2.1. Psidium guajava

*Psidium guajava* (Guava), belonging to the Family of Myrtaceae, which is originated in Mexico and extends all over the South America, Europe, Africa and Asia. Based on archaeological data, it has been used widely and known in Peru since pre-Columbian times. *Psidium guajava* grows in all the tropical and subtropical areas of the world, adapts to different climatic conditions but prefers dry climates (Stone, 1970). The main habitual use known is as an anti-diarrhoeal. Other reported uses include gastroenteritis, dysentery, antibacterial colic pathogenic germs of the intestine.

2.2. Use in traditional medicine

Medicinal plants are an essential constituent of the indigenous medical systems around the world. These resources are part of their traditional knowledge. Up to date ethnopharmacological studies show that *Psidium guajava* is used in many parts of the world for the treatment of a number of diseases, e.g. as an anti-inflammatory, used in the treatment of gastroenteritis, diarrhea, dysentery, diabetes, hypertension, caries, wounds, pain relief and reducing fever (Table 1). Some of the countries with a long history of traditional medicinal use of guava include Mexico and other Central American countries including the Caribbean, Africa and Asia. In addition to the medicinal uses, *Psidium guajava* is also employed as food, in carpentry, in construction of houses and in the manufacture of toys (Table 2).

2.3. Phytochemistry of leaves

*Psidium guajava* leaves contain essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, cineol, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene (Zakaria and Mohd, 1994; Li et al., 1999). Arima and Danno (2002) isolated flavonoids and saponins from the leaves and its combined with oleanolic acid. Nerolidiol, β-sitosterol, ursolic, crategolic, and guayavolic acids have also been identified (Iwu, 1993).
<table>
<thead>
<tr>
<th>Place, Country</th>
<th>Part(s) used</th>
<th>Ethno medical uses</th>
<th>Preparation(s)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colombia, Mexico</td>
<td>Leaves</td>
<td>Gastroenteritis, diarrhoea, dysentery, rheumatic pain, wounds, ulcers, and toothache</td>
<td>Decoction and poultice</td>
<td>Heinrich et al. (1998), Aguilar et al. (1994)</td>
</tr>
<tr>
<td>Indigenous Maya, Nahuatl, Zapotec and Popoluca of the region Tuxtlas, Veracruz, Mexico</td>
<td>Leaves</td>
<td>Cough, diarrhoea</td>
<td>Decoction or infusion</td>
<td>Heinrich et al. (1998), Leonti et al. (2001)</td>
</tr>
<tr>
<td>Latin America, Mozambique</td>
<td>Leaves</td>
<td>Diarrhoea, stomach ache</td>
<td>Infusion or decoction</td>
<td>Pontikis (1996)</td>
</tr>
<tr>
<td>Mexico</td>
<td>Shoots, leaves, bark and leaves mixed, rip fruits</td>
<td>Febrifuge, expel the placenta after childbirth, cold, cough hypoglycaemic, affections of the skin, caries, vaginal haemorrhage, wounds, fever, dehydration, respiratory disturbances</td>
<td>Decoction, poultice</td>
<td>Martinez and Barajas (1991), Argueta et al. (1994), Linares and Bye (1990), Leonti et al. (2001), Heinrich et al. (1998)</td>
</tr>
<tr>
<td>Panama, Cuba, Costa Rica, México, Nicaragua, Panamá, Perú, Venezuela, Mozambique, Guatemala, Argentina</td>
<td>Leaves</td>
<td>Anti-inflammatory</td>
<td>Externally applied hot on inflammations</td>
<td>Pardo (1999), Jansen and Méndez (1990), Valdizán and Maldonado (1972)</td>
</tr>
<tr>
<td>South Africa</td>
<td>Leaves</td>
<td>Diabetes mellitus, hypertension</td>
<td>Infusion or decoction</td>
<td>Oh et al. (2005), Ojewole (2005)</td>
</tr>
<tr>
<td>Caribbean</td>
<td>Leaves</td>
<td>Diabetes mellitus</td>
<td>Infusion or decoction</td>
<td>Mejía and Rengifo (2000)</td>
</tr>
<tr>
<td>China</td>
<td>Leaves</td>
<td>Diarrhoea, antiseptic, Diabetes mellitus</td>
<td>Infusion or decoction</td>
<td>Teixeira et al. (2003)</td>
</tr>
<tr>
<td>Philippines</td>
<td>Leaf, bark, unripe fruit, roots</td>
<td>Astringent, ulcers, wounds, diarrhoea</td>
<td>Decoction and poultice</td>
<td>Smith and Nigel (1992)</td>
</tr>
<tr>
<td>India</td>
<td>Leaves, shoots</td>
<td>Febrifuge, antispasmodic, rheumatism, convulsions, astringent</td>
<td>Decoction or infusion</td>
<td>Hernandez (1971)</td>
</tr>
<tr>
<td>Ghana</td>
<td>Shoots</td>
<td>Convulsions, astringent</td>
<td>Decoction or infusion</td>
<td>Hernandez (1971)</td>
</tr>
<tr>
<td>Peru</td>
<td>Flower buds,</td>
<td>Heart and constipation,</td>
<td>Infusion or decoction</td>
<td>Cabieses (1993)</td>
</tr>
<tr>
<td>Location</td>
<td>Plant Parts</td>
<td>Conditions</td>
<td>Preparation</td>
<td>Source</td>
</tr>
<tr>
<td>-------------------------------</td>
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<td>-----------------------------------</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td>Kinshasa, Congo</td>
<td>Leaves, bark</td>
<td>Diarrhoea, antiamoebic</td>
<td>Infusion or decoction, tisane</td>
<td>Tona et al. (1999)</td>
</tr>
<tr>
<td>Senegal</td>
<td>Shoots, roots</td>
<td>Diarrhoea, dysentery</td>
<td>Infusion or decoction</td>
<td>Tona et al. (1999)</td>
</tr>
<tr>
<td>Uruguay</td>
<td>Leaves</td>
<td>Vaginal and uterine wash, especially in leucorrhoea</td>
<td>Infusion or decoction</td>
<td>Conway (2002)</td>
</tr>
<tr>
<td>Fiji</td>
<td>Leaves, roots, ripe fruit</td>
<td>Diarrhoea, coughs, stomach-ache, dysentery, toothaches, indigestion, constipation</td>
<td>Juice, the leaves are pounded, squeezed in salt water</td>
<td>Word Health Organization (1998)</td>
</tr>
<tr>
<td>Tahiti, Samoa</td>
<td>Whole plant, shoots</td>
<td>Skin tonic, painful menstruation, miscarriages, uterine bleeding, premature labour in women, wounds</td>
<td>Infusion or decoction, paste</td>
<td>Word Health Organization (1998)</td>
</tr>
<tr>
<td>Cook Islands</td>
<td>Leaves</td>
<td>Sores, boils, cuts, sprains</td>
<td>Infusion or decoction</td>
<td>Word Health Organization (1998)</td>
</tr>
<tr>
<td>Trinidad</td>
<td>Leaves</td>
<td>Bacterial infections, blood cleansing, diarrhoea, dysentery</td>
<td>Infusion or decoction</td>
<td>Word Health Organization (1998)</td>
</tr>
<tr>
<td>Latin America, Central and West Africa, and Southeast Asia</td>
<td>Leaves</td>
<td>Gargle for sore throats, laryngitis and swelling of the mouth, and it is used externally for skin ulcers, vaginal irritation and discharge</td>
<td>Decoction</td>
<td>Mejía and Rengifo (2000)</td>
</tr>
<tr>
<td>Panama, Bolivia and Venezuela</td>
<td>Bark and leaves</td>
<td>Dysentery, astringent, used as a bath to treat skin ailments</td>
<td>Decoction</td>
<td>Conway (2002)</td>
</tr>
<tr>
<td>Brazil</td>
<td>Ripe fruit, flowers, and leaves</td>
<td>Anorexia, cholera, diarrhoea, digestive problems, dysentery, gastric insufficiency, inflamed mucous membranes, laryngitis, mouth (swelling), skin problems, sore throat, ulcers, vaginal discharge</td>
<td>Mashed, Decoction</td>
<td>Holetz et al. (2002), Cybele et al. (1995)</td>
</tr>
<tr>
<td>USA</td>
<td>Leaf</td>
<td>Antibiotic and diarrhoea</td>
<td>Decoction</td>
<td>Smith and Nigel (1992)</td>
</tr>
</tbody>
</table>
Table 2 Commercial applications of *Psidium guajava*

<table>
<thead>
<tr>
<th></th>
<th>Application</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruit</strong></td>
<td>Food: juice, jelly nectar, concentrated, stuffed of candies, gelatines, pastes, tinned products, confectionery, etc.</td>
<td>All the countries</td>
<td>Jimenez <em>et al.</em> (2001)</td>
</tr>
<tr>
<td><strong>Wood and leaves</strong></td>
<td>Carpentry and turnery use the leaves to make a black dye for silk</td>
<td>Malaya</td>
<td>Rodarte (1994)</td>
</tr>
<tr>
<td><strong>Wood</strong></td>
<td>Engravings</td>
<td>India</td>
<td>Rodarte (1994)</td>
</tr>
<tr>
<td><strong>Wood</strong></td>
<td>Spinning tops</td>
<td>Guatemala</td>
<td>Morton (1987)</td>
</tr>
<tr>
<td><strong>Wood</strong></td>
<td>Hair combs</td>
<td>El Salvador</td>
<td>Morton (1987)</td>
</tr>
<tr>
<td><strong>Wood</strong></td>
<td>Construction of houses</td>
<td>Nigeria</td>
<td>Lucas <em>et al.</em> (2006)</td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td>Employed to give a black colour to cotton</td>
<td>Southeast Asia</td>
<td>Rodarte (1994)</td>
</tr>
<tr>
<td><strong>Leaves</strong></td>
<td>Serve to dye matting</td>
<td>Indonesia</td>
<td>Rodarte (1994)</td>
</tr>
<tr>
<td><strong>Bark</strong></td>
<td>Dyes, stains, inks, tattoos and mordants</td>
<td>Africa</td>
<td>Burkill (1985)</td>
</tr>
<tr>
<td><strong>Wood flowers</strong></td>
<td>The tree may be parasitized by the mistletoe, <em>Psittacanthus calyculatus</em> Don, producing the rosette-like malformations called “wood flowers” which are sold as ornamental curiosities. Also the tree is seeded to give shade to the coffee and its wood is used in the construction</td>
<td>Mexico</td>
<td>Argueta <em>et al.</em> (1994)</td>
</tr>
</tbody>
</table>
Furthermore, the leaves contain triterpenic acids as well as flavonoids; avicularin and its 3-1-4-pyranoside (Oliver, 1986), fixed oil 6%, 3.15% resin, and 8.5% tannin, and a number of other fixed substances, fat, cellulose, tannin, chlorophyll and mineral salts (Nadkarni and Nadkarni, 1999). Guavanoic acid, guavacoumaric acid, 2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol d and β-sitosterol-3-O-β-D-glucopyranoside also have been isolated from the leaves of *Psidium guajava* (Begum et al., 2002a, b).

Two triterpenoids, 20β-acetoxy-2α, 3β-dihydroxyurs-12-en-28-oic acid (guavanoic acid), and 2α, 3β-dihydroxy-24-p-z-coumaroyloxyurs-12-en-28-oic acid (guavacoumaric acid), along with six known compounds 2α-hydroxyursolic acid, jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol d and β-sitosterol-3-O-β-D-glucopyranoside, have been isolated from the leaves of *Psidium guajava* (Begum et al., 2002a). Two new triterpenoids, guajavolide (2α-, 3β-6β-, 23-tetrahydroxyurs-12-en-28, 20β-olide, and guavenoic acid, were also isolated from fresh leaves of *Psidium guajava* (Begum et al., 2002b).

### 2.4. Biological activity

Scientific research on the medicinal properties of guava dates back to the 1940s. A summary of the findings of these studies performed is presented below.

#### 2.4.1. Antimicrobial

Fighting to microbes has become an increasingly important and pressing global problem. Man used the antimicrobial drugs against microbes since times immemorial. The employment and development of the drugs against microbes continued throughout civilizations until the modern era. Traditional medicine (including herbal medicine) could be effective alternative source for much therapeutics, particularly after the recent remarkable failures of antibiotics against multi-drug resistant microorganisms.

Iwu (1993) tested the extract of *Psidium guajava* showed *in vitro* antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Shigella dysenteria*. Vieira et al. (2001) have reported the antibacterial effect of guava
leaves extracts and found that they inhibited the growth of the *Staphylococcus aureus*. The methanolic plant leaf extracts of *Psidium guajava* and barks of this plant have antimicrobial activity. The organism inhibited was *Salmonella species*, *Bacillus species*, and the concentrations vary according to the organisms (Abdelrahim *et al.*, 2002).

*Psidium guajava* leaf and bark tinctures were subjected to *in vitro* sensitivity tests by serial dilution at concentrations ranging from 5% to 15% against six test dermatophytes, viz., *Trichophyton tonsurans*, *Trichophyton rubrum*, *Trichosporon beigelii*, *Microsporum fulvum*, *Microsporum gypseum* and *Candida albicans* (Neira and Ramirez, 2005). Sanches *et al.* (2005) evaluated the antibacterial activities of *Psidium guajava* against Gram positive and negative bacteria testing ethanol:water extract of *Psidium guajava* leaves, stem bark and root, and aqueous extract against *Staphylococcus aureus* and it was found to be more active by using ethanol:water extract than with just aqueous extract.

The *in vitro* antibacterial activity of *Psidium guajava* leaf extract on *Staphylococcus aureus* was possibly due to protein degrading activity of the extracts (Belmtougri *et al.*, 2006). Perez *et al.* (2008) study were shown to inhibit both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Bacillus cereus*, *Proteus species*, *Shigella species* and *Escherichia coli*.

Medicinal plants are rich in a numerous variety of secondary metabolites of antimicrobial properties such as saponines, tannins, alkaloids, alkenyl phenols, glycoalkaloids, flavonoids, sesquiterpenes lactones, terpenoids and phorbol esters (Tiwar and Singh, 2004; Lewis and Ausubel, 2006).

The leaves of *Psidium guajava* are rich in tannin, and have antiseptic properties (Hernandez, 1980). The microbicidal activity of *Psidium guajava* was attributable to guajaverine and to psydiolic acid. The active flavonoid compound guaijaverin extracted from leaves of same plant was reported to have high potential antiplaque activity (Limsong *et al.*, 2004; Brotz-Oesterhelt *et al.*, 2005). Furthermore, the leaves contain large amounts of tannin, riterpernoids (crateogolics, guaijavolic, oleanolics and ursolic acid).

The flavone derivatives isolated were reported to inhibit the growth of *Staphylococcus aureus* in a dilution of 1:10,000 (Oliver, 1986). The γ-terpinene and γ-pinene obtained by hydro distillation showed antimicrobial activity against *Propionibacterium acnes* (Athikomkulchai et al., 2008). In previous study, *Psidium guajava* leaves' flavonoids were also shown to have bacteriostatic effects on fish pathogenic bacteria (Rattanachaikunsopon and Phumkhachorn, 2007). These findings explain the folkloric use of the extracts as bactericide, in cough, diarrhea, gargles to relieve oral ulcers and inflamed gums wound.

### 2.4.2. Anti-hyperglycemic

Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now (American Diabetes Association, 2010). In India, the prevalence rate of diabetes is estimated to be 1-5% (Rao et al., 1989). This rapidly increasing diabetes mellitus is becoming a severe threat to human health in all parts of the world. The management and treatment of diabetes and its complications mainly depend on the chemical or biochemical agents. Scientific data implies that the herbal remedies could stand for culturally important complementary or alternative treatments, as well as serve in the search for new antidiabetic agents with hypolipidaemic potentials.

The effect of *Psidium guajava* bark, leaves and fruit as anti-diabetic agents has been studied by several authors. Grover et al. (2002) evaluated some medicinal plants of India for anti-diabetic potential. They assessed some medicinal plants and *Psidium guajava* is one among those which has anti-diabetic activity. They administered the aqueous extract of leaves of 1gm/Kg for 30 days. It showed a significant reduction in blood glucose, urea, body weight, liver glycogen and serum cholesterol. These were estimated in alloxan induced experimental rats and they were contrasted with control and also with insulin management.
In another study, a decoction of *Psidium guajava* leaves was assessed for hypoglycaemic activity on alloxan-induced diabetic rats. In both acute and sub-acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycaemic activity (Mukhtar *et al.*, 2004). Maryna *et al.* (2008) screened and scored of 11 plants traditionally used in South Africa for anti-diabetic activity. On the evaluation *Psidium guajava* was also assessed mainly for its anti-diabetic activity. It showed that the organic leaf extract that were active in fat and muscle cells also in liver. And they also observed an *in vitro* alpha-glucosidase inhibition that slowed down the digestion of carbohydrate *in vivo*.

Prasad *et al.* (2009) tested a group of rats for the anti-diabetic activity of some herbal plants in diabetes-induced rats. Diabetes was induced by streptozotocin in rats and 6 streptozotocin induced diabetic rats was used. The *Psidium guajava* aqueous extract of leaves at a dosage of 500 mg/Kg body weight everyday for 15 days given once a time orally was administered. On observation, after oral dosage of leaf extract, there occurred a significant reduction of sugar in blood and calculated as 43.59% and glibenclamide as 47.74%.

Due to increased blood sugar level, there occurs more protein glycation and so, early glycation products and glycation end products (AGEs) are said to be major complication in diabetic patients. Ju-Wen *et al.* (2009) study was done to inhibit such a glycation process in albumin/glucose ratio model and it was compared with the extract of polyphenon 60, a polyphenol product extracted from green tea and also with standard antiglycation agent, aminoguanidine. Reports showed that *Psidium guajava* leaf extracts inhibition upon is calculated to be 95% at 50Lg/ml. Such as, the leaf extract of *Psidium guajava* consists of many compounds and among it, the phenolic compound showed effective inhibition on glycation of albumin and mainly quercetin showed 95% inhibition at 100 Lg/ml.

Tannins, flavonoids, pentacyclic triterpenoids, guaijaverin, quercetin, and other chemical compounds present in the plant are speculated to account for the observed hypoglycaemic and hypotensive effects of the leaf extract (Ojewole, 2005; Wang *et al.*, 2010).
Wang et al. (2007) showed in their study as there occurred a significant inhibition of alpha-glucosidase activity in small intestine and that inhibition causes slowing down the uptake of carbohydrates from digestion. The effects of guava extracts could be due to the different composition in phenolics compounds or other non-phenolic components. Quercetin, quercetin-derived glycosides, galloycatechin, gallic acid and ferulic acid have been identified from guava leaf extracts (Chen and Yen, 2007; Lozoya et al., 1994; Matsuo et al., 1994).

All phenolic constituents, except ferulic acid, markedly suppressed the formations of α–dicarbonyl compounds and advanced glycation end products. Some authors reported the medicinal value of all parts of Psidium guajava with mainly leaf extract having anti-hyperglycemic effect. In the streptozotocin induced diabetes rats, the leaf extract of Psidium guajava induced glucose utilization in liver tissues (Gutierrez et al., 2008; Shen et al., 2008).

Psidium guajava is an excellent anti-LDL (low density lipoprotein) glycative agent whose potential therapeutic uses can be extended to the prevention of a variety of cardiovascular and neurodegenerative diseases associated with glycations (Hsieh et al., 2007). Oh et al. (2005) demonstrated that the methanol extract from Psidium guajava leaves exhibited significant inhibitory effect on PTP1B (protein tyrosine phosphatase 1B) in Lepr[db]/Lepr[db] mice homozygous for the diabetes spontaneous mutation (Leprdb) become identifiably obese around 3–4 weeks of age. These homozygous mutant mice are polyphagic, polydipsic, and polyuric. Blood glucose lowering effect of the methanol extract was observed after i.p. dose of 10 mg/kg, with an anti-diabetic effect via the inhibition of PTP1B.

Anti-LDL glycative agents were investigated using aqueous decoctions of Psidium guajava fruit ripe at concentrations of 0.01–0.625 mg/ml (Hsieh et al., 2005). The results have revealed that guava fruits exhibit excellent antiglycation effect, being a rather powerful and effective inhibitor of LDL glycation in both glucose and glyoxal induced models. The antiglycation activities of guava fruit were relevantly and directly related to its polyphenolic content (extractable polyphenols 2.62–7.79%), yet it seemed to
us that *Psidium guajava* fruit also possesses a rather specific and somewhat different degree of free-radical scavenging ability, thus it was speculated that the reaction mechanism of guava might have occurred in the initiation rather than the propagation phase, a mechanism being quite different from the conventional free-radical scavenging by the polyphenolics. This problem is indeed worth exploring in further studies.

### 2.4.3. Cardiovascular, hypotensive effects

CVD is common in the general population, affecting the majority of adults, past the age of 50 years. Conde *et al.* (2003) studied the effect of an aqueous leaf extract of *Psidium guajava* on myocardial injury in the model of global ischemia followed by reperfusion. High-energy phosphates and malondialdehyde (MDA) in the reperfused hearts were significantly reduced with the plant extract. In another study, aqueous leaf extract of *Psidium guajava* exhibited cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts. Augmentation of endogenous antioxidants, maintenance of the myocardial antioxidant status and significant restoration of most of the altered hemodynamic parameters may have contributed to its cardioprotective effect (Yamashiro *et al.*, 2003).

Ojewole (2005) evaluated the hypoglycemic and hypotensive effects of *Psidium guajava* leaf extract. They administered intravenously as *Psidium guajava* leaf extract of 50–800mg/Kg. According to variation in doses, there occurred a significant reduction in systemic arterial blood pressure and heart rates of hypertensive, dahl–salt–sensitive rats. While evaluating by using cholinergic mechanisms, the extract caused a hypotension in mammalian experimental animal model. Belemtougri *et al.* (2006) observed that aqueous and ethanolic leaf extracts of *Psidium guajava* inhibits intracellular calcium release. A guava leaf extract may therefore be beneficial for the prevention of CVD, also since its traditional use in hypertension is well established.

### 2.4.4. Hepatoprotective effects

The liver is the key organ regulating homeostasis in the body. The liver is expected not only to achieve physiological functions but also to defend against the hazards of harmful drugs and chemicals. In spite of tremendous scientific progression
in the field of hepatology in recent years, liver problems are on the rise. In India plentiful medicinal plants used for the treatment of liver disorders.

The hepatoprotective effect of an aqueous leaf extract of *Psidium guajava* was examined on rat liver damage induced by CCl₄ by monitoring serum transaminase [aspartate amino transferase (AST) and serum alanine amino transferase (ALT)], alkaline phosphatase (ALP), serum cholesterol, serum total lipids and histopathological alterations. The leaf extract at doses of 500 mg/kg produced significant hepatoprotection (Roy et al., 2006).

Pretreatment with asiatic acid (a triterpenoid extracted from *Psidium guajava* leaves and fruit) at doses of 25 mg/kg, 50 mg/kg or 100 mg/kg significantly blocked the LPS (lipopolysaccharide) and (D-galactosamine) D-GalN-induced increases in both serum AST and serum ALT levels, showing improved nuclear condensation, ameliorated proliferation and less lipid deposition (Gao et al., 2006). Several studies have indicated the ability of guava to reduce several parameters associated with liver injury.

Flavonoids are natural phenolic substances present in *Psidium guajava* that can act as antioxidants in biological systems. Quercetin, one of the most abundant flavonoids in *Psidium guajava* and is a potent oxygen free radicals scavenger and a metal chelator (Middleton, 1998). Increasing evidence shows that quercetin can protect liver from injury induced by hepatotoxins (Ghosh et al., 2009; Bhatt and Flora, 2009; Vicente-Sanchez et al., 2008; Kebieche et al., 2009). Scientific evidence also suggests *Psidium guajava* contain quercetin and therefore it may be used to protect the liver from toxic injury.

2.4.5. Antioxidant, free radical scavenger and radioprotective activities

Free radicals are natural by-products of our own metabolism. These molecules attack our cells, tearing through cellular membranes to react and create destruction with the nucleic acids, proteins, and enzymes present in the body. These attacks by free radicals, collectively known as oxidative stress, are capable of causing cells to lose their structure, function and can eventually destroy them. Since free radicals play such an important role in the disease scenario of an individual, a thorough understanding of the
various physiologically significant free radicals is of paramount importance before the search of the radical scavengers or the antioxidant principles to treat the physiological disorders caused by them.

Plants represent a source of natural antioxidants that might serve as leads for the development of novel drugs. In fact, several anti-inflammatory, digestive, antinecrotic, neuroprotective, and hepatoprotective drugs have recently been shown to act through an antioxidant and/or radical-scavenging mechanism as part of their activity (Conforti et al., 2008). Dried leaves of *Psidium guajava* were extracted with hot water. The total phenolic content in the extract was determined spectrophotometrically according to Folin–Ciocalteu’s phenol method and calculated as gallic acid equivalent (GAE). A remarkably high total phenolic content 575.3±15.5 were obtained (Qian and Nihorimbere, 2004).

The antioxidant activity of lyophilized leaf extracts was determined using free radical DPPH (2, 2-diphenyl-1-picrylhydryzyl) scavenging. The results obtained showed that ascorbic acid was a substantially more powerful antioxidant than the extracts from guava leaf (Qian and Nihorimbere, 2004; Thaipong et al., 2005). These antioxidant properties are associated with its phenolic compounds such as protocatechuic acid, ferulic acid, quercetin and guavin B (Thaipong et al., 2005), quercetin, ascorbic acid, gallic acid and caffeic acid (Jimenez et al., 2001). Guava leaf extracts are a potential source of natural antioxidants (Qian and Nihorimbere, 2004). Other studies show that guava fruits also exert antioxidant and radioprotective activity in the assay with technetium-99m [(99m) Tc] (Abreu et al., 2006).

Wu et al. (2009) have reported potent antioxidant activity in guava leaf extracts and attributed it to the phenolic compounds. Zhang et al. (2006) also identified gallic acid, chlorogenic acid, kaempferol, procatechuic acid, ferulic acid, caffeic acid, quercetin and rutin in acetone extract from guava leaf. The antioxidant, free radical scavenging effects and protection from UVB–induced oxidation of aqueous extracts from *Psidium guajava* leaf were also stronger than that of water soluble extracts of some nutraceutical herbs including *Camellia sinensis* (gamma–aminobutyric acid (GABA tea, a functional tea
by fermenting fresh tea leaves under nitrogen gas), or *Toona sinensis* Roem. and *Rosemarinus officinalis* L. (Chen et al., 2007). Although the contents of total phenolic compounds and flavonoids in *Psidium guajava* leaf extracts were lower than that of aqueous rosemary extracts, *Psidium guajava* leaf extracts showed the strongest antioxidant activity in most of the tested methods.

Soman et al. (2010) investigate the antioxidant as well as antiglycative potential of ethyl acetate fraction of *Psidium guajava* leaves. Oral administration of the extract at different doses showed a significant decrease in blood glucose level. It also showed an improved antioxidant potential as evidenced by decreased lipid peroxidation and a significant increase in the activity of various antioxidant enzymes such as CAT, SOD, GPx and GSH-R. *In vitro* studies also support the antioxidant as well as antiglycative potential of *Psidium guajava* leaves.

Ling et al. (2010) evaluated thirteen Malaysian plants for antioxidant capacity and cytotoxicity. *Psidium guajava* is one among those which has antioxidant capacity. The ethanolic extracts were better free radical scavengers than the aqueous extracts and some of the tested extracts were even more potent than a commercial grape seed preparation. Similar results were seen in the lipid peroxidation inhibition studies. Their findings also showed a strong correlation of antioxidant activity with the total phenolic content and conclude that although traditionally these plants are used in the aqueous form, its commercial preparation could be achieved using ethanol since a high total phenolic content and antioxidant activity is associated with this method of preparation.

### 2.4.6. Antigenotoxic and antimutagenic effects

Generation of DNA damage is considered to be an important initial event in carcinogenesis. A considerable battery of assays exists for the detection of different genotoxic effects of compounds in experimental systems, or for investigations of exposure to genotoxic agents in environmental or occupational settings. Treatment with the aqueous whole plant extracts of *Psidium guajava* afforded protection (anti-genotoxic activity) against mitomycin C, nalidixic acid and H$_2$O$_2$ (three genotoxins) (Bartolome et al., 2006). In another study, a pre-treatment with an aqueous guava leaf extract was
found to be effective in inactivating the mutagenicity of direct-acting mutagens 4-nitro-o-phenylenediamine and 2-aminofluorene in the tester strains of *Salmonella typhimurium*. Therefore aqueous leaf extracts of *Psidium guajava* show promising antigenotoxic/antimutagenic activity (Grover and Bala, 1993).

### 2.4.7. Anticancer/antitumour effects

Cancer is one of the most dreaded diseases of the 20th century and spreading further with persistence and increasing incidence in 21st century. By 2030, the global burden is expected to grow to 21.4 million new cancer cases and 13.2 million cancer deaths simply due to the growth and aging of the population, as well as reductions in childhood mortality and deaths from infectious diseases in developing countries (Ferlay *et al.*, 2008).

An aqueous extract of *Psidium guajava* leaves inhibited (the viability) of the cancer cell line DU-145 in a dose-dependent manner. At 1.0 mg/ml, the extract reduced the viability of PCa DU-145 (the androgen independent PCa cells) to 36.1% and 3.6%, respectively after 48 h and 72 h of incubations (Chen *et al.*, 2007). Essential oil extracted from leaves of *Psidium guajava* was highly effective in reducing the growth of human mouth epidermal carcinoma (KB) and murine leukemia (P388) cell lines when they were treated with different concentrations of the oil ranging from 0.019 mg/ml to 4.962 mg/ml.

Guava leaf oil showed the highest anti-proliferative activity with an IC$_{50}$ value of 0.0379 mg/ml (four times more potent than vincristine) on P388 cell lines (Manosroi *et al.*, 2006); an effect mostly attributed to the monoterpenes present in the essential oil (Citó *et al.*, 2003). A chemopreventive effect was also demonstrated in another study of a methanol leaf extract on mice-induced cancer inoculated with B16 melanoma cells. A significant decrease in the incidence and average number of animals with cancer was found compared to the control group (Fernandes *et al.*, 1995). These findings suggest that *Psidium guajava* aqueous leaf extracts are efficacious for the prevention of tumour development by depressing Tr cells and subsequently shifting to Th1 cells (Seo *et al.*, 2005).
Furthermore, jacoumaric acid (isolated from guava seeds) was evaluated for its antitumour effect; it was found to significantly reduce the incidence of tumours (Numata et al., 1989). Phytochemical investigations of the acetone extract of *Psidium guajava* seeds has led to the isolation of phenyl-ethanoid glycosides (1-O-3, 4-dimethoxy-phenylethyl-4-O-3, 4-dimethoxy cinnamoyl-6-O-cinnamoyl-beta-D-glucopyranose and O-3, 4-dimethoxyphenylethyl-4-O-3, 4-dimethoxy cinnamoyl-beta-D-glucopyranose) which showed cytotoxic activities *in vitro* against Ehrlich Ascites Cells (EAC) and leukaemia P3888 cells (Salib and Michael, 2004). These finding suggested that *Psidium guaijava* extracts have the potential to be developed as new chemotherapeutic agents to prevent or to inhibit the growth of tumours and cancers.

### 2.5. Flavonoids - Quercetin

Flavonoids are a large group of phenolic plant constituents. To date, more than 6000 flavonoids have been identified, although a much smaller number is important from a dietary point of view. Flavonoids are potent antioxidants *in vitro*, and therefore one of the main interests in the compounds has involved protection against CVD. Antioxidation is, however, only one of the many mechanisms through which flavonoids could exert their actions.

Quercetin [3, 30, 40, 5, 7-pentahydroxyflavone, CAS no. 117-39-5] is one of several naturally-occurring dietary flavonol compounds belonging to a broad group of polyphenolic flavonoid substances (Fig. 1). Flavonoids are characterized by a phenyl benzo(c)pyrone-derived structure consisting of two benzene rings (A and B in Fig. 1) linked by a heterocyclic pyran or pyrone ring (C in Fig. 1) (Kuhnau, 1976; Morand et al., 1998). In plants, the flavonol aglycone is most commonly present conjugated at the 3-position of the unsaturated C-ring with a sugar moiety, forming O-β-glycosides such as quercitrin or rutin (Merck, 2001). Quercetin can be obtained from plants via extraction of the quercetin glycosides followed by hydrolysis to release the aglycone and subsequent purification.

Flavonols exhibit numerous biological and pharmacological effects, including antioxidant, chelation, anti-carcinogenic, cardioprotective, bacteriostatic, and secretory
Fig. 1 Chemical structure of quercetin
properties (Gross et al., 1996; Middleton et al., 2000; PDRNS, 2001). In plants, these compounds are involved in energy production (Theoharides et al., 2001) and exhibit strong antioxidant properties, possibly protecting plants against harmful ultraviolet rays (Wiczkowski et al., 2003).

The Joint FAO/WHO Expert Committee on Food Additives evaluated quercetin for use in food in 1977 (JECFA, 1977), but the limited amount of toxicity data available at the time of the evaluation precluded establishing an acceptable daily intake (ADI). Subsequently, in 1998 the International Agency for Research on Cancer evaluated quercetin for its potential carcinogenic risk to humans, assigning an overall Group 3 classification (i.e., not classifiable as to its carcinogenicity to humans) (IARC, 1999).

Based on the specified use-levels of quercetin in foods such as breakfast cereals, chewing gum, fats and oils, frozen dairy desserts and mixes, grain products and pastas, hard and soft candies, milk and plant protein products, beverages and beverage bases, and processed fruits and fruit juices, it was calculated that under a worst-case scenario of estimating intake, a heavy-end consumer of quercetin (90th percentile) would not be exposed to more than 4.70 mg quercetin/kg body weight/day (226 mg quercetin/person/day) from the intended addition of quercetin to foods. In Japan, quercetin is permitted as a food additive under the List of Existing Food Additives (MHLW, 1996).

Because of the prevalence of quercetin in the diet and its potential clinical and food applications, the safety of quercetin has been evaluated extensively in a variety of genotoxicity assays and a full range of acute, subchronic, chronic, and reproductive toxicity studies. In an attempt to reconcile the differences observed between in vitro results demonstrating quercetin-related mutagenic activity and the absence of carcinogenicity in vivo, several reviews of some of the data available for quercetin were conducted.

Most recently, Okamoto (2005) provided an extensive overview of the safety data available for quercetin. Presently, the bioavailability and antioxidant properties of quercetin appear to be two areas of intense research. Specifically, validation of quercetin
as a potent antioxidant \textit{in vivo}, but also realizing its potential for pro-oxidant activity following oxidation, are of prime interest in an effort to determine its clinical applicability and acceptability for use in food.

Moreover, the pro-oxidant state of quercetin, as a consequence of its potent antioxidant activity, may provide insight into its apparent \textit{in vitro} mutagenicity. Regardless, the available data suggest that \textit{in vivo} protective mechanisms adequately limit any potential for adverse effects related to quercetin pro-oxidant activity. The implications of the \textit{in vitro} results and their usefulness in determining the potential for quercetin toxicity \textit{in vivo}, in light of the largely negative results obtained in animal studies, are assessed, with special emphasis placed on the prooxidant properties of quercetin as a potential mechanism for its \textit{in vitro} mutagenicity.

2.6. Natural dietary occurrence of quercetin

Flavonols occur ubiquitously in the human diet as glycosides, with wide distribution in the edible portions of food plants, including berries, citrus, and various other fruits, leafy vegetables, roots, tubers and bulbs, herbs and spices, legumes, and cereal grains, as well as in tea and cocoa (Brown, 1980). Fruits and vegetables, particularly apples, cranberries, blueberries, and onions, are the primary sources of naturally-occurring dietary quercetin of the typical Western diet and contain the flavonol at levels as high as ~350 ppm (expressed as the aglycone) (Day and Williamson, 1999; Harnly \textit{et al.}, 2006). Black tea, as well as red wine and various fruit juices, also were identified as rich dietary sources of quercetin (Hertog \textit{et al.}, 1993a, b; Sampson \textit{et al.}, 2002).

In the United States, the average daily intake of all flavonoids (flavanones, flavones, flavonols, anthocyanins, catechins, and biflavans) from a normal mixed diet is estimated at ~1 g/day [expressed as quercitrin equivalents, where one biflavan molecule is equal to 2 molecules of quercitrin], of which, depending on seasonal variations, 160-175 mg/day is accounted for by flavanones, flavones, and flavonols (Ku’hnau, 1976; Brown, 1980). It is estimated that flavonol glycosides, expressed as quercetin
equivalents, are consumed at levels of up to ~100 mg/day (Brown, 1980; Jones and Hughes, 1982; NTP, 1992; Rimm et al., 1996; USDA, 2000; Sampson et al., 2002).

National dietary record-based cohort assessments (e.g., from Australia, the Netherlands, Finland, Italy, Croatia, Japan, and the United States) of the intake of quercetin from the habitual diet indicated mean consumption levels in the range of less than 5 mg to ~40 mg quercetin/day (Hertog et al., 1995; Rimm et al., 1996; Knekt et al., 1997; Kimira et al., 1998; Johannot and Somerset, 2006; Lin et al., 2006); however, daily levels of quercetin as high as 200–500 mg may be attained by high-end consumers of fruits and vegetables, especially in cases where the individuals consume the peel portion of quercetin-rich fruits and vegetables, such as tomatoes, apples, and onions (Jones and Hughes, 1982; USDA, 2000).

2.7. Metabolic fate of quercetin
2.7.1. Absorption and metabolism

Since the potential toxicity of quercetin, as well as any of its putative beneficial pharmacological effects are largely dependent on its bioavailability following oral administration, the absorption, distribution, metabolism, and excretion of quercetin have been extensively studied in laboratory animals and humans. As depicted in Fig. 2, quercetin may be O-methylated, primarily resulting in the formation of 3’-O-methylquercetin (isorhamnetin) and to a smaller extent, 4’-O-methylquercetin (tamaraxetin), sulfated, or glucuronidated at one of the hydroxyl groups in the absorptive cells of the intestinal epithelium following ingestion of the aglycone (Crespy et al., 1999; Murota et al., 2000; Rechner et al., 2002; Murota and Terao, 2005; Graf et al., 2006).

Subsequently, the resulting quercetin derivatives and any remaining unmetabolized quercetin are released into the circulation via the hepatic portal vein. Alternatively, Murota and Terao (2005) recently demonstrated that quercetin may be absorbed systemically from the gastrointestinal tract via the lymph. In the liver, quercetin and its derivatives are further subjected to conjugation, resulting in the formation of sulfate and/or glucuronide derivatives (Shali et al., 1991; Morand et al.,
Fig. 2 Schematic representation of the absorption, metabolism and excretion of quercetin in mammals

Adapted from Day and Williamson (1999)
1998; Oliveira and Watson, 2000; Boersma et al., 2002). Additionally, the catechol-O-methyltransferase (COMT) enzymes of the liver and kidneys also may participate in further methylation of quercetin or its derivatives (Onishi et al., 1982; Zhu et al., 1994; De Santi et al., 2002; O’Leary et al., 2003; Graf et al., 2006).

Alternatively, quercetin may be degraded to one of several different phenolic acids (e.g., 3, 4-dihydroxyphenylacetic acid) and carbon dioxide (CO₂) by the colonic microflora (heterocyclic ring fission) (Murray et al., 1954; Booth et al., 1956; Deeds, 1968; Krishnamurty et al., 1970; Stelzig and Ribeiro, 1972; Ueno et al., 1983; Gross et al., 1996; Pietta et al., 1997; Braune et al., 2001; Justesen and Arrigoni, 2001; Rechner et al., 2002; Olthof et al., 2003; Weldin et al., 2003). Chen et al. (2005) specifically evaluated the relative contribution of the gut and liver in the metabolism of quercetin in rats. More than 90% of an orally administered dose of quercetin was metabolized in the gut prior to absorption, while metabolism in the liver accounted for a further 3%.

Following administration of an oral dose of radiolabeled quercetin to male ACI rats, only ~20% of the radiolabel was estimated to be absorbed (Ueno et al., 1983). More recently, Chen et al. (2005) demonstrated absorption of ~60% of total quercetin (i.e., free and conjugated quercetin and its metabolites) and 5% of unchanged quercetin following oral administration of a single dose of 10 mg/kg body weight of quercetin to male Sprague-Dawley rats. Evaluated in ileostomy patients, 24% of total quercetin was absorbed following ingestion of 100 mg of the quercetin aglycone (Hollman et al., 1995, 1997), while in healthy subjects provided 100 mg of radiolabeled quercetin, up to 53% of the total administered radioactivity was absorbed (Walle et al., 2001).

In plasma samples collected from laboratory animals and humans following quercetin administration or consumption of quercetin-rich foods or herbal extracts, sulfate and glucuronide conjugates of quercetin and its O-methylated derivatives were identified (Zhu et al., 1994; Manach et al., 1995, 1997, 1998, 1999; Morand et al., 1998, 2000a, b; Ader et al., 2000; Morrice et al., 2000; Oliveira and Watson, 2000; Day et al., 2001; Moon et al., 2001). One hour following single intragastric administration of quercetin (50 mg/kg body weight) to male Sprague-Dawley rats, 93% of quercetin was metabolized,
with quercetin glucuronides, sulfoglucuronides, and sulfates, as well as isorhamnetic conjugates identified as the major metabolites (Justino et al., 2004).

### 2.7.2. Bioavailability

Following single or repeat oral administration of 75–1000 mg quercetin (~300–4000 mg/kg body weight), total plasma quercetin concentrations (i.e., free and conjugated quercetin and its metabolites) between 12.2 and up to 100 nmol/mL were detected in Wistar and Lister rats (Manach et al., 1997, 1999; Nakamura et al., 2000; Carbonaro and Grant, 2005). Although free quercetin was not detected in the plasma with single dosing (1 g/kg body weight), unconjugated quercetin was present at levels of 0.56 nmol/mL (0.17 μg/mL) in male Wistar rats following repeat-dose administration (10 days at 1 g/kg body weight) (Nakamura et al., 2000).

In a more recent study, free quercetin was found in the plasma, albeit only at a concentration of 0.9 l mol/L (0.27 μg/mL), following single intragastric treatment of male Sprague–Dawley rats with 50 mg quercetin/kg body weight (Justino et al., 2004). Conversely, in weanling rats administered quercetin in the diet at 0.45% for a period of 6 weeks (equivalent to doses of up to 58.5 mg/day during the last week of treatment), plasma samples did not contain any detectable levels of the aglycone (Graf et al., 2006). While low concentrations of free quercetin were identified in liver and kidney tissues (i.e., less than 8% of total quercetin identified in these tissues), the authors suggested that this may have occurred as a result of ex vivo hydrolysis of the quercetin metabolites.

In humans, total plasma quercetin levels (i.e., quercetin, quercetin glycosides, glucuronides, and sulfates) between 29 and 248 ng/mL were attained following ingestion of single meals consisting of quercetin-rich foods (~50 mg quercetin) (Hollman et al., 1996; de Vries et al., 1998; McAnlis et al., 1999); however, daily ingestion of 114 mg quercetin from onions for 7 continuous days resulted in total quercetin plasma levels of 453 ng/mL (Janssen et al., 1998).

While in earlier human trials the unconjugated quercetin aglycone was not identified in plasma samples following oral administration of the flavonol (Manach et al.,
1998; Erlund et al., 2000; Walle et al., 2000; Graefe et al., 2001), more recent trials utilizing improved detection methods have demonstrated trace quantities of the aglycone in the plasma of one subject ingesting fried onions (Mullen et al., 2004) and mean plasma levels of up to 8 ng/mL in another study in which participants were provided 500 mg quercetin three times daily (t.i.d.) for a period of 5 days (120–350 ng/mL of total quercetin) (Wang and Morris, 2005).

Following single-dose administration of 10 mg quercetin per 70 kg of body weight dissolved in different beverages (i.e., vegetable juice, white wine, or grape juice), mean plasma aglycone levels of up to 25 ng/mL were identified, with the highest plasma levels observed when quercetin was provided in wine (Goldberg et al., 2003). Some variability in plasma concentrations of the quercetin aglycone, as observed between the Wang and Morris (2005) and Goldberg et al. (2003) studies is expected as a result of differences in methods of analysis. Also, the food matrix in which quercetin is administered appears to play a significant role in the bioavailability of the free aglycone following ingestion (Goldberg et al., 2003). While both animal and human studies have demonstrated that following oral consumption of quercetin as much as 60% of the dose is absorbed (as total quercetin), extensive metabolism as a result of the first-pass effect ensures that the free unconjugated quercetin aglycone circulates in plasma at extremely low concentrations.

2.7.3. Distribution

Several authors reported on the distribution of quercetin following oral administration to determine possible target organs of the antioxidant activity of quercetin. Although acute treatment of Wistar rats with quercetin resulted in equal distribution of systemically-absorbed quercetin across all major tissues (Abrahamse et al., 2005), in rats fed quercetin in the diet for a period of 11 weeks, quercetin was observed to concentrate in several organs (i.e., lung, testes, kidney, thymus, heart, liver), with the highest concentrations of quercetin and its methylated derivatives, particularly isorhamnetin, detected in the pulmonary tissue (de Boer et al., 2005). Quercetin was primarily identified in its conjugated form in the tissue samples; however, free quercetin was extracted from some organs (i.e., lungs, liver, kidney, and testes) at concentrations
up to 40% of total extracted quercetin. As noted by the authors, although tissue β-
glucuronidase deconjugation of quercetin may occur in vivo, conversion of conjugated
quercetin to the aglycone upon extraction also could not be fully discounted.

2.7.4. Excretion

Systemically-absorbed quercetin may be eliminated in the urine (Gugler et al.,
1975; Onishi et al., 1982; Ueno et al., 1983; de Vries et al., 1998; Young et al., 1999;
Nakamura et al., 2000; Wang et al., 2003a, b) or alternatively, may be secreted into the
bile and excreted in the feces (Ueno et al., 1983). Also, as previously indicated, quercetin
can undergo microbial degradation in the colon to phenolic acids and CO₂, which is
exhaled in the breath (Ueno et al., 1983; Walle et al., 2001; Abrahamse et al., 2005).
Unabsorbed quercetin and the phenolic acid microfloral degradation products are
eliminated in the feces (Gugler et al., 1975; Ueno et al., 1983; Nakamura et al., 2000).

In experimental rat models, absorbed quercetin (~20% of the administered dose)
was excreted as expired CO₂ (35%), or via the feces (45%) and urine (10%) as
glucuronide or sulfate conjugates following oral administration (Ueno et al., 1983).
Excretion of quercetin in the urine and feces in human subjects accounted for only 3.3–
5.7% and 0.21–4.6% of an orally administered dose (100 mg), with the majority of the
radioactivity recovered as CO₂ (i.e., 41.8–63.9%) (Walle et al., 2001). Given its extensive
metabolism, quercetin is mostly recovered in the form of various metabolic products;
however, Wang and Morris (2005) demonstrated that small quantities of unchanged
quercetin (15.5–74.9 μg) were excreted in the urine within an 8 h period by human
subjects ingesting quercetin supplements (500 mg X t.i.d.).

The biliary secretion of quercetin in rats is suggestive of a potential for
enterohepatic circulation following deconjugation of the quercetin conjugates back to the
aglycone in the lower intestine (Ueno et al., 1983). Although the potential for biliary
recirculation could theoretically contribute to an extended bioavailability (Erlund, 2004;
Abrahamse et al., 2005), it is dependent on the colonic deconjugation of the quercetin
derivatives to the aglycone. Furthermore, subsequent degradation of the quercetin
aglycone to phenolic acids may reduce the amount of quercetin re-entering the
circulation (Crespy et al., 1999). Following ingestion of quercetin (100 mg), half-lives in the range of 31–50 h were observed in humans, with peak plasma levels observed at 30 min and again at 8 h post-treatment (Walle et al., 2001).

Although such an extended half-life and biphasic elimination (Gugler et al., 1975; Hollman et al., 1996; Ader et al., 2000; Skibola and Smith, 2000; Walle et al., 2001; Khaled et al., 2003) are supportive of enterohepatic recirculation, it also might be indicative of the binding of quercetin to plasma proteins presumably following initial enzymatic conversion (Walle et al., 2001; Carbonaro and Grant, 2005).

In both rats and humans, quercetin and its derivatives were shown to possess strong affinity for serum albumin (Manach et al., 1995; McAnlis et al., 1999; Sengupta and Sengupta, 2003). Furthermore, although recent studies conducted in rats confirm excretion of quercetin via the bile in the form of glucuronide/sulfate and methylate conjugates, enterohepatic recirculation was considered unlikely due to a similar metabolic profile of quercetin in rats with and without bile duct cannulation (Chen et al., 2005).

2.8. Beneficial effects of quercetin

Quercetin has been shown to be an excellent in vitro antioxidant. Within the flavonoid family, quercetin is the most potent scavenger of ROS, including O2•− (Hanasaki et al., 1994; Cushnie and Lamb, 2005), and RNS like NO• (van Acker et al., 1995; Haenen and Bast, 1999) and ONOO− (Haenen et al., 1997; Heijnen et al., 2001). Furthermore, it has been shown in vitro that quercetin also possesses anti-fibrotic (Lee et al., 2003), anti-coagulative (Bucki et al., 2003), anti-bacterial (Cushnie and Lamb, 2005), anti-atherogenic (de Whalley et al., 1990; Perez-Vizcaino et al., 2006), anti-hypertensive (Duarte et al., 2001; Perez-Vizcaino et al., 2006) and anti-proliferative properties (Kuo, 1996; Orsolic et al., 2004; Orsolic et al., 2004; Gulati et al., 2006).

Furthermore, quercetin is reported to directly modulate the gene expression of enzymes involved in biotransformation (Walle et al., 1995; Pacifici, 2004; Schwarz et al., 2005; Moon et al., 2006) and to inhibit cell proliferation by interacting with estrogen
binding sites (Piantelli et al., 1995; Caltagirone et al., 1997). Altogether, these studies indicate that quercetin may exert health-beneficial capacities via various damage modulating effects. However, most of these studies have been performed with immortalized or cultured cell lines only and are thus not easy to extrapolate to the in vivo human situation.

2.9. Biological functions ascribed to quercetin

As cited by Middleton et al. (2000) and Erlund (2004), antioxidant, anti-carcinogenic, anti-inflammatory, and cardioprotective properties are several key biological functions ascribed to quercetin. Middleton et al. (2000) stressed the anti-carcinogenic properties of quercetin and other flavonoids. Galati and O’Brien (2004) also reviewed the ability of certain flavonoids to prevent tumor development and also raised the possibility of flavonoid–drug interactions. It remains to be determined whether these properties of quercetin are effected independently or share a common mechanism of action. Several authors have related the anti-carcinogenic and anti-inflammatory effects to the antioxidant and free radical scavenging properties of quercetin (Stavric, 1994; Formica and Regelson, 1995).

The antioxidant properties are largely a function of the chemical structure of quercetin, particularly the presence and location of the hydroxyl (–OH) substitutions and the catechol-type B-ring (Rice-Evans et al., 1996; Wang et al., 2006). Bors et al. (1990) elucidated the structural features of an effective antioxidant, which consisted of the presence of (i) an ortho-dihydroxy or catechol group in the B-ring, (ii) a 2,3-double bond, and (iii) hydroxyl substitution at positions 3 and 5. Several authors have pointed out that quercetin, which is characterized by a hydroxylation pattern of 3, 5, 7, 30, and 40 and a catechol B-ring, possesses all the structural elements characteristic of an antioxidant (Silva et al., 2002; Rietjens et al., 2005); however, by exerting its antioxidant activity, quercetin may be converted into reactive products (Metodiewa et al., 1999; Boots et al., 2003).