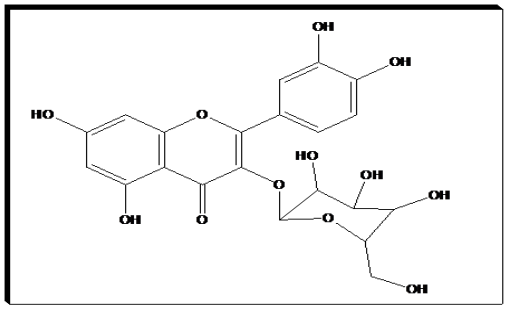

Figure 1. Quercetin-3-O-ß-glucoside (52).

d- The antioxidant properties of quercetin

Quercetin is considered to be a strong antioxidant due to its ability to scavenge free radicals and bind transition metal ions. These properties of quercetin allow it to inhibit lipid peroxidation [53, 54]. Lipid peroxidation is the process by which unsaturated fatty acids are converted to free radicals via the abstraction of hydrogen.

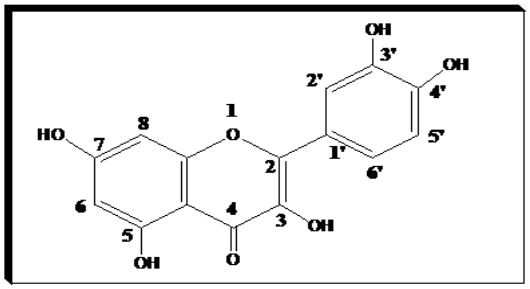

Figure 2. Quercetin

The subsequent free radicals are oxidized by molecular oxygen to create lipid peroxy radicals. This process is propagated by the resulting lipid peroxy radicals extracting hydrogen from other unsaturated fatty acid molecules to create more free radicals. It is catalyzed, in part, by the presence of trace amounts of transition metal ions. Lipid peroxidation can create deleterious effects throughout the body, such as cardiovascular and neurodegenerative diseases; however, it can be terminated by antioxidants, like quercetin, which interfere by reacting with the radicals formed [55].

The oxidation of low-density lipoproteins (LDL) can result in the formation of atherosclerotic plaques, leading to cardiovascular disease [53]. However, several studies have illustrated quercetin's ability to inhibit LDL oxidation. Graf and co-workers found a 21% reduction in cardiovascular disease mortality when the intake of quercetin was greater than 4mg/dl [56]. Chopra [57] gave one group of males 30mg/d of quercetin and another group 1g of red wine powdered extract for two weeks, prior to which there had been a placebo period for all participants so that each could be their own control. The red wine extract was in the form of a powder and contained several flavonoids, among which quercetin constituted 3.5 mg per gram of powder. Every participant was required to keep a journal of their intake of specific foods, including: fruits, vegetables, chocolates, fruit juices, milk, and alcohol. Vitamins C and E plasma concentrations were also measured along with flavonoids. They reported that the red wine extract and quercetin inhibited LDL and there was no effect on plasma concentrations of vitamin C and E. However, plasma concentrations of LDL remained constant. Chopra and co-workers suggested that LDL-cholesterol is only lowered by quercetin in hyperlipidemic patients; otherwise, quercetin inhibits LDL oxidation [57].

The vulnerability of brain lipid membranes to lipid peroxidation is thought to lead to neurodegenerative disease, such as Alzheimer's and Parkinson's disease [58]. Balazs and Leon [58] found that oxidative stress occurring in the brain membrane lipids is associated with the extracellular accumulation of amyloid beta-peptide, which precedes neural losses in Alzheimer's patients. Yet, formation of amyloid plaques can be prevented by taking antioxidants [59, 60]. In this situation, quercetin does not only stop the propagation of lipid peroxidation, but also increases glutathione (GSH) levels [59]. GSH is part of the neuron's defense against oxidative damage. When the superoxide radical is formed, the radical can be converted to the hydrogen peroxide radical by superoxide dismutase; however, GSH can convert hydrogen peroxide to oxygen and water, preventing the formation of free radicals [58].

Quercetin can also reduce inflammation by scavenging free radicals. Free radicals can activate transcription factors that generate pro-inflammatory cytokines, which are often found elevated in patients that suffer from chronic inflammatory diseases [61]. Chronic prostatitis is not well understood, but it is thought that the disease inflammates the genital tract. Alexander [62] examined semen samples taken from normal men and men with chronic prostatitis, measuring the levels of the cytokines tumor necrosis factor-alpha and interleukin-1 beta. The results showed that men with the disease had higher levels of both pro-inflammatory cytokines in seminal plasma. In another study, Shoskes and co-workers administered men with chronic prostatitis 500mg of quercetin twice a day for one month. As a result, 67% of the men had a 25% improvement in symptoms.

Oxidative stress can cause cell death by means of prolonged elevations of intracellular Ca^{2+} concentrations [54]. Elevated levels of Ca^{2+} concentrations lead to an increase in energy expenditure and subsequent initiation of cytoskeletal degradation, which can lead to strokes and acute neuronal losses [64]. However, quercetin can protect cells suffering oxidative stress and thus prevent Ca^{2+}-dependent cell death [54]. In a 15 year study following 550 middle-aged men, those with a flavonol intake greater than 30mg/d had a 60% reduction in their risk for strokes [53].

Quercetin can also protect against the more obvious environmental causes of free radicals, such as smoking. Cigarette tar is a source of free radicals, which has been found to damage erythrocyte membranes. Begum and Terao [65] found that the quercetin aglycone and its conjugate metabolites (quercetin-3-*O*-β-glucuronide and quercetin-3-*O*- β -glucoside) could protect erythrocytes from the membranous damage that is caused by smoking. The control used in the study was flavone, which has the basic structure of quercetin but no hydroxyl groups, and it had no effect on the erythrocytes. This indicated that the hydroxyl groups are important to the antioxidant properties of quercetin.

e- Structural Criteria for the Antioxidant Action of quercetin.

Intensity of the antioxidant activity of a quercetin strongly depends on its chemical structure. There is a great deal of discussion and contradiction regarding the structure antioxidant activity relationships of flavonoids [66, 67].

However, it is well-accepted that the antioxidant activity of flavonoids is markedly influenced by the number and position of hydroxyl groups on the B and A rings, and by the extent of conjugation between the B and C rings [68-77]. On the basis of many previous and recent findings [78, 91, 92-98], it seems that favourable general structural requirements for effective radical scavenging and/or the antioxidative potential of quercetin follow the famous three Bors' criteria [99]:

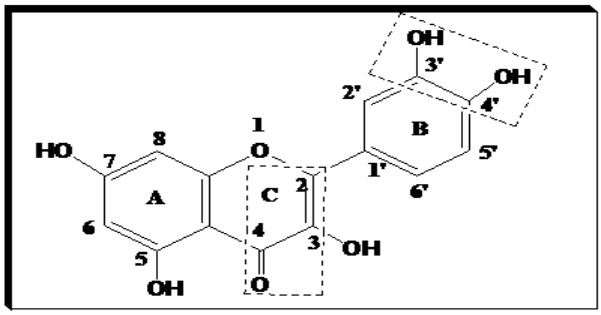

Figure 3. Structural features of quercetin with high antioxidant activity.

a) The *o*-dihydroxy (3',4'-diOH, *i.e.,* catechol) structure in the B ring, which confers high stability to the quercetin phenoxyl radical *via* hydrogen bonding or by expanded electron delocalization;
b) The C2-C3 double bond (in conjugation with the 4- oxo group), which determines the coplanarity of the heteroring and participates in radical stabilization *via* electron delocalization over all three ring systems;

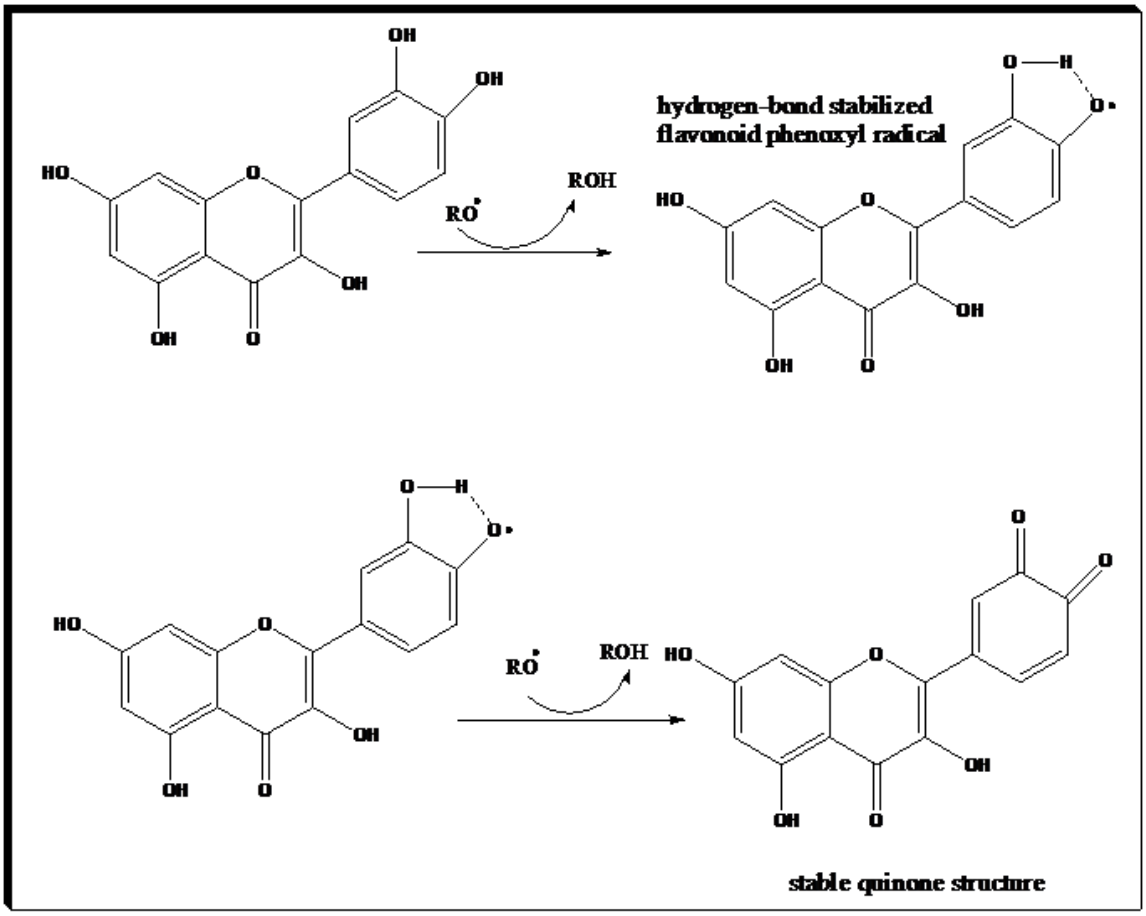

Figure 4a. Mechanism of antioxidant action of 3',4'-diOH quercetin

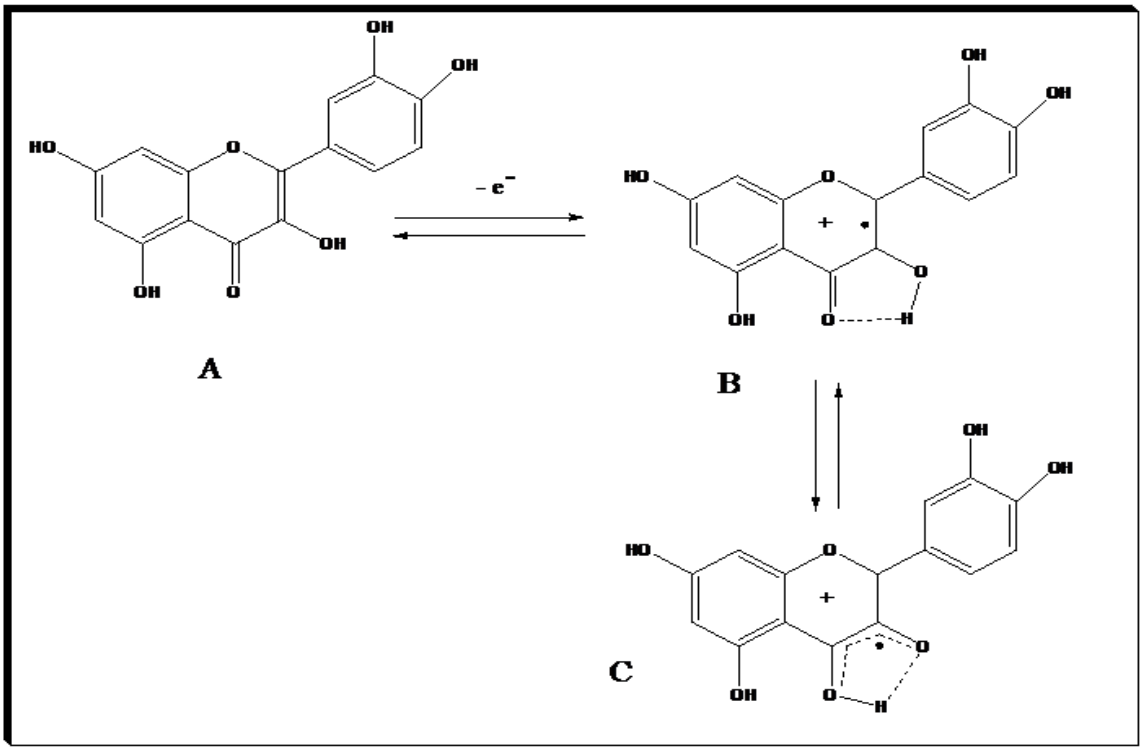

Figure 4b. Mechanism of antioxidant action of C3-OH or C5-OH quercetin.

c) The presence of both 3-OH and 5-OH groups for the maximal radical scavenging capacity and the strongest radical absorption. Moreover, an additional criterion could be added:
d) In the absence of *o*-dihydroxy structure in the B ring, hydroxyl substituents in a catechol structure on the A ring are able to compensate and become a larger determinant of flavonoid antiradical activity [100-106]. According to van Acker [66], the basic quercetin structure does not seem to be essential for good antioxidant activity. It becomes important only when the catechol moiety is not present.

f- Mechanisms of the Antioxidant Action of quercetin

Mechanisms of the antioxidant action of quercetin can include direct scavenging of reactive free radicals, chelating of trace metal ions involved in free radical formation, inhibition of enzymes involved in free radical production, and regeneration of membrane-bound antioxidants such as α- tocopherol [81, 70, 107].

The antioxidant action of flavonoids can arise from direct scavenging of reactive oxygen species. It is generally considered that the primary mechanism of the radical scavenging activity of flavonoids is hydrogen atom donation. These antioxidants may also act by single-electron transfer [108]. Structural requirements for the H-donating antioxidant activity include *ortho*-dihydroxy substitution in the B ring, C2-C3 double bond, and C-4 carbonyl group in the C ring [99, 109]. In the hydrogen atom transfer mechanism, hydroxy groups donate hydrogen to a radical, stabilizing it and giving rise to a relatively stable flavonoid phenoxyl radical (Fig. **4a,b**). The quercetin phenoxyl radical may react with a second radical ($RO^{\bullet}$), acquiring a stable quinone structure. The electron donation mechanism may be valid for the monohydroxyflavones, where hydrogen atom donation by other hydroxyl moieties is not an option. For 3-OH and/or 5- OH hydroxyflavones, the strong hydrogen bond of their OH moiety with the oxygen atom of the C-4 carbonyl group may prevent not only their efficient deprotonation, but also their radical scavenging action by means of hydrogen atom donation. The proposed mechanism of the antioxidant action of C3-OH or C5-OH hydroxyflavones is shown in Fig. **4a,b**

Structure A is the parent neutral molecule of 3-hydroxyflavone, B is the initial radical cation (resulting from electron abstraction from the neutral molecule), and C is its more stable tautomeric form. The tautomeric form C of the radical cation results from the initial radical cation B and the proton transfer from C3-OH to the C-4 carbonyl group.

A number of flavonoids efficiently chelate trace metal ions, such as Fe^{2+} and Cu^{+} that play an important role in oxygen metabolism and free radical formation [78]. Free iron(II) and copper(I) help the formation of reactive oxygen species, as exemplified by the reduction of hydrogen peroxide (Fenton reaction) with generation of the highly aggressive hydroxyl radical:

$$H_2O_2 + Fe^{2+} (Cu^{+}) \rightarrow HO^{\bullet} + OH^{-} + Fe^{3+} (Cu^{2+})$$

The proposed binding site for trace metal ions to quercetin is the 3',4'-diOH moiety in the B ring. In addition, C-3 and C-5 OH groups and the 4-carbonyl group also contribute to metal ion chelation (Fig. **5**). Besides scavenging free radicals directly and chelating transition metal ions by masking their prooxidant actions, flavonoids also behave as antioxidants through inhibition of prooxidant enzymes. This mechanism seems to be responsible for their *in vivo* effects [110]. Non-antioxidant mechanisms of flavonoid action, such as modulation of signalling pathways and gene expression, could also contribute to protective properties of quercetin [111, 109, 98].

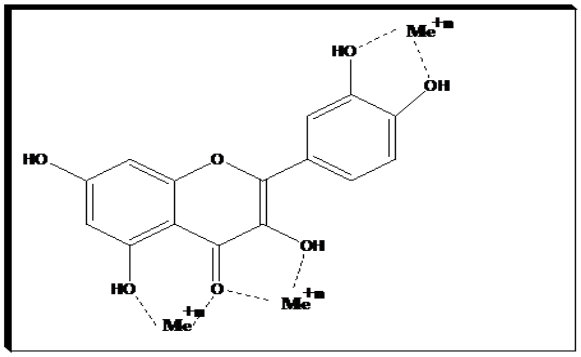

Figure 5. Binding sites for trace elements [78].

Conclusion

There is increasing evidence to suggest that antioxidant action is not the fundamental process that leads to any health benefits of Jasonia montana polyphenols. It seems likely that any activity occurs at a more fundamental level related to enzyme interaction and gene expression. Quercetin exhibits

a range of activities. Antioxidant activity is unique in that rather than describing a physiological function it describes a chemical reactivity based on the oxidizability of these compounds. The question is whether this reactivity is expressed in physiological conditions and, if so, what are the oxidation products and, perhaps more importantly, what is the physiological significance of such reactivity? Similar questions can be asked of the products of quercetin metabolism in the liver and by intestinal microflora. On the other hand, the reactivity of polyphenols as reflected in their antioxidant potential means that certain quercetin derivatives can bind with proteins impacting their behavior. Although polyphenol concentrations in plasma are below levels expected to show bioactivity, non-covalent interaction with protein provides a means of delivery to target organs/tissues where their localized concentrations may exert the observed effects.

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