The cardioprotective power of leaves

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Abstract

Lack of physical activity, smoking and/or inappropriate diet can contribute to the increase of oxidative stress, in turn affecting the pathophysiology of cardiovascular diseases. Strong anti-oxidant properties of plant polyphenolic compounds might underlie their cardioprotective activity. This paper reviews recent findings on the anti-oxidant activity of plant leaf extracts and emphasizes their effects on blood platelets, leukocytes and endothelial cells – the targets orchestrating the development and progression of cardiovascular diseases. We also review the evidence linking supplementation with plant leaf extracts and the risk factors defining the metabolic syndrome. The data point to the importance of leaves as an alternative source of polyphenolic compounds in the human diet and their role in the prevention of cardiovascular diseases.

Key words: anti-oxidants, cardiovascular diseases, leaf extracts, polyphenols.

Introduction

Cardiovascular disease (CVD) is one of the leading causes of mortality in developed countries. Cardiovascular disease may be a result of heart and/or blood vessels abnormalities, such as atherosclerosis, coronary heart disease, cerebrovascular disease, peripheral artery disease, congenital heart disease, rheumatic heart disease, pulmonary embolism and deep vein thrombosis. The main cause of CVD is inappropriate lifestyle, such as unhealthy diet, smoking, and the lack of physical activity. These factors contribute to the development of oxidative stress, atherosclerosis, chronic inflammation and metabolic syndrome (MS) [1]. Epidemiological studies indicate that a diet rich in polyphenols may reduce the risk of CVD without changes in lifestyle [2, 3]. Among patients with established CVD, polyphenols can diminish the effects of risk factors and improve parameters disturbed by the development of disease [4]. *In vitro* studies suggest that the mechanism of action of phenolic compounds is a result of their anti-oxidative properties and their ability to interfere with blood platelets, the immune system and endothelial cell signalling [5–7]. Polyphenols are secondary metabolites distributed in edible as well as inedible parts of plants. Leaves, flowers and woody parts, such as stems or bark, are a rich source of flavonoids, phenolic acids, stilbenes, tannins and lignans. Fruits, especially berries, are the most popular source of polyphenols beneficial to the vascular system, including anthocyanins,

proanthocyanidins, flavonols, catechins and hydroxylated derivates of benzoic and cinnamic acid [8]. However, there is evidence that extracts from other parts of plants, e.g. leaves, are also a rich source of phenolic compounds, which may have cardioprotective potential due to their strong free-radical scavenging, anti-oxidant and/or anti-peroxidative properties towards lipids. Leaves from strawberry, red and black raspberry, as well as thornless blackberry, appeared to have even higher total polyphenol content and exhibit higher antioxidant capacity than their fruits [9]. Also, blackcurrant and apple leaves have higher content of polyphenols than their fruits and demonstrate elevated antioxidant activity [10, 11]. Leaves have been widely used in traditional medicine, e.g. *Rubus* spp. (*Rosaceae*) leaves have been used as antimicrobial, anticonvulsant and muscle-relaxing agents. *Morus alba* (*Moraceae*) leaves have been applied in Chinese medicine as a remedy for fever, as a hepatoprotective agent and as an agent lowering blood pressure [12]. These examples clearly demonstrate that leaves can be at least equally interesting as fruits or other parts of plants. Importantly, they are also a much more accessible source of polyphenols than fruits.

Literature search strategy

We searched for the papers cited in this review in May to November 2013, using the electronic databases PubMed, Google Scholar and Research Gate. The main search keyword phrases were: "leaves" AND "polyphenols", "leaves" AND "platelet", "leaves" AND "metabolic syndrome", "leaves" AND "inflammation", "leaves" AND "endothelium", "leaves" AND "atherosclerosis", "leaves" AND "cardiovascular disease", "leaves" AND "anti-oxidant". When the essential pieces of information were cited in the relevant papers (e.g. in the Discussion) found according to the keywords mentioned above, we additionally used the particular Latin names of the eligible plants as search keywords.

Anti-oxidative properties of leaf extracts

Consequences of radical production *in vivo*

The imbalance between free-radical production during metabolic reactions and their removal from cells by anti-oxidative systems causes oxidative stress, which underlies the pathogenesis of numerous chronic disease, including atherosclerosis, diabetes, Alzheimer disease, carcinogenesis and inflammation [13–15]. Excessive reactive oxygen species (ROS) are released during activation of membrane NADPH oxidase, arachidonic acid metabolism, cyclooxygenase and lipoxygenase pathways [16]. The most significant oxygen radical is the anion superoxide (O_2^-) and derivative products of its conversion, such as hydroxyl radical (HO^-), hydrogen peroxide (H_2O_2), and peroxynitrite ($ONOO^-$) [17, 18]. In small quantities, ROS are important for physiological processes, but they become toxic for cells and lead to their death at higher concentrations [19]. This pathogenic effect of ROS on cells is a result of changes in cellular compounds, i.e. protein oxidation, lipid peroxidation and nucleic acid damage. Changes in amino acid residues, splitting of the polypeptide chain, creation of protein dimers and aggregates are some consequences of protein oxidation. These processes lead to inactivation of membrane transporters, enzymes and regulatory proteins [20, 21]. Major damage to proteins is caused by O_2^-, but the action of H_2O_2 oxidizes –SH residues and $ONOO^-$ decreases the activity of some enzymes [22, 23]. Hydroxyl radical and singlet oxygen are the main factors responsible for strand breaks in DNA and RNA, as they destabilize phosphodiester and hydrogen bonds and damage nitrogenous bases [24]. Lipid peroxidation is initiated by anion superoxide and the hydroxyl radical, resulting in disintegration of polyunsaturated fatty acids and inactivation of membrane enzymes and transporting proteins [25]. Compounds with anti-oxidative activity, including natural plant extracts, have the ability to eliminate free radicals, protect cells from ROS, prevent lipid and protein oxidation, and reduce DNA damage.

Methods for the detection of anti-oxidant capacity

Anti-oxidative activity of various compounds *in vitro* can generally be measured using 2 approaches, as a hydrogen atom transfer (HAT) and a single electron transfer (SET).

Oxygen radical absorbance capacity (ORAC) is a commonly applied HAT method based on the monitoring of linearly decreased fluorescence of a molecular probe (usually β-phycoerythrin or fluorescein), caused by free radicals, and referred to as the Trolox equivalent anti-oxidant capacity (TEAC) [26]. Trolox is a synthetic, water soluble, vitamin E derivative with strong anti-oxidant properties, commonly applied as a standard in anti-oxidant activity assays [27]. Most frequently, the applied SET method measures the ability of anti-oxidant to scavenge the stable synthetic radical 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Upon reduction, DPPH changes colour from purple to yellow, which can be measured spectrophotometrically. The interpretation of results is based on the calculation of EC50 (the concentration of anti-oxidative agent that decreases the initial DPPH concentration by 50%). The main disadvantage is that DPPH as a hydrophobic agent cannot be used to examine water soluble anti-oxidants

[28]. The alternative method that allows for examining not only hydrophobic substances, but also those dissolved in water, involves the ABTS (2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) radical. The ABTS assay depends on reaction time, anti-oxidant concentration and its activity. Its reduction in a solution is observed as the fading of a blue-green colour proportionally to anti-oxidant concentration. Anti-oxidant content is often expressed as TEAC per capacity or weight unit [29]. The FRAP (ferric reducing ability of plasma) assay directly measures anti-oxidative properties of substances. This method is based on measuring the formation of intense blue ferrous tripyridyl triazine complex reduced from its colourless ferric form and read at 593 nm. Anti-oxidative properties of a given substance are determined as a change in sample absorbance in comparison to the change in an Fe (II) standard, which is directly proportional to anti-oxidant concentration. One FRAP unit is equivalent to the reduction of 1 mol/l Fe^{3+} to Fe^{2+} [27, 30].

Anti-oxidant activity of plant extracts in biological systems: focus on leaf extracts

Since polyphenols became known as strong reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators, they have become widely applied in dietary supplements in order to protect cells against the consequences of oxidative stress.

In many studies using DPPH and ABTS assays, polyphenolic compounds present in leaves have proved to exhibit significant anti-oxidant potential, revealing them as a source of natural anti-oxidants. *Withania somnifera* (*Solanaceae*), which is commonly used in Indian traditional medicine as a remedy for several diseases including gastrointestinal and neurological disorders, is an abundant source of polyphenols with antimicrobial properties. *Withania* also demonstrated some cardioprotective action *in vivo*, when studied in the model ischaemia and reperfusion injury induced in Wistar rats [31, 32]. Alam *et al.* found that among extracts obtained from different parts of *W. somnifera* (fruits, roots and leaves), leaf extract was the most abundant source of polyphenols and flavonoids, whereas root extract had the lowest concentrations. The anti-oxidative properties also differed significantly among extracts. Leaf extract was found to be the strongest DPPH radical inhibitor (92%), while the root extract was the weakest (56%). In addition, leaf extract was a better lipid peroxidation inhibitor and more powerful Fe (III) reducer than root extract. HPLC analysis of all extracts allowed 6 compounds to be identified in leaf extract (catechin, gallic, syringic, benzoic, p-coumaric and vanillic acids), 3 in fruit extract (catechin, naringenin, kaempferol), and 2 in root extract (catechin, benzoic acid). Better anti-oxidative properties of leaf extract were explained by a higher content of polyphenols and stronger ability to inhibit the DPPH radical [33, 34].

The leaves and seeds of *Abelmoschus moschatus* belonging to the *Malvaceae* family have been applied in traditional medicine for the treatment of digestive system disorders or skin diseases. Seeds are also used as antispasmodic and cardiotonic agents. Gul *et al.* compared anti-oxidative potential and polyphenolic content between seeds and leaf extracts of this species. The data indicated that extracts from leaves were a more abundant source of polyphenols (9.5–13.8 mg expressed as gallic acid content) and significantly stronger anti-oxidants (total anti-oxidant activity was in the range 13.3–21.5 ascorbic acid equivalent (AAE)/g dry weight) than those obtained from seeds. Polyphenolic content of seed extract ranged from 1. 6 to 3.7 mg, whereas total anti-oxidant activity was 8.1–10.8 AAE/g dry weight. Further experiments showed that phenolic compounds contained in leaves were stronger DPPH radical scavengers (IC_{50} 43–176 µg of gallic acid equivalent (GAE)/ml) than those from seeds (IC_{50} 38–39 µg of GAE/ml). What is more, aqueous leaf extract inhibited superoxide radical formation by up to 67% and hydroxyl radical-mediated deoxyribose degradation by up to 99% [35].

The plant family *Fabaceae* comprises > 18,000 species, among which some plants are used in traditional medicine and have anti-cancer, anti-inflammatory, antimicrobial and antidiabetic bioactivity [36–38]. For instance, *Glycyrrhiza uralensis* has been shown to induce apoptosis and G1 cell cycle arrest in human breast cancer cells [36]. Isoflavonoids isolated from *Erythrina variegata* appear to possess antimicrobial properties against methicillin-resistant *Staphylococcus aureus* [37]. Among the plants of the *Fabaceae* family there are also some species with cardioprotective properties, e.g. *Trifolium pallidum* and *Trifolium scabrum* have been shown to reduce thrombin-induced platelet adhesion to fibrinogen and platelet aggregation, whereas black soybean (*Glycine max*) extract has revealed inhibitory activity on collagen-induced platelet aggregation in isolated human platelets [39, 40]. Chew *et al.* assessed total polyphenolic content (TPC) and anti-oxidant activity among leaf and flower extracts obtained from 9 *Fabaceae* species: *Acacia auriculiformis, Bauhinia kockiana, Bauhinia purpurea, Caesalpinia pulcherrima*, *Calliandra tergemina*, *Cassia surattensis, Leucaena leucocephala*, *Peltophorum pterocarpum*, and *Samanea saman*. Leaf extracts from *B. purpurea*, *C. pulcherrima*, *C. tergemina*, *P. pterocarpum* and *S. saman* had significantly higher TPC than extracts

of flowers of the same species. The highest TPC was observed in *C. pulcherrima* leaf extract (5,030 ±602 mg GAE/100 g), whereas the lowest was observed in *B. purpurea* (1310 ±124 mg GAE/100 g). TPC of these extracts was also positively correlated with its free-radical scavenging activity, which varied from 7690 ±618 mgAA/100 g (IC_{50} = 50 µg/ml) for *C. pulcherrima* to 1010 ±122 mgAA/100 g for *B. purpurea* (IC_{50} = 384 µg/ml) [41].

Another species from *Fabaceae*, *Desmodium adscendens*, which grows in the Amazonian rainforest of Peru, other South American countries and the West Coast of Africa, is also worth mentioning. Leaves of this plant are widely used in traditional medicine to treat leucorrhoea, body aches, pain, ovarian inflammation, excessive urination, gonorrhoea, diarrhoea, asthma, fever and epilepsy. A positive effect of *D. adscendens* on hepatic infections was also proven *in vivo*, but still relatively little is known about its cardioprotective action [42]. *Desmodium adscendens* leaves are a rich source of polyphenols and contain 11.2 mg/g of phenolic compounds (GAE), 12.8 mg/g of flavonoid compounds, 0.018 mg/g of anthocyanins and 0.39 mg/g of condensed tannins. HPLC analysis of water extract from *D. adscendens* leaves showed the presence of gallic acid, protocatechuic acid, catechin, rutin, quercetin glucoside and dihydrate, and cinnamic acid. Methanolic extracts did not contain gallic and protocatechuic acid, but chlorogenic acid was detected. ABTS and DPPH tests showed that the extract from *D. adscendens* leaves exhibited scavenging anti-oxidant activity, which was relevant to 12.8 and 8.5 mg of vitamin C equivalent per g dry weight (VCE/g) for ABTS for DPPH tests, respectively (the IC_{50} value for *D. adscendens* leaves extract was 4 µg/ml). Anti-oxidative properties of *D. adscendens* leaf extract were also examined in cell tests with the fluorescent probe 2,7-diacetate dichlorofluorescein. The extract significantly inhibited ROS generation in murine neutrophils treated with exogenous H_2O_2 by up to 83% [43].

Helichrysum longifolium is a species in the *Asteraceae* family, the leaves of which are used as dressings for wounds after circumcision, bruises, cuts and also to cure stress-related diseases, but experimental studies focused on this plant are scarce [44]. Photochemical analysis showed that *H. longifolium* extract is a source of tannins, flavonoids, steroids and saponins. Total phenolic content of aqueous leaf extract was 0.5 mg GAE/g dry weight, whereas the total flavonoid and proanthocyanidin content was respectively 0.71 and 0.005 mg GAE/g dry weight. It was found that bioactive compounds encountered in *H. longifolium* leaves are potent free-radical scavengers; aqueous extract significantly reduced ABTS concentration to 75%, hydrogen peroxide concentration to 72%, superoxide anion radical to 76%, DPPH radical to 65% and nitric oxide radical to 67% [45].

Overall the above data indicate that there are some significant differences in the distribution of polyphenols between leaves and other parts of plants. Some polyphenols are synthesized only in leaves, e.g. rutin and chlorogenic acid, which was detected in *Bauhinia kockiana* and *Cassia surattensis*. The differences in phenolic distribution are reflected in extracts with stronger anti-oxidant activity obtained from leaves. This divergence in anti-oxidative potency of polyphenols contained in leaves may have an evolutionary background and be due to high oxidative stress, which these parts of a plant experience in the course of photosynthesis [46]. Absorption of excessive light energy by the leaf tissue and transformation of absorbed light energy in chlorophyll is connected with the reduction of highly reactive chemical species. Such conditions cause the need for agents which are able to quench and remove ROS and to minimize damage related to oxidative stress [47]. Thus, strong anti-oxidative properties of leaf extracts make them promising natural agents for CVD prevention and treatment by effective reduction of oxidative stress.

Anti-inflammatory and immunomodulatory properties of polyphenolic extracts

Inflammation is an essential process for tissue protection and homeostasis. It is a defensive host response to infection, injury and irritation. Inflammation in a healthy organism is a self-limiting process that enables affected tissue to return to homeostasis. When immune cells are unable to manage with their inflammatory factors, they produce excessive amounts of cytokines and free radicals that result in acute inflammation or chronic disease [48]. Atherosclerosis is a slowly progressing pathological change in arteries that underlies coronary artery disease; it has its origin in endothelium injuries and low-density lipoprotein deposition in the arterial wall. These factors cause plaque formation and activation of an innate as well as an adaptive immune response, which leads to chronic inflammation. Immune cells influence the initiation and progression of atherosclerotic lesions via infiltration of the arterial wall, and cytokine and free-radical production [49–51]. Cytokines act in 2 ways, as pro-inflammatory factors (interleukins: 1, 2, 6, 7, 8, tumor necrosis factor α (TNF-α) and interferon γ (IFN-γ)) and anti-inflammatory agents (interleukins: 4, 10, 13, IFN-α, transforming growth factor β (TGF-β)). In coronary artery disease (CAD), the levels of both anti- and pro-inflammatory factors, i.e. IL-10, IL-2, and TNF-α, are raised [52]. Pro-inflammatory factors, such as TNF-α and IL-1β, enhance adhesion

molecule expression and endothelial permeability, which causes LDL deposition in the arterial wall, monocyte and lymphocyte infiltration, elevation of the inflammatory response, disintegration of fibrin filaments, and finally plaque rupture, which can cause for example a stroke [53]. The main cytokine in atherosclerosis progression is IL-6, released by the majority of cells which build up plaque, such as macrophages, foam cells, smooth muscle cells and activated endothelial cells [54]. IL-6 promotes early plaque formation and destabilization by up-regulation of IL-1β, TNF-α level, leukocyte infiltration and activation, lipid deposition, smooth muscle cell proliferation and down-regulation of enzymes involved in collagen synthesis enzymes [55–59]. An atherosclerotic plaque is stabilized by balanced collagen synthesis and decomposition in a fibrous cap. TNF-α destabilization is due to intensification of LDL oxidation; in cooperation with IL-6, it stimulates liver production of C-reactive protein (CRP) [60]. Other factors released in significant quantities by immune cells, including free radicals, intensify the immune response, adhesive molecule expression, platelet activation and tissue damage. The T lymphocyte major cytokine INF-γ also increases the immune response and destabilizes plaque by decreasing collagen synthesis in the fibrous cap [61].

Polyphenols not only limit excessive inflammation by quenching oxidative stress, but may also decrease inflammatory cytokine production, immune cell activation and inflammatory gene expression [62].

Leaf extracts as immunomodulators

The effects of polyphenolic compounds on immune and inflammatory cell function have been investigated in *in vitro* studies, animal models and clinical trials.

Young leaves of *Abelmoschus esculentus* (*Malvaceae*), *Hibiscus acetosella* (*Malvaceae*), *Manihot esculenta* (*Euphorbiaceae*) and *Pteridium aquilinum* (*Dennstaedtiaceae*) are common polyphenolic sources in Western and Central Africa. They are essential dietary components, also widely applied in folk medicine; e.g. *Abelmoschus*, *Hibiscus* and *Manihot* leaves are used to treat fever, headache, rheumatism, haemorrhoids, tumours, conjunctivitis, sores and abscesses. *Abelmoschus esculentus* also has a beneficial impact on the cardiovascular system. Sabitha *et al*. demonstrated that *A. esculentus* peel and seed powder reduced the blood glucose level and improved the lipid profile level in diabetic male Wistar rats [63]. Aqueous extracts prepared from young leaves of these plants were shown by Tsumbu *et al*. to influence neutrophils and monocytes under conditions pivotal for the first line of host immune defence. After 10 min incubation, the extracts not only decreased production of reactive nitrogen species by phorbol myristate acetate (PMA) stimulated equine neutrophils in a concentration-dependent manner (1–10 µg/ml), but also diminished neutrophil degranulation and myeloperoxidase (MPO) release into the extracellular milieu. The most powerful ROS scavengers were extracts obtained from *Pteridium* and *Hibiscus*, which also contained the highest amounts of polyphenol, phenolic acid and flavonoid. *Abelmoschus* and *Pteridium* extracts at 10 µg/ml were the most efficient inhibitors of MPO release. Extracts from *Pteridium* and *Manihot* at 10 µg/ml also significantly inhibited nitration-peroxidase activity of MPO [64]. Moreover, all these extracts decreased ROS production via HL-60 monocytes activated with PMA in a concentration-dependent manner. *Manihot* and *Pteridium* were the strongest inhibitors [65].

Tea (*Camellia sinensis*, Theaceae), the most important non-alcoholic beverage in the world, has been extensively studied for its putative disease preventive effects. Tea leaves are well known as an abundant source of polyphenols with strong anti-oxidant properties [66]. Regular tea intake prevents cancer and vascular disorders, and regulates the digestive system [67–69]. Animal as well as human studies point to the cardioprotective effect of black tea by lowering of cholesterol level. Also, the ability of black tea to decrease some inflammatory markers and mediators expressed by endothelium clearly points to the beneficial prosperities of this plant towards the vascular system [70]. Polyphenols contained in green tea, mainly catechins and flavonols, influence the immune system. Lymphocytes isolated from IL-2-deficient mice with inflammatory bowel disease have lower INF-γ and TNF-α production after 6 weeks' oral ingestion of water with green tea polyphenol extract [71]. Green tea extract also reduces the secondary response in an experimental model of spinal cord trauma. Administration of 24 mg/kg at 1 and 6 h after injury caused IκBα degradation and decreased the level of nuclear factor κB (NF-κB) and its phosphorylation at the site of injury. Severe neutrophil infiltration is associated with spinal cord injury. Green tea extract decreased TNF-α and IL-1β concentration and MPO activity compared to the control group, which suggests that the extract restricted neutrophil infiltration. Western blot and immunohistochemical analysis showed that the extract prevented inducible nitric oxide synthase (iNOS) expression and attenuated oxidative stress, which was measured as nitrotyrosine formation, lipid peroxidation and protease-activated receptor (PAR) formation [72].

Immunomodulatory effects of leaf extracts were also shown *in vivo* in a randomised crossover

study on purple sweet potato leaves (*Ipomoea batatas*, *Convolvulaceae*), which are common diet constituents in Asian cuisine. Because of the wide tolerance under severe environmental conditions (diseases, pest infestation, flooding), high polyphenolic content (2–14 g/100 g dry weight) and anti-oxidative activity, sweet potato leaves can be an important source of nutrients for people living in poorly resourced areas [73]. Some findings suggest that peripheral blood mononuclear cells (PBMC) isolated from blood of healthy humans who consumed daily 200 g of purple sweet potato leaves (PSPL) for 2 weeks showed increased proliferative responsiveness and elevated secretion of IL-2 and IL-4. Dietary supplementation with sweet potato leaves increased the lytic properties of natural killer (NK) cells and salivary IgA secretion [74]. The data contradict *in vitro* outcomes which indicate that flavonoids have immunosuppressive activity and decrease cytokine secretion, NK lytic function and lymphocyte proliferation [75]. Purple sweet potato leaves are also a promising supplement for athletes. Meals prepared from these leaves modulated release of inflammatory cytokines during exercise-induced oxidative stress. Analysis of blood taken from healthy individuals after 1 h of running revealed that those on a 1-week PSPL diet had a lower level of lipid peroxidation products and the inflammatory cytokine IL-6 compared to the control group [76].

In this regard, polyphenols contained in leaves modulate production of cytokines (IL-1β, TNF-α, IL-6, INF-γ), adhesive molecule expression and neutrophil infiltration and down-regulate oxidative stress by reducing ROS release and iNOS expression. As this is important in the progression and pathogenesis of arteriosclerosis, this leaf extract is a promising agent in the prevention of CVD and heart failure.

Beneficial effects of polyphenolic extracts in relation to metabolic syndrome

Metabolic syndrome is a group of concurrent risk factors including insulin resistance, hyperinsulinaemia, impaired glucose tolerance, centrally distributed obesity, high levels of triglycerides, low levels of HDL cholesterol, elevated blood pressure, and pro-inflammatory and prothrombotic states [77, 78]. Occurrence of MS is associated with the development of cardiovascular disease, type 2 diabetes, non-alcoholic fatty liver disease, obstructive sleep apnoea, renal disease and cancer [79–83]. Conditions that underlie MS remain unclear, but this syndrome is associated with physical inactivity, ageing and hormonal imbalance, such as polycystic ovary syndrome and testosterone insufficiency [84–87]. Nuclear peroxisome proliferator-activated receptors (PPAR) can be involved in the development of MS; they participate in β-oxidation of fatty acids, adipogenesis, glucose homeostasis and lipid metabolism, which is why their activation can improve some metabolic parameters, such as glucose and lipid levels [88, 89].

Activity of leaf extracts in metabolic syndrome

The therapeutic potential of polyphenols from leaves to treat many of the symptoms of MS has been seen in suitable animal models. Olive (*Olea europaea, Oleaceae*) leaves have been known for their medicinal properties since ancient times; tea made from them have been used to heal malaria and associated fevers. Extracts of olive leaves have strong antimicrobial, anti-oxidant and hypoglycaemic activity and cardioprotective properties [90–92]. Olive leaf extract increased the proportion of living cells, protected insulin secretion and not only reduced ROS production but also facilitated the excessive antioxidant defence in an insulin-producing β-cell line after pre-incubation with cytokines inducing toxicity [93]. Olive oil leaf extract has also demonstrated strong antimicrobial activity against *Campylobacter jejuni*, *Helicobacter pylori* and *Staphylococcus aureus* (including methicillin-resistant *S. aureus*) [92]. HPLC analysis showed that an ethanolic olive leaf extract (OLE) is rich in oleuropein (13.0 g/l) and hydroxytyrosol (2.7 g/l). Other polyphenols present in the extract are tyrosol, aesculin, hydroxypinoresinol-glycoside, luteolin 7-glucoside, and oleoside. Oleuropein and hydroxytyrosol are particularly important components of OLE that can reverse both chronic inflammation and oxidative stress, both contributing to cardiovascular, hepatic, and metabolic symptoms in a rat model of diet-induced obesity and diabetes. Male Wistar rats fed with a high fat (high-cholesterol high fat (HCHF)) or carbohydrate cornstarch diet for 8 weeks demonstrated attenuated fat deposition after supplementation with 3% OLE for a further 8 weeks in comparison with those fed for 16 week with a HCHF or cornstarch diet without OLE. Rats on a HCHF diet supplemented with OLE have also shown lower plasma total cholesterol, triglycerides, oxidative stress markers and improved oral glucose tolerance compared to those without supplementation. A high fat diet led to pathological changes in Wistar rat heart (left ventricle inflammation, interstitial collagen deposition), liver (inflammatory cell infiltration, lipid accumulation and portal fibrosis), and coronary vessels (decreased vasorelaxation). Olive leaf extract supplementation markedly reduced these symptoms [91].

In traditional Chinese medicine, leaves of mulberry (*Morus* sp., *Moraceae*) have been used to cure diabetes and inflammation. The extract of mulberry possesses anti-oxidant activity, sup-

presses lipoxygenase, is cytotoxic to cancer cells and inhibits their migration [94, 95]. It was found that some mulberry anthocyanins, cyanidin 3-rutinoside and cyanidin 3-glucoside inhibited migration and invasion of human lung cancer cells. Prenylflavonoids, cudraflavone B, cudraflavone C and oxyresveratrol extracted from *Morus alba* exhibited a DPPH free radical scavenging effect and hepatoprotective effects on tacrine-induced cytotoxicity in human liver-derived Hep G2 cells. Mulberry polyphenols also act cardioprotectively, e.g. the extract of mulberry root bark significantly inhibited collagen- and arachidonic acid-induced platelet aggregation and thromboxane formation in cultured platelets [96, 97]. *Morus* leaves are rich in polyphenolic constituents such as quercetin and kaempferol, which have anti-diabetic action and can ameliorate hyperglycaemia and dyslipidaemia [98]. *In vivo* experiments showed that an extract from *Morus* leaves ameliorated hyperlipidaemia in high fat diet male Wistar rats. The extract significantly decreased plasma triglycerides and non-esterified fatty acid levels. Rats given *Morus* leaf extract had an up-regulated PPAR signalling pathway and down-regulated androgen, oestrogen and butanoate metabolism, bile acid biosynthesis and synthesis, and degradation of ketone bodies. Lipid metabolism and B-oxidation of fatty acids were up-regulated in mulberry-treated rats in contrast to lipid and steroid biosynthetic processes, which were down-regulated. *Morus* leaf extract not only regulated the genes responsible for lipid and fatty acid metabolism, but also up-regulated genes involved in the response to oxidative stress [99].

Sasa quelpaertensis (*Poaceae*) leaves have been used in traditional medicine as tea with anti-diabetic, diuretic and anti-inflammatory properties, but scientific data concerning the molecular basis underlying the possible benefits of *S. quelpaertensis* for health are scarce. Ryou *et al.* studied ovariectomised Sprague-Dawley rats fed on a *Sasa quelpaertensis* leaf powder diet (the leaf powder comprised 10% of the diet) and found that these animals were characterized by significantly lower daily weight gain, although the effect of such powder is not clearly associated with cholesterol, triglyceride or glucose levels, or with aggregation of blood platelets, compared to sham-operated controls [100]. Otherwise, the leaves of persimmon (*Diospyros kaki*; *Ebenaceae*), commonly consumed as a tea, possess an evident anti-diabetic activity. Kawakami *et al.* found that the addition of the powder concentrate of persimmon leaves, rich in proanthocyanidin oligomers, to the diet of male Wistar rats resulted in a decreased blood glucose level in a concentration-dependent manner [101]. In folk medicine, dandelion (*Taraxacum officinale*; *Asteraceae*) has been used to treat hepatic disorders and inflammation with its choleretic, diuretic and anti-rheumatic properties. Dandelion is a source of flavonoids, caffeic acid, chlorogenic acid, luteolin, and luteolin 7-glucoside [102]. Oral administration of dandelion leaves improved parameters in metabolic syndrome of high-cholesterol fed male New Zealand white rabbits. Administration of *T. officinale* leaf extract for 4 weeks significantly increased HDL cholesterol, and lowered levels of triglycerides and LDL in comparison to the control group. Dandelion supplementation increased activity of the hepatic anti-oxidant enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx). Haematoxylin and eosin staining of representative aortic sections showed that supplementation with dandelion leaves limited lipid deposition and formation of atherosclerotic lesions within the aortic intima [103]. Cardioprotective action of fruits of *Vitis vinifera* is broadly described in the literature, e.g. polyphenolic fractions obtained from grape skin have been shown many times to inhibit platelet aggregation and LDL oxidation *in vitro* [104]. In traditional medicine, also *Vitis vinifera* leaves are known as a remedy for hypertension, haemorrhages and inflammatory disorders. Extract from *V. labrusca* leaves had hepatoprotective, cardioprotective, and renal protective effects in Wistar rats. The major phenolic compounds in its leaves are flavonoids and hydroxycinnamic acids. Polyphenols from *V. labrusca* leaves restored liver and kidney superoxide dismutase and heart catalase activity, and decreased lipid and protein damage in Wistar rat tissues treated with H_2O_2 [105].

Polyphenols modulate platelet and endothelial function

Platelet and endothelial dysfunction are among the leading factors responsible for CVD. In normal physiological conditions, the endothelium prevents adhesion and activation of platelets through secretion of nitric oxide (NO) and prostacyclin (PGI_2) [106, 107]. Pathological changes (diabetes, hyperlipidaemia and hypertension) diminish production of anti-aggregatory factors, increase the release of vasoconstrictors (endothelin-1), and lead to collagen exposure. Platelet adherence to the exposed collagen is connected with their activation and secretion of pro-coagulatory factors, e.g. ADP, calcium, thromboxane A_2 [108, 109].

Effect of leaf extracts on platelet function

Polyphenolic compounds contained in plant leaf extracts can attenuate platelet hyper-reactivity and reverse endothelial dysfunction by modulating cellular signalling. In traditional medicine,

Urtica dioica (*Urticaceae*) roots and leaves are a remedy for hypertension, diabetes, prostate hyperplasia and cancer [110–112]. *Urtica dioica* possesses anti-inflammatory, anti-hyperglycaemic, antimicrobial, anti-oxidant, anti-ulcer and analgesic activity [113–115]. *Urtica dioica* leaf extract, when administered before glucose loading, has demonstrated strong ability to decrease glucose level in alloxan-induced diabetic rats [113]. Extract from *U. dioica* also appears to be an effective scavenger of free radicals, including superoxide anion radicals and hydrogen peroxide. Moreover, *Urtica* leaf extract has revealed antimicrobial activity against nine different microorganisms, antiulcer activity against ethanol-induced ulcerogenesis and an analgesic effect on acetic acid-induced stretching [115]. This herb also demonstrates hypotensive and diuretic actions [116]. The cardioprotective effect *of U. dioica* has been demonstrated in male Wistar rats fed on a high-cholesterol diet, as significantly decreased levels of total cholesterol, low-density lipoprotein cholesterol, liver enzymes and body weight [117]. Investigating the influence of 3 different *U. dioica* leaf extracts (in water, methanol or ethyl acetate) on thrombin-induced aggregation of washed platelets in Wistar rats showed that only the ethyl acetate extract possessed significant anti-platelet activity. Further investigation indicated that this distinction in the action of the extract was a result of higher concentrations of flavonoids in ethyl acetate extract [118]. *Artemisia dracunculus* (*Asteraceae*) is commonly used in Iranian folk medicine as an anti-coagulant and anti-hyperlipidaemic agent. *In vitro* studies on *A. dracunculus* methanolic leaf extract indicated its ability to significantly inhibit thrombin-induced platelet aggregation by 60%, platelet adhesion to laminin coated plates by 50%, and protein secretion from thrombin-activated platelets by 50% [119]. Numerous studies indicate that garlic *Allium sativum* (*Amaryllidaceae*), known since ancient times for its healing properties, may be a beneficial agent for the treatment of CVD [120, 121]. *Allium sativum* can normalize plasma lipids, enhance fibrinolytic activity, inhibit platelet aggregation, and reduce blood pressure and blood glucose level. In experiments on platelet aggregation evoked by ADP, collagen or arachidonic acid, Hiyasat *et al.* compared the anti-platelet activity of methanolic and aqueous extracts isolated from the leaves of *A. ursinum* and *sativum*. Alcoholic extracts of both species and an aqueous extract of *A. sativum* most efficiently inhibited ADP-induced platelet aggregation, while an aqueous extract of *A. ursinum* inhibited platelet aggregation, but the effect did not depend on the type of platelet agonist [122]. Olive leaf extract has an anti-aggregatory influence on platelets, seen in a randomised single-blinded study involving healthy male volunteers given supplements of OLE containing 5.4 mg/ml of oleuropein. *In vitro*, OLE used at 5.4–54 µg/ml inhibited blood platelet aggregation and ADP release in a dose-dependent manner, but significant changes occurred only at the highest concentration of OLE [123].

Effect of leaf extracts on endothelial cells

Numerous reports deal with the influence of leaf extracts on endothelial cells. The main action is the improvement of NO-dependent vasorelaxation, an effect achieved with leaf extracts of *Fragaria vesca* (*Rosaceae*), *Tanacetum vulgare* (*Asteraceae*) and *Mansoa hirsuta* (*Bignoniaceae*) [124–126]. Among leaves, those from *Ginkgo* (*Ginkgoaceae*) and *Morus* species seem to be well confirmed. Extracts of the leaves of *Ginkgo biloba* are a source of flavonoids (ginkgo flavone glycosides, bioflavonoids) and terpenoids (ginkgolides and bilobalide) and have anti-tumour, anti-aging, hepatoprotective and cardioprotective properties [127–130]. *Ginkgo* extract decreases the activities of serum marker enzymes and lipid peroxidation in carbon tetrachloride-induced hepatotoxicity in male Wistar rats. Such a hepatoprotective effect has been ascribed to anti-oxidative properties of this extract, which have been associated with increased levels of glutathione, as well as increased activities of superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase [129]. *Ginkgo* extract also demonstrates cardioprotective activity, which has been demonstrated in an experiment with $HgCl_2$-induced oxidative damage in Wistar albino male and female rats. While $HgCl_2$ has been shown to significantly increase thromboplastic activity and malondialdehyde levels or decrease glutathione levels in serum and tissue samples, this effect has been effectively reversed by *Ginkgo* leaf extract [130]. It has also been used to treat dementia and vaso-occlusive and cochleovestibular disorders [128, 131]. Ou *et al.* found that *Ginkgo biloba* leaf extract (GbE) attenuated endothelial cell dysfunction induced by oxidized low-density lipoprotein (oxLDL). Pre-treatment of human umbilical vein endothelial cells (HUVECs) with GbE before exposure to oxLDL dramatically decreased the level of ROS generation (96% inhibition at 100 µg/ml) in comparison to Trolox (104% inhibition at 2.5 µg/ml). HUVECs treated with oxLDL for 24 h had reduced endothelial nitric oxide synthase (eNOS) protein expression, which stimulated THP-1 cells to increasingly adhere to HUVECs and show enhanced expression of adhesion molecules; however, incubation of HUVECs with GbE for 2 h significantly reduced these tendencies. Moreover, GbE inhibited oxLDL-induced cytotoxicity of HUVECs

[132]. The effect of *Ginkgo* leaf extract has also been investigated in randomised clinical trials in patients with early stage diabetic nephropathy. After 8 weeks of *Ginkgo* supplementation, patients had less von Willebrand factor and increased NO plasma levels [133].

Adhesion molecules and cytokines are well-recognized markers and mediators of endothelial dysfunction. Therefore, they are often the targets for studying vascular protective activity of plant extracts. Using Western blot analysis, water extract from another commonly investigated species (*Morus alba* leaves) may suppress expression of vascular cell adhesion molecule-1 (VCAM-1), intracellular adhesion molecule-1 (ICAM-1) and E-selectin after 6 weeks of supplementation in rats fed an atherogenic diet [134]. Also, in experiments *in vitro*, *Morus alba* leaf extract decreased expression of the adhesion molecule resistin. It is a cytokine that increases the expression of P-selectin and monocyte adhesion to human endothelial cells. Methanolic extract from *M. alba* leaves significantly reduces P-selectin expression and inhibits monocyte adhesion to endothelium previously exposed to resistin [135]. *Dicksonia sellowiana* (*Dicksoniaceae*) is a common tree in Central and South America; its leaves are used in a folk medicine to treat scabies, pruritus, parasitic diseases and asthma. Hydroalcoholic extract of *D. sellowiana* (HEDS) decreases hypertension and induces endothelium-dependent relaxation in spontaneously hypertensive rat (SHR) aortic rings. Hydroalcoholic extract of *D. sellowiana* induces aortic relaxation by activation of muscarinic receptors and stimulation of the NO pathway in SHR rat aortic endothelium. In porcine coronary artery rings, HEDS also causes endothelium-dependent relaxation via redox-sensitive activation of the endothelial PI3-kinase/Akt pathway, which leads to eNOS phosphorylation [136].

Chemical structure, bioavailability and functionality of polyphenols contained in leaf extracts

Data presented in this paper suggest that the main acting agents in the investigated leaf extracts are phenolic acids and flavonoids (Table I). However, numerous pieces of evidence indicate that also tannins, terpenoids, saponins and steroids, commonly occurring components in leaf extracts, may exhibit a significant pharmacological influence, very often overlapping with that attributed to polyphenolic agents. Derivatives of hydroxybenzoic acid (e.g. gallic acid, protocatechuic acid) and hydroxycinnamic acid (e.g. caffeic acid, chlorogenic acid) are among the phenolic acids most commonly detected in leaves. Their chemical structures include a single aromatic ring containing the functional groups of either hydroxybenzoic or hydroxycinnamic acid, and possible substitutions at the positions of R_1, R_2 and R_3 include hydrogen, hydroxyl or methoxy residues (Table II). Flavonoids detected in leaf extracts belong mainly to anthocyanins, proanthocyanidins (procyanidin B_1, procyanidin B_2), flavanols (catechin and epigallocatechin), flavones (luteolin) and flavonols (kaempferol, quercetin) (Tables I and II). Their chemical structures consist of two aromatic rings (C_6), one of which is fused with a heterocyclic pyran or hydropyran, whereas the second is substituted at positions 3, 4 and 5 with hydrogen, hydroxyl or methoxy residues. In general, biological and chemical properties of polyphenols are largely determined by their aromatic chemical structure, as well as the number, type and locations of functional groups in a molecule [137]. On the other hand, polyphenolic content of plant extracts depends on environmental factors, such as pedoclimatic (soil type, sun exposure, rainfall) and agronomic conditions (cultures in greenhouses or fields, biological cultures, hydroponic cultures). Also, the exposure to light, and the degree of ripeness (fruits) or maturity (leaves) may influence concentrations and proportions of various polyphenols [138, 139]. Biological activities of extracts from plant specimens belonging to the same species may significantly differ, mainly because they are determined by the chemical composition of the plant tissue (which is dependent on cultivation conditions) or the method of its extraction/preparation [140–142]. The discrepancies between degree of relationship and biological activity are indicated in Figure 1, illustrating the clustering of the discussed species in agglomerates showing the highest similarities. Interestingly, the plant extracts discussed in this review are ascribed to three main agglomerates concerning their biological activity (Figure 1 B), which do not correspond to agglomerates created on the basis of their taxonomic relationships (Figure 1 A). It would indicate that various related plant species may demonstrate different or even divergent biological properties of their extracts originating from differentiated chemical compositions of the extracts. It seems likely that environmental conditions (including plant habitats) may matter much more in determining such biological properties of the extracts than the taxonomic relationships between the plants themselves. Biological activity *in vivo* is also strongly dependent on bioavailability of consumed polyphenolic compounds. Plasma concentrations of polyphenol metabolites may vary greatly, from 0 to 4 µmol/l. Of the polyphenols commonly occurring in leaf extracts, gallic acid and isoflavones are most efficiently absorbed. Also catechins, flavanones and

Table I. Characteristics of leaf extracts from 34 selected plants described in the review

Latin scientific name (and common/ abbreviated name)	Family	Types of common extracts	Class of active ingredients	Active agent	Mode of action
Abelmoschus esculentus [64, 65]	*Malvaceae*	Aqueous	Phenolics	Not defined	Anti-radical
			Flavonoids		Anti-inflammatory
			Tannins		Modulatory
Abelmoschus moschatus [35]	*Malvaceae*	Aqueous	Phenolics	Not defined	Anti-oxidative
		Ethanolic	Flavonoids		Antimicrobial
					Anti-proliferative
Acacia auriculiformis [41]	*Fabaceae*	Methanolic	Terpenoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Saponins		Antimicrobial
			Steroids		
Allium sativum [122]	*Amaryllidaceae*	Aqueous	Alliins	Not defined	Antiplatelet
		Methanolic	Allicins		
			Saponosides		
Allium ursinum [122]	*Amaryllidaceae*	Aqueous	Alliins	Not defined	Antiplatelet
		Methanolic	Allicins		
			Saponosides		
Artemisia dracunculus [119]	*Asteraceae*	Methanolic	Phenolics	Not defined	Antiplatelet
			Flavonoids		
			Coumarins		
Bauhinia kockiana [41]	*Fabaceae*	Methanolic	Flavonoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Steroids		Antimicrobial
			Tannins		
Bauhinia purpurea [41]	*Fabaceae*	Methanolic	Terpenoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Saponins		Antimicrobial
			Steroids		
Caesalpinia pulcherrima [41]	*Fabaceae*	Methanolic	Flavonoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Terpenoids		Antimicrobial
			Tannins		
Calliandra tergemina [41]	*Fabaceae*	Methanolic Dichloromethane: methanol	Flavonoids	Not defined	Anti-oxidative
			Terpenoids		Antimicrobial
			Tannins		
			Saponins		
Camellia sinensis [66, 70, 71]	*Theaceae*	Aqueous	Phenolics	Epigallocatechin-3-gallate	Anti-oxidative
			Flavonoids	Epicatechin-3-gallate	Antimicrobial
				Epigallocatechin	Anti-inflammatory
				Epicatechin	

Table I. Cont.

Latin scientific name (and common/ abbreviated name)	Family	Types of common extracts	Class of active ingredients	Active agent	Mode of action
Desmodium adscendens [43]	*Fabaceae*	Aqueous	Phenolics	Gallic acid	Anti-oxidative
		Methanolic	Flavonoids	Protocatechuic acid	
			Anthocyanins	Catechin	
			Condensed tannins	Rutin	
			Hydroxycinnamic acids	Quercetin glucoside	
				Quercetin dihydrate	
				Chlorogenic acid	
				Cinnamic acid	
Dicksonia sellowiana (HEDS) [43]	*Dicksoniaceae*	Ethanolic	Phenolics	Gallic acid	Activation of NO pathway
			Hydroxycinnamic acids	Protocatechuic acid	
				Chlorogenic acid	
				Coumaric acid	
				Ferulic acid	
				Sinapic acid	
				Cinnamic acid	
Diospyros kaki [101]	*Ebenaceae*	Aqueous	Phenolics	Catechin	α-Amylase inhibition
		Ethyl acetate	Flavonoids	Epigallocatechin	
			Proanthocyanidins	Epigallocatechin-3-O-gallate	
				Epicatechin	
				Epicatechin-3-O-gallate	
				Prodelphinidin	
Fragaria vesca [124]	*Rosaceae*	Aqueous	Phenolics	Catechin	Improvement of NO-dependent vasorelaxation
			Flavonoids	Epicatechin	
			Procyanidins	Epigallocatechin	
			Stilbenoids	Epicatechin-3-gallate	
				Quercetin-4'-glucoside	
				Procyanidin B1	
				Procyanidin B2	
				Piceid	
				Astringin	
				Trans-resveratrol	
Ginkgo biloba (GbE) [127–133]	*Ginkgoaceae*	Commercial	Flavonoids	Ginkgo flavones	Anti-tumour
			Terpenoids	Glycosides	Anti-aging
				Bioflavonoids	Hepatoprotective
				Ginkgolides	Cardioprotective
				Bilobalide	

Table I. Cont.

Latin scientific name (and common/ abbreviated name)	Family	Types of common extracts	Class of active ingredients	Active agent	Mode of action
Helichrysum longifolium [45]	*Asteraceae*	Aqueous	Phenolics	Not defined	Anti-oxidative
			Flavonoids		
			Tannins		
			Steroids		
			Saponins		
			Proanthocyanidins		
Hibiscus acetosella [64, 65]	*Malvaceae*	Aqueous	Phenolics	Phenolic acid	Anti-radical
			Flavonoids		Anti-inflammatory
			Tannins		Modulatory
Ipomoea batatas (PSPL) [73]	*Convolvulaceae*	Fresh leaf	Phenolics	Not defined	Immunomodulatory
			Flavonoids		
Leucaena leucocephala [41]	*Fabaceae*	Methanolic	Flavonoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Tannins		Antimicrobial
			Steroids		
			Saponins		
Manihot esculenta [64, 65]	*Euphorbiaceae*	Aqueous	Flavonoids	Not defined	Anti-radical
					Anti-inflammatory
					Modulatory
Mansoa hirsuta [126]	*Bignoniaceae*	Ethanolic	Phenolics	Proanthocyanidin B_2	Improvement of NO-dependent vasorelaxation
			Tannins		
			Proanthocyanidins		
Morus alba [94–99]	*Moraceae*	Aqueous	Phenolics	Epicatechin	Anti-inflammatory
		Ethanolic	Flavonoids	Myricetin	Anti-diabetic
				Quercetin hydrate	Anti-oxidative
				Luteolin	
				Kaempferol	
Olea europaea (OLE) [90–93]	*Oleaceae*	Ethanolic	Phenolics	Tyrosol	Antimicrobial
			Flavonoids	Hydroxytyrosol	Anti-oxidant
			Hydroxycinnamic acids	Ligstroside	Hypoglycaemic activity
				Dimethyl oleuropein	Cardioprotective properties
				Oleoside	
				Oleuropein	
				Apigenin	
				Kaempferol	
				Luteolin	
				Caffeic acid	
Peltophorum pterocarpum [41]	*Fabaceae*	Methanolic	Tannins	Not defined	Anti-oxidative
		Dichloromethane: methanol	Terpenoids		Antimicrobial
			Saponins		
			Steroids		

Table I. Cont.

Latin scientific name (and common/ abbreviated name)	Family	Types of common extracts	Class of active ingredients	Active agent	Mode of action
Pteridium aquilinum [64, 65]	*Dennstaedtiaceae*	Aqueous	Polyphenols	Phenolic acid	Anti-radical
			Flavonoids		Anti-inflammatory
			Tannins		Modulatory
Samanea saman [41]	*Fabaceae*	Methanolic	Terpenoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Steroids		Antimicrobial
Sasa quelpaertensis [100]	*Poaceae*	Leaf powder	Flavonoids	Tricin	Tyrosine
			Hydroxycinnamic acids	Isoorientin	Hydroxylase inhibitor
				P-coumaric acid	
				Chlorogenic acid	
Senna suratensis (Cessia suratensis) [41]	*Fabaceae*	Methanolic	Flavonoids	Not defined	Anti-oxidative
		Dichloromethane: methanol	Tannins		Antimicrobial
Tanacetum vulgare [125]	*Asteraceae*	Aqueous	Terpenoids	Not defined	Improvement of no-dependent vasorelaxation
			Tannins		
			Steroids		
Taraxacum officinale [102, 103]	*Asteraceae*	Fresh leaf	Flavonoids	Luteolin	Anti-diabetic
			Hydroxycinnamic acids	Luteolin 7-glucoside	Diuretic
				Caffeic acid	Anti-inflammatory
				Chlorogenic acid	
Urtica dioica [113–118]	*Urticaceae*	Aqueous	Flavonoids	Genins	Antiplatelet
				Heteroside	
				Flavonoids	
Vitis labrusca [105]	*Vitaceae*	Ethanolic	Phenolics	Resveratrol	Hepatoprotective
			Flavonoids		Cardioprotective
			Stilbenoids		Renal-protective
					Anti-oxidative
Withania somnifera [31–34]	*Solanaceae*	Methanolic	Phenolics	Gallic acid	Anti-oxidant
			Flavonoids	Syringic acid	Antimicrobial
			Hydroxycinnamic acids	Benzoic acid	
				Catechin	
				Vanillic acid	
				P-coumaric	

Table II. Basic structures of the typical active components of polyphenols most commonly occurring in leaf extracts

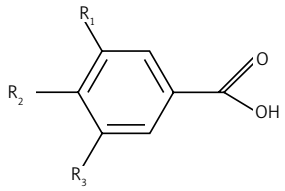
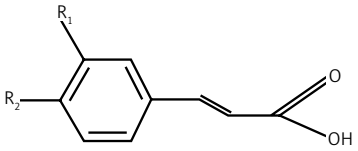
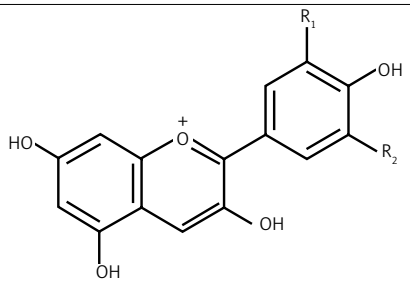
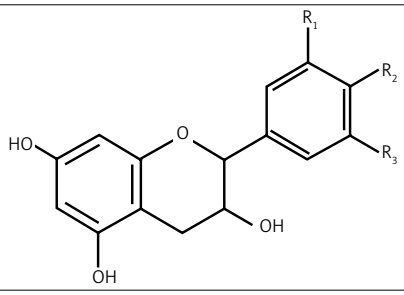
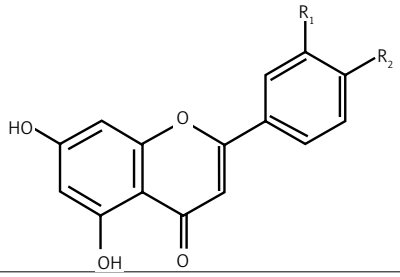
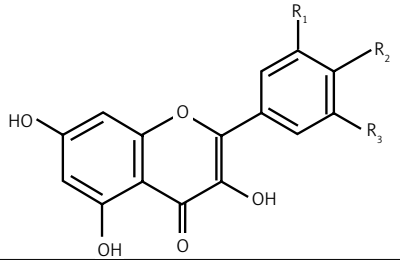
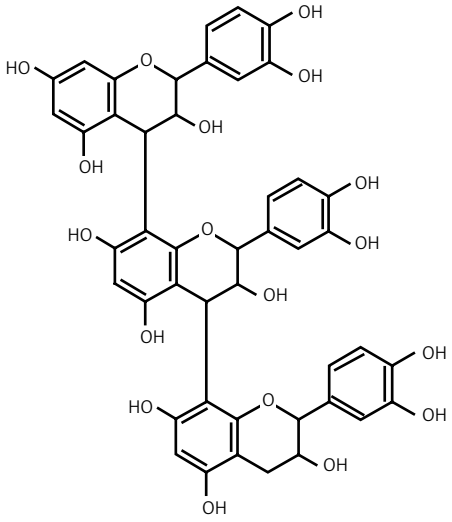
Group		Structure	Common residues and representatives
1	Phenolic acids		
1.1	Hydroxybenzoic acids		$R_1 = R_2 = OH$, $R_3 = H$ Protocatechuic acid $R_1 = R_2 = OH = R_3 = OH$ Gallic acid
1.2	Hydroxycinnamic acids		$R_1 = OH$ Coumaric acid $R_1 = R_2 = OH$ Caffeic acid $R_1 = OCH_3$, $R_2 = OH$
2	Flavonoids		
2.1	Anthocyanins		
2.2	Flavanols		$R_1 = R_2 = OH$, $R_3 = H$ Catechin $R_1 = R_2 = R_3 = OH$ Gallocatechin
2.3	Flavones		$R_1 = R_2 = OH$ Luteolin
2.4	Flavonols		$R_1 = R_2 = OH$, $R_3 = H$ Quercetin $R_2 = OH$, $R_1 = R_3 = H$ Kaempferol

Table II. Cont.

Group		Structure	Common residues and representatives
2.5	Proanthocyanidins		Trimeric Procyanidin

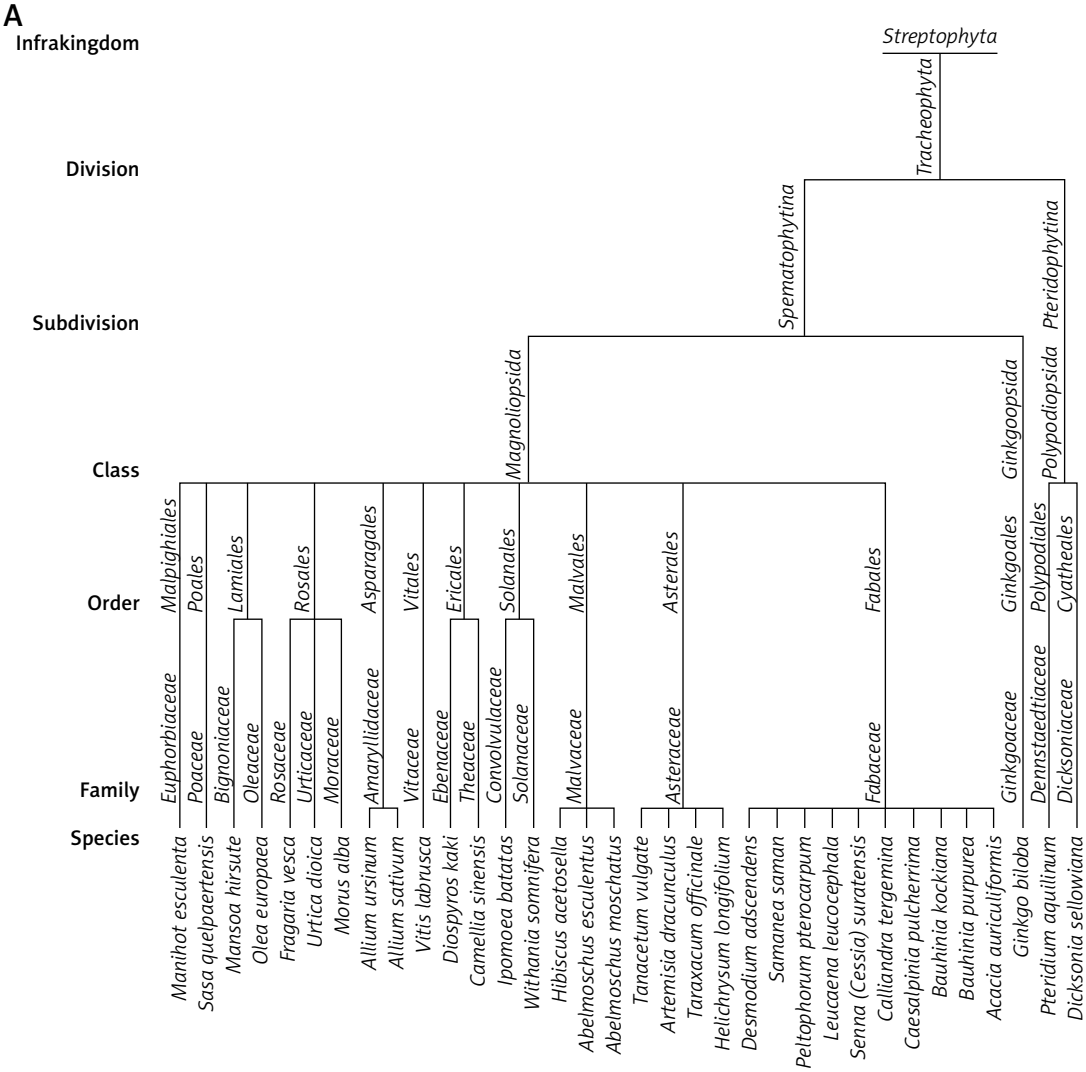


Figure 1 A. Taxonomic relationships between plant species and similarities among them based on their biological activities. – The plant species discussed in this review were agglomerated in clusters using a single linkage method based on Euclidean distances estimated according to plants belonging to the following taxa: species, family, order, class, subdivision, division, infrakingdom (Source: http://www.itis.gov).

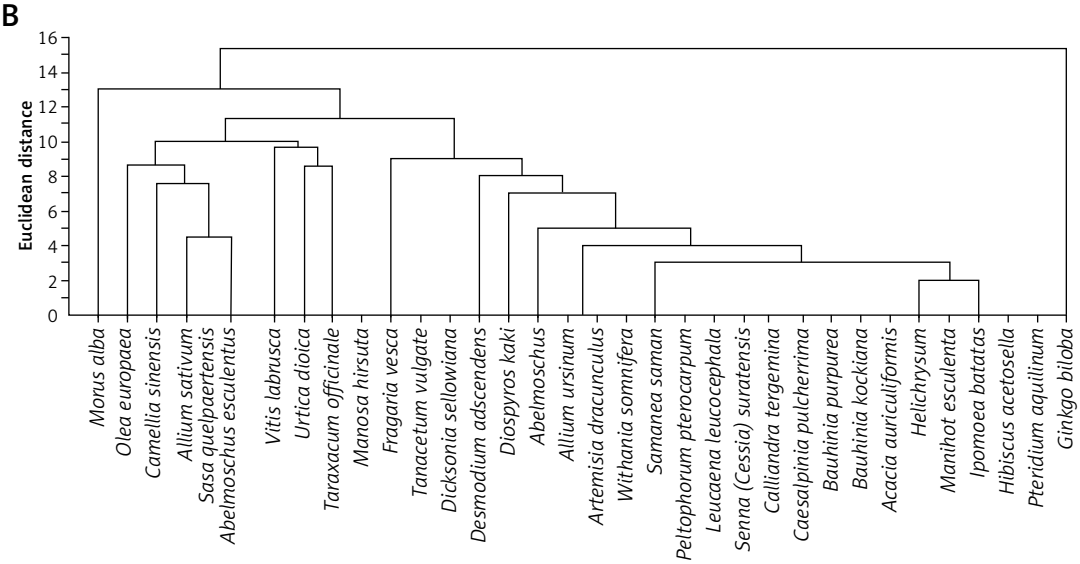


Figure 1 B. Taxonomic relationships between plant species and similarities among them based on their biological activities. – The plants were agglomerated according to their biological activity including: anti-oxidative, hepato-protective, renal-protective, anti-inflammatory, antimicrobial, antiplatelet, anti-cancer, anti-aging, anti-diabetic, NO release-propagating, lipid profile improving and adhesion molecule expression lowering properties. Agglomeration analysis was performed using the single linkage method based on Euclidean distances estimated according to distributions of plant leaf extract properties (Source: all essential information is included in Table I)

quercetin glucosides demonstrate bioavailability more favourable than other polyphenols. On the other hand, polyphenols with particularly low availability are proanthocyanidins, galloylated tea catechins and anthocyanins. Following their absorption, these compounds are further metabolized *in vivo* mainly via the glucuronic acid pathway [143]. Low bioavailability is the cause of much reduced in-organism effectiveness of these flavonoids, as validated by the revealed discrepancies between the activities of certain polyphenolic compounds demonstrated under *in vitro* and *in vivo* conditions. The reason for relatively low bioavailability is that polyphenols occur in plants in the form of esters or glycosides that cannot be absorbed without the contribution of intestinal enzymes or colonic gastrointestinal microflora [143]. Moreover, diet in general, as well as regional dietary habits in particular, may considerably influence bioavailability of polyphenols. An example that nicely illustrates this phenomenon refers to the differences between concentrations of catechins and flavonols in black tea brew and black tea customarily served with milk in the UK [70].

It is also worth mentioning that general cardioprotective effects of all kinds of polyphenols seem to be largely based on their anti-oxidative action, which results in quenching of blood ROS and preventing their formation by inhibition of enzymes responsible for oxidative stress, such as cyclooxygenases, lipoxygenases or NADPH oxidases [96].

Conclusions

In this review, we have focused on the actions of leaf extracts on various pathophysiological phenomena involving impairments in the cardiovascular system. Apart from their anti-oxidative and anti-inflammatory activities, other beneficial effects of leaf extracts have also been discussed in relation to the metabolic syndrome. *In vivo* and *in vitro* studies have clearly indicated that polyphenolic extracts from leaves exert a plethora of effects on cellular functions due to their strong anti-inflammatory, anti-oxidative, anti-aggregatory, vasorelaxant, hypolipaemic and hypoglycaemic properties. Thus, polyphenolic extracts from edible and inedible leaves are promising dietary supplements in preventing and treating cardiovascular disease, and probably deserve to be considered with not lesser enthusiasm than extracts obtained from other parts of plants.

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Conflict of interest

The authors declare no conflict of interest.

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