

## MINI REVIEW

## PLANT PHENOLICS AS DRUG LEADS – WHAT IS MISSING?

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**Abstract:** Low molecular weight phenols of plant origin are undoubtedly semiochemicals although not all of them can be easily classified as typical allelochemicals, which straightforwardly benefit the releaser. We have selected and surveyed this particular class of secondary metabolites, which shares high chemical reactivity with intrinsic biocompatibility and affinity for variety of molecular targets gained through evolution, because their suitability as prospective lead compounds for medicinal chemistry seems high but relatively unexplored. In particular, plant phenolics could be perceived as a natural product library, which contains privileged scaffolds, as evidenced by examples of endogenous phenols, phytochemicals containing aryl hydroxyl groups and phenolic synthetic drugs. It is postulated that application of bio-chemo-informatic tools to such library can be helpful in pulling out new drug candidates as well as in validating ADMET compatibility and suitability of the old ones. After short survey of structural diversity represented by plant phenolics, we focus on the compounds which either have obvious dietary significance or rich record of pharmacological studies, or both. It can be seen that apart from growing use of phytochemicals in dietary supplements, slow progress through clinical trials towards new drug registration is observed in that category of natural products. Such waste of resources on the way of transformation from renewable materials to high tech/high value products aimed for improved human healthcare is deplorable and should be reformed in name of sustainability. We attempt to answer the question why popular plant phenolics with well established health benefits and reasonably well recognized molecular pharmacology (such as: catechins, curcumin, resveratrol, quercetin and its glycosides, genistein, silymarin) have difficulties in attaining registered drug or even IND level.

**Keywords:** low molecular weight phenols of plant origin, catechins, curcumin, genistein, quercetin, resveratrol, silymarin

**Abbreviations:** ADMET – absorption, distribution, metabolism, excretion, toxicity, AP-1 – activator protein 1, API – active pharmaceutical ingredient, BA – bioavailability, BHT – 2,6-di-*tert*-butyl-6-methylphenol, EGCG – (–)-epigallocatechin-3-gallate, GLP – good laboratory practice, GT – glutathione, GTE – green tea extract, HTS – high throughput screening, IND – investigational new drug, IP – intellectual property, MAPK – mitogen-activated protein kinases, MS – mass spectrometry, NDA – new drug application, NF- $\kappa$ B – nuclear factor – kappaB, PAL – phenylalanine ammonia lyase, PK – pharmacokinetics, PUFA – poly-unsaturated fatty acids, ROS – reactive oxygen species, SM – secondary metabolite, RNS – reactive nitrogen species, ROS – reactive oxygen species, SOD – superoxide dismutase

Although it is generally agreed that majority of contemporary drugs are derived, directly or through semi-synthesis, from natural sources, lasting inspiration from unsurpassed structural diversity of secondary metabolites (SM) did not radically improve unsatisfactory low output of drug discovery process in recent years. The reasons for this rather deplorable situation are, in general terms, discussed in innumerable medicinal chemistry reviews and monographs (1–8). In order to offer more specific comments, we would like to limit the field of discussion to certain category of natural

products, which are ubiquitous, yet easy to define, distinguish and determined by analytical means. Let us first observe that even natural compounds with exceptionally rich ethnopharmacological tradition, like active components of herbals used in traditional Chinese or Ayurvedic medicine, often performed poorly in western clinical trials, indicating serious problems either with pharmacokinetic (PK) parameters [and consequently in bioavailability (BA)] or with overall efficacy. Therefore, a choice of SM category for discussion of prospective drug leads is very important. To gain more

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critical view of SM as drug leads we have focused in this paper on examples of compounds of plant origin which are phenolic by structure and chemical characteristic. In authors opinion there are sound rational arguments for such choice: phenol function is encountered in pharmacophores of both: natural, as well as synthetic origin; phenolics constitute a large group of secondary metabolites, estimated at tens of thousands of individual entities, which are encountered in practically all higher plants, therefore, they are of interest from the point of view of ethnopharmacology as well as human nutrition and toxicology. Phenolic compounds of plant origin belong to reactive chemical species and they share certain characteristics, like antioxidant activity, which is considered beneficial for human health; they are seldom pharmacologically inert and last but not least, they are known to share certain metabolic pathways, by which they are conjugated and excreted, like other xenobiotics, from human body (9–12). Plant phenolics are in fact well suited for a concept of reverse pharmacology, describing activities in which the time arrow points from the state of proven safety and efficacy of an agent towards study of molecular mechanism of its action. What is probably most important, vast knowledge (chemical and biological) on individual members of the class has been already accumulated but needs consolidating. Therefore, treatment of plant phenolics as a structurally related library for data management and retrieval, would enable quick multi-parametric evaluation of a sizable collection of chemical enti-

ties, which is already rich with validated targets and elements of biological activity verification.

### Structural diversity of plant phenolics

It is estimated that ca. 40% of organic carbon is bound in form of polymeric structures containing phenolic functionality. For the purpose of this article plant phenolics are defined as low molecular weight (roughly, less than 50 atoms heavier than hydrogen, or < 700 D) secondary metabolites, which contain in their structure at least one hydroxylated aromatic ring. Since phenols can be found in many categories of biologically relevant compounds, let's quote some examples: some endogeneous amino acids and biogenic amines (tyrosine, dopamine, serotonin, epinephrine); steroids (estradiol), some synthetic drugs (e.g., entacapone, etilefrin, fenoterol, isoprenaline, paracetamol, salicylates, salbutamol), phytochemicals turned into well known drugs (morphine, etoposide, sylibin, topotecan) and many antibiotics, can be classified as low molecular weight phenols (13–15). Obviously, a single phenolic function by itself does not render any particular pharmacological advantage (although all phenols can be considered antioxidants) but low molecular weight plant phenolics, which share high reactivity with biocompatibility, can be considered as a library of natural products featuring privileged structural fragments and reach in terms of already collected data describing their properties (16–18). In biological and phytochemical literature another term: polyphenols is frequently encountered, for describing similar collection of SM, but without molecular weight restriction. The focus on multiplic-

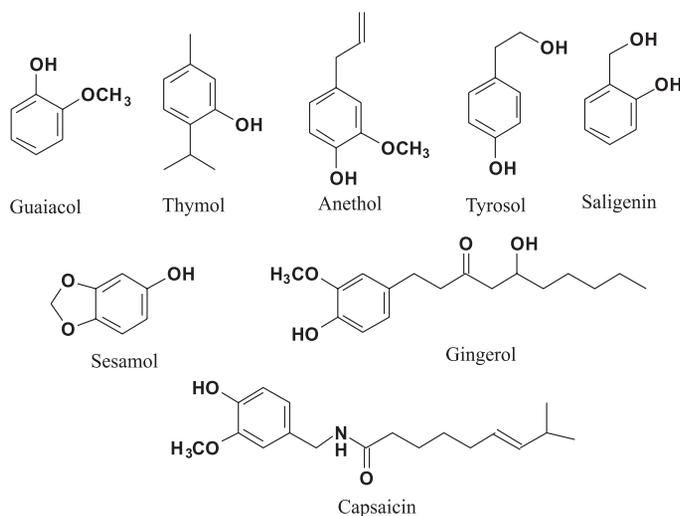


Figure 1a. Simple phenols



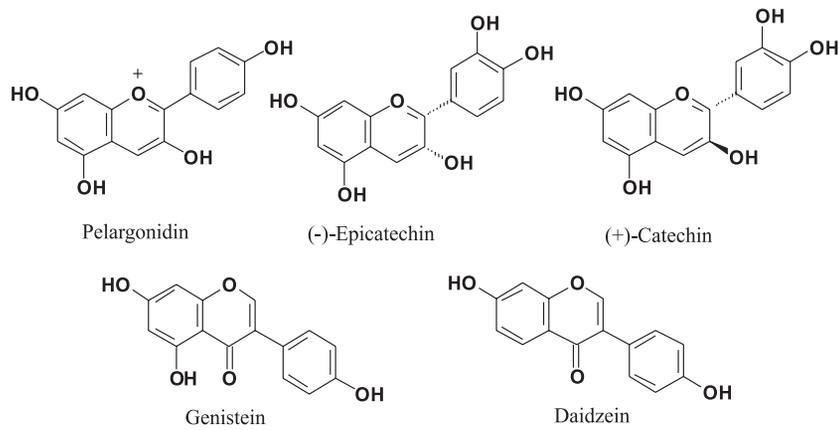


Figure 1f. Anthocyanidins, catechins, isoflavones

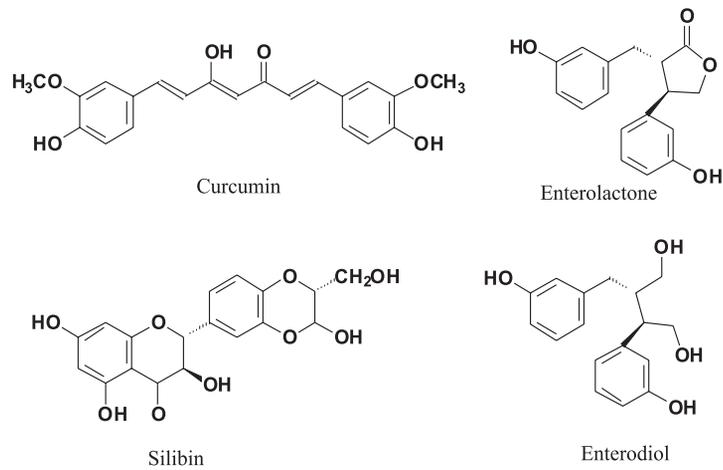


Figure 1g. Curcuminoids, lignans

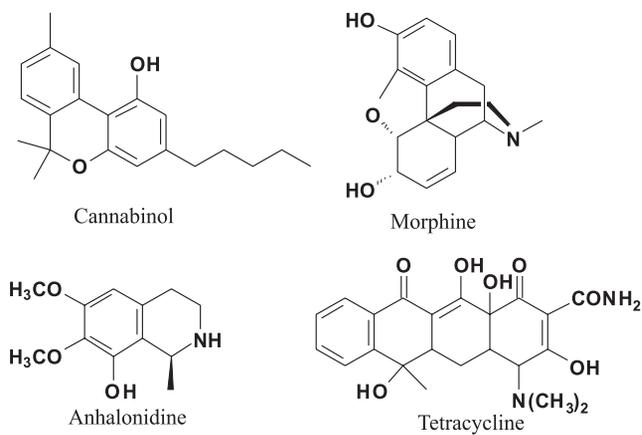


Figure 1h. Miscellaneous



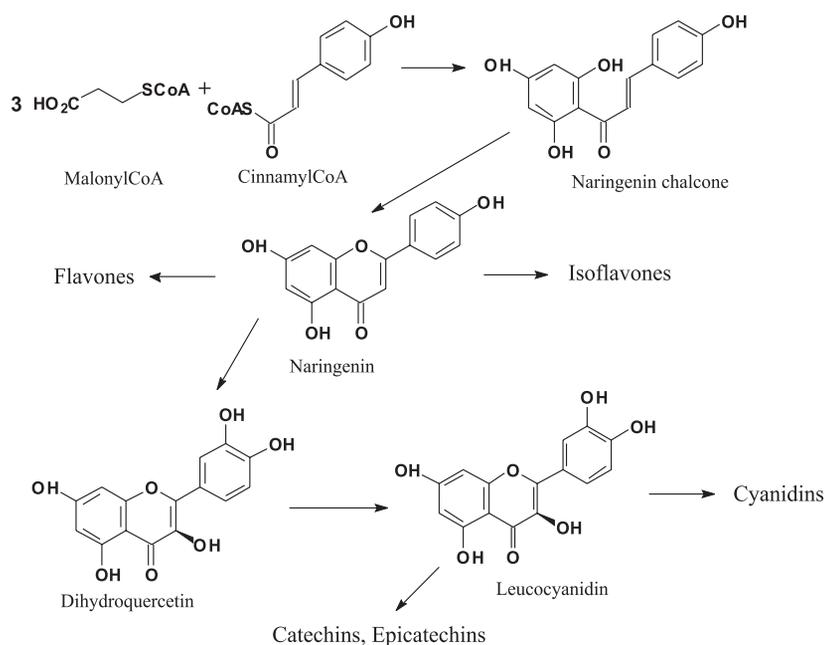


Figure 3. Biosynthesis of flavonoids

relatively high concentrations, as exemplified by toxic alkaloids which function as deterrents for herbivores, but even in such cases molecules in question are in dynamic equilibrium with their precursors and degradation products.

#### Plant phenolics as constituents of human diets and prospective medicines

Vacuolar plants biomass contain, apart from water, mainly carbohydrate polymers which serve as a principal construction material, but high molecular weight phenolics such as lignans are second in the mass balance. It follows, that formation of low molecular weight phenolics is common in metabolic turnover and some of them stay in that category throughout plant life cycle. Human vegetable food has always contained considerable amount of phenolics, which in higher proportion can affect the taste, adding sensation of bitterness and astringency, which is acceptable at least in spices and herbs used for food preservation and curative purposes. Today, owing to well developed analytical methods, individual phenolic constituents can be easily quantified in food and their metabolites in body fluids, so phenolic intake levels are known in detail for various populations, differentiated by geographic region or dietary habits. SMs of phenolic character, listed in the first part of this paper, are usually considered as markers of fruit and vegetable intake, recommended

by all medical authorities as a base of healthy diet. The recommendation is based on both ethnobotanic tradition and modern pharmacognosy, which attempts to make clear-cut distinction between edible and medicinal plants, the latter often containing toxic substances. It is well established that human body contain numerous phenolics, both: endogenous (catecholamines, dopamine derivatives, serotonin, estradiol, vitamin E type compounds) and xenobiotic (flavonoids, catechins, lignans), the latter in doses which can exceed one gram per day level. Many important aspects of the xenobiotics in diet are still poorly recognized, for example the role of native phenolic glycosides in biological activity of better studied phenolic aglycones, or mutual importance of human metabolism *versus* metabolism of bacteria which colonize intestinal tract of the host. Such factors can obviously considerably influence individual biological effects of plant phenolics intake. The compounds are present in practically all fruits and vegetables, fresh and processed, as well as in popular beverages: tea, coffee, cocoa, wines and beer (7, 9, 13, 18). Presently, plant phenolic category covers entire spectrum of biocompatibility, medical acceptance and market status: from quercetin and its glycosides generally recognized as safe, with several ongoing clinical trials, through genistein and diosmin, which acquired OTC drug status, some lignans recognized as useful drug leads under investigation,

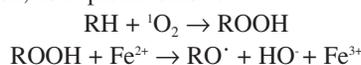
up to well established in medicinal practice but strictly controlled substances as morphine and its analogs.

### Plant phenolics as antioxidants

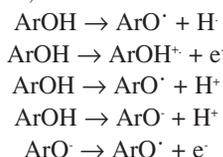
Life in an aerobic environment is a risky business – not only the molecular oxygen (triplet state;  $^3\text{O}_2$ ; formally a bi-radical) is toxic at high concentrations – it also can attain several other reactive oxygen species (ROS), which are even more noxious: ozone ( $\text{O}_3$ ), singlet oxygen ( $^1\text{O}_2$ ), oxygen anions (I) and (II) ( $\text{O}^-$  and  $\text{O}^{2-}$ ) superoxide anion ( $\text{O}_2^{\cdot-}$ ), superoxide radical anion ( $\text{O}^{\cdot-}$ ), hydroxyl radical ( $\cdot\text{OH}$ ) and hydroperoxy radical ( $\cdot\text{OOH}$ ). There is a physiologically accepted low level of ROS, which are needed to fight pathogens, but disturbance of oxidative-reductive equilibrium to the point of lipid peroxidation or DNA damage is called an oxidative stress and is recognized as a pathology. Peroxidation and autooxidation of organic compounds, particularly unsaturated, was studied since ca. 1940 in connection with both: chemical industrial processes and biological chemistry. In last decades, particular importance of radical chemistry has been stressed and chemical reactivity parameters for ROS has been measured allowing for their classification as agents causing hydrogen abstraction from various organic substrates, and starting radical chain reactions by homolytic cleavage of an O – H or C – H bonds. Phenols and particularly vicinal diphenols (catechols), are susceptible towards free radicals attack, which results in formation of relatively stable phenolic radicals ( $\text{ArO}^{\cdot}$ ), therefore they are considered effective antioxidants and believed to serve as protectants against ROS (19, 20).

Oxidative stress results from the presence of reactive oxygen or nitrogen species (ROS and RNS, respectively) which stem either from endogenous (mitochondria, peroxisomes, inflammatory cells) or exogenous (radiation, oxygen, xenobiotics) sources. This constant challenge is normally kept in check by biological defense systems, composed of two essential parts: enzymatic (catalase, superoxide dismutase, glutathione peroxidase) and nonenzymatic, to which many plant phenolics can significantly contribute (vitamin E, vitamin C, glutathione, flavonoids) (21, 22).

Typical ROS (alkoxyl and peroxy radicals) are generated by activated oxygen in the presence of transition metal ions, from biogenic polyunsaturated fatty acids (PUFA), starting from hydroperoxide formation, as depicted below.



Antioxidants, in general, and phenols, in particular, quench reactive radical intermediates in an array of molecular processes involving intact ArOH or variety of radical species generated in electron transfer reactions, as illustrated below.



In general, cells are well equipped against oxidative stress, which is practically a nonspecific, multitarget assault. There are designated enzymes, which react with superoxide radical anion (superoxide dismutase; SOD), peroxides, including hydrogen peroxide [catalases, glutathione peroxidases (GPx) and peroxyredoxins]. Besides, nonenzymatic cellular antioxidants operate in both: aqueous (ascorbate, glutathione) and lipid (tocopherols) phase and there is also a considerable amount of exchange of reductive potential at the interphase. They are all able to neutralize relatively slow reacting radicals, breaking radical propagation chains. Hydroxyl radical should be mentioned here, as an exceptionally reactive species, characterized by diffusion controlled reactivity, therefore, not amenable to protection by regular antioxidants. Another important issue, frequently missed in discussion of physiological red-ox equilibrium, is metal catalysis. Towards the end of XIX century H.J.H. Fenton observed efficient oxidation of tartaric acid by hydrogen peroxide in the presence of Fe (II) salts and the reagent composition was soon recognized as having wide scope for organic synthesis. In 1934, F. Haber and J. Weiss postulated that Fenton's reagent is a source of highly reactive hydroxyl radicals (23, 24), but importance and relevance of this idea for biological chemistry was not realized until recently. The most prolific ROS: superoxide radical anion, which may be generated by variety of enzymes, including cytochromes, oxidases, peroxidases and dehydrogenases, can reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  and the latter is capable of breaking down hydrogen peroxide molecule into hydroxide anion ( $\text{OH}^-$ ) and hydroxide radical ( $\text{HO}^{\cdot}$ ), with regeneration of  $\text{Fe}^{3+}$  (iron catalyzed Haber–Weiss reaction). These findings can easily account for lipid, DNA and protein damage within physiological oxidation potential framework, with an assumption that free radical damage by hydroxide radical can be a localized event, not necessarily connected to a high cellular level oxidative stress. The role of phenolics and their radicals, in an environment which allows them to adopt a function of antioxidant, prooxidant or

metal ion complexing agent is difficult to evaluate, especially in case when redox cycles, involving hydroquinone – quinone forms are possible.

It is generally believed that dietary antioxidants of plant origin are beneficial for human well being. Experiments with synthetic antioxidants of phenolic nature (e.g., BHT, Trolox) clearly indicate, that under oxygen stress they are consumed with various velocity. Although free radical scavenging properties of antioxidants are relatively simple to measure, overall biological effects of their action is not so obvious. Products of the primary free radical transformations, e.g., phenolic radicals ( $\text{ArO}\cdot$ ) are relatively slow reacting species but kinetics of their recombination can vary so much, depending on individual phenols, that although most of them qualify as terminators of a radical chain reactions, some can also be considered as radical propagating agents. Moreover, covalent adducts resulting from phenolic radical recombination reactions, remain in most cases hypothetical and their toxicities remain to be studied. In recent years, measurements of antioxidant activity of plant derived materials and individual secondary metabolites became very popular and many new tests for this purpose have been developed, marking visible switch from spectrophotometric methods to more specific electrochemical assays. The most popular are: ORAC (oxygen radical absorbing capacity); F-C

(Folin–Ciocalteu phenolic assay) and TEAC (trolox equivalent antioxidant capacity) assays and their scope and limitations are repeatedly reviewed (23–28). Regardless of their accuracy and reliability it has to be stressed, that results of antioxidant capacity measurements of plant phenolics has practically no bearing to their hypothetical function in red-ox equilibrium of a cell, which constitutively contains ca.  $10^7$  molecules of glutathione, while xenochemicals can hardly exceed  $10^3$  molecules per cell number. Besides, phenolics are used up and excreted in metabolic processes, while GT is capable of replenishing glutathione stores, according to current needs. It can be argued that antioxidant action of phenolics is practically outside of pharmacological interest because of very low specificity and need for concentrations which are not attainable within pharmaceutical intervention limits. In any case, it seems that antioxidant properties of plant phenolics will remain of interest in domain of food and nutrition, perhaps with some focus on prophylactics and prevention, rather than clinical therapy (25, 26, 28). In this connection it may be of interest that one of popular tests for radical scavenging properties, namely DPPH (based on a stable organic radical) has recently been coupled to planar chromatography technique (TLC), which renders much more selectivity to the antiradical and antioxidant directed analysis (29, 30).

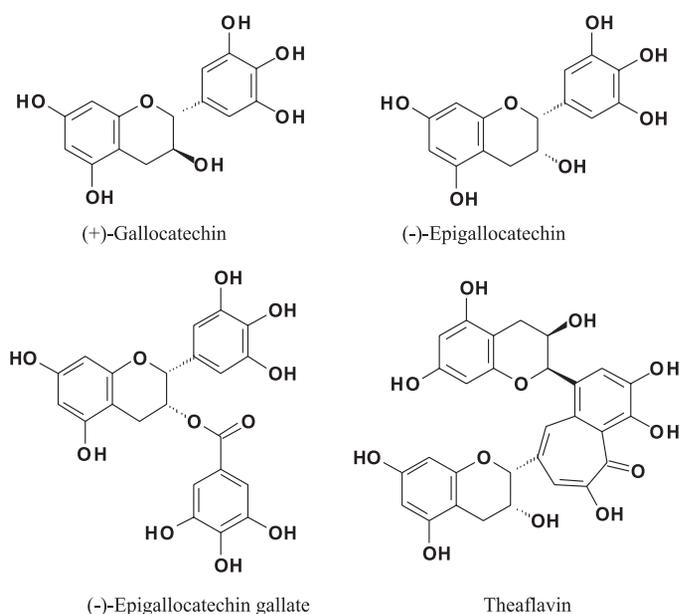


Figure 4. Gallocatechins and theaflavin

### Plant phenolics in molecular pharmacology perspective; some examples

Modern chemoinformatics can apply only very limited resources for consideration of the entire 3D chemical space, which accommodates all possible chemical structures. Reasonable estimates predict ca.  $10^{60}$  compounds which can be assigned to low molecular weight category and it is reasoned that within this astronomical number of structures, clusters of drug-like compounds exist, which are in principle distinguishable by a set of molecular descriptors, including certain types of ring arrangements and scaffolds, as well as hydrogen donating and hydrogen accepting functional groups. Since more than half of existing drugs derive their structures from secondary metabolites, it is reasonable to assume that medicinal chemistry sought clusters and privileged natural products occupy the same areas of the tri-dimensional chemical space. As a mental exercise in structure/property clustering, we select phenolics as a phenomenological privileged category of chemical entities, which have already undergone evolutionary selection and attained position of proven ligands for variety of target proteins. In particular, they are biogenetically products of specific enzymes and they have evolved towards variety of molecular targets, which are themselves most likely to be proteins. This kind of biocompatibility, usually lacked by synthetic compounds, should be very useful as a starting point for medicinal chemistry consideration. As pointed out above, plant phenolics represent wide structural diversity, and from the point of view of currently applied molecular descriptors, there is no general indication how to design an efficacious mimetic of a plant phenolic for a particular medical need.

Nevertheless, apart from numerous clinical trials performed on native MS, there is continuous effort to modify their structures, in order to attain better PK and PD parameters. Generally, in contrast to synthetic compounds aiming at HTS, plant phenolics follow the rules of “reverse pharmacology” – with their biocompatibility and safety beyond reasonable doubt, the remaining concerns of efficacy focus mainly on: correction of physicochemical properties; design of pro-drugs; or elaboration of a suitable pharmaceutical formulation. The following examples offer some indication how seemingly well advanced drug leads from plant phenolic category are progressing in their development from “established traditional use” level towards IND and NDA status.

### Catechins

There is little doubt that flavonoids represent the most important category of plant phenolics, when their effects for human health is considered, because of widespread occurrence and relatively high content in edible fruits and vegetables, as well as in plants used as spices or for beverage preparation. Flavanols, such as catechins represented in Figure 4 are particularly abundant in tea (*Camellia sinensis*), which is also exceptionally rich in gallic acid. Phenolics make up to 36% of the dry weight of tea leaves (for comparison – different varieties of tea contain 3–6% of caffeine, traditional marker of stimulating properties). (–)-Epigallocatechin-3-gallate (EGCG) is the main phenolic constituent of the green tea (nonfermented), accounting for ca. 65% of the total catechin content. Since tea is the most popular beverage, worldwide interest in its constituents biological activity is well grounded. Three main

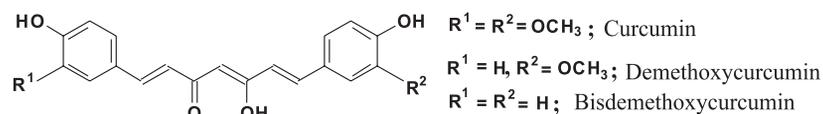


Figure 5. Curcuminoids

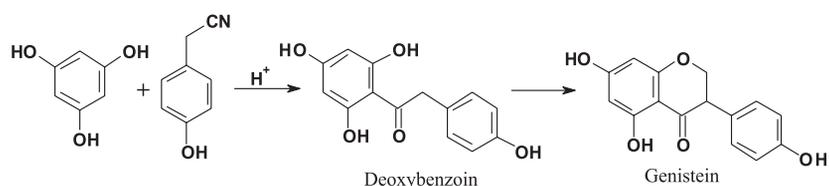


Figure 6. Synthesis of genistein

types of tea (black, highly fermented, ca 75% of the global consumption; green, low fermentation product consumed mainly in Asia; and oolong, intermediate fermentation product, accounting for ca. 2% of the global use) considerably differ in composition of phenolics, but they all are believed to decrease risk of cardiovascular episodes, particularly myocardial infarction. Tea consumption also improves endothelial function and can also reverse some endothelial dysfunction, probably by increasing nitric oxide production. Catechins are difficult objects to study, because their chemical instability. For example, EGCG can dimerize to theaflavin, which in turn gives rise to high molecular weight oligomers of not fully explored structures (31, 32).

Apart from well known stimulating, and recently much popularized antioxidant properties of tea secondary metabolites, there is an accumulating evidence for anticancer and cancer preventive activity of the flavanol constituents. In particular, it has been demonstrated that EGCG inhibits activity of mitogen-activated protein kinases (MAPK), activation of activator protein 1 (AP-1), nuclear factor-kappaB (NF- $\kappa$ B), topoisomerase I, and other cancer-related targets which control apoptosis, proliferation, migration and differentiations of cells (32). It has to be stressed that black teas, in contrast to green ones, contain little monomeric catechins, which undergo biotransformation during fermentation process. Standardized green tea catechin extract (GTE; ca. 75% total catechin content) has been examined as dietary supplement in a randomized double-blind placebo-controlled trial carried out for 3 months on obese, hypertensive patients (33). Both groups (GTE and placebo) had extensive multiparameter baseline characteristics, which facilitated efficacy of the intervention. GTE supplementation was demonstrated to exert positive effect on blood pressure, carbohydrate metabolism and lipid profile, warranting more studies on the subject.

Pure EGCG (98%) is commercially available but its susceptibility to degradation under stress makes any stable product development based on that substance as API rather problematic.

### Curcuminoids

Asian spice turmeric (*Curcuma longa* L.; a component of curry powder) has been known for millennia as a source of yellow pigment, to which Ayurvedic medicine ascribed numerous curative properties. Its main constituent, curcumin, was assigned diferuoylmethane structure (Fig. 5) by S. Kostanecki and its synthesis, based on malonate ester chemistry, was completed by Wiktor Lampe

soon after (34, 36). Hundred years later, turmeric is an important agricultural commodity (ca 850 000 tons manufactured annually in India alone), and its main phenolic constituents (curcumin, demethoxycurcumin and didemethoxycurcumin) evoke great interest as biologically active compounds, as evidenced with thousands of publications recorded in scientific literature over recent decades (34–36). Convincing evidence exists that curcumin interacts with exceptionally wide array of molecular targets. In particular, it inhibits peroxidation of lipids and production of pro-inflammatory cytokines, inducible nitrogen oxide synthase, and thioredoxin reductase. Among other inhibited enzymes, a group of protein kinase should be mentioned (cAK, ERK, FAK, JAK, PKA, PKB, PKC, MAPK), along with ATPase, DNA polymerase, and telomerase. In the other target categories, receptors, growth factors, transcriptional factors, cell adhesion molecules and gene expression modulators were studied, and significant downregulation of their activity has been observed (34). In traditional medicinal chemistry such an abundance of targets would probably disqualify the compound as a drug candidate on the target promiscuity ground but contemporary approach is different, at least in the field of oncology, where multitarget agents are valued, particularly if their systemic toxicity is low.

There is a special interest in curcumin affinity to various proteins, in connection with its ability to inhibit pathological aggregation of amyloids in the brain, which may prove significant for treatment of Alzheimer's disease. The main factor, which seems to hamper development of curcumin into therapeutic drug, is its negligible bioavailability upon oral administration.

### Genistein

Isoflavone genistein was first identified as a secondary metabolite of dyer's broom (*Genista tinctoria* L.) and was later blamed for reproductive problems of Australian sheep feeding on subterranean clover. Proven phytoestrogen, and selective ligand of ER $\beta$ , it is presently used as an active ingredient of innumerable dietary supplements, based on soy isolates and concentrates (37, 38). Two other findings from the second part of the last century made genistein one of best studied secondary metabolites: inhibition of protein kinase C activity and epidemiological evidence indicating that soy rich diet practiced in Asian populations inversely correlates with tumor mortality from hormone dependent cancers (37, 38). More recent studies linked genistein to lipid metabolism and molecular targets involved in metabolic

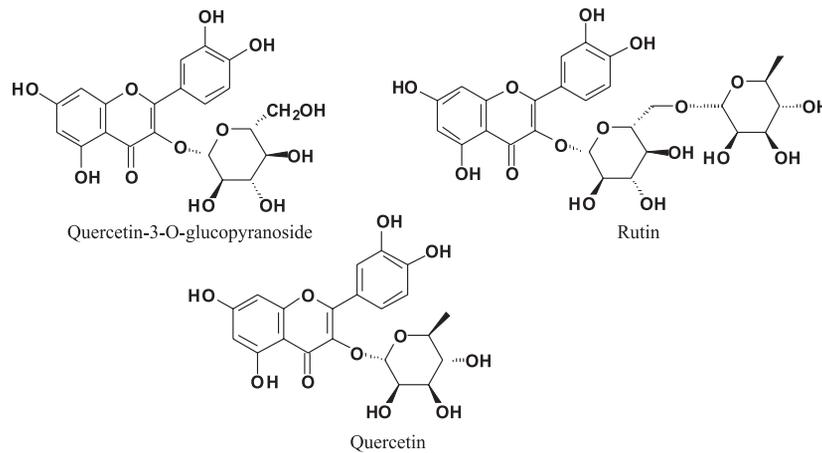


Figure 7. Quercetin glycosides

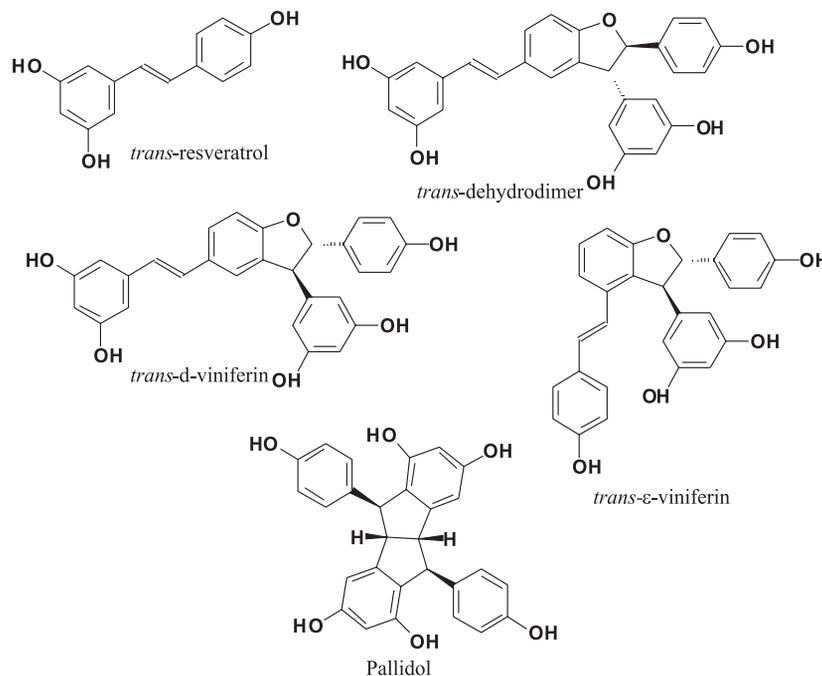


Figure 8. Resveratrol and its dimers

syndrome. Genistein remains also of vivid interest to human nutrition as a soybean constituent, considering the fact that soy is one of the principal agricultural crop in the global scale. The plant is cultivated as GMO, selected for quality of oil and protein, but protein fraction (soy flour) contains also some isoflavons and saponins, because their physicochemical characteristic is similar to oligopeptides. Because of relative stability of the substance and its availability from chemical synthesis (Fig. 7), rela-

tively large number of its new derivatives have been synthesized and studied, which in turn indicated new targets and new mechanisms of action (39). Both: genistein and its 7-O-D-glucopyranoside – genistin – can inhibit storage of glucosaminoglycan plaque in CNS, in case of glucosaminases deficiency resulting in genetically determined neurodegeneration (40). Unexpectedly, some derivatives of genistein, exhibited influence on the cell cycle by interference in tubulin dynamics and organization of the mitotic

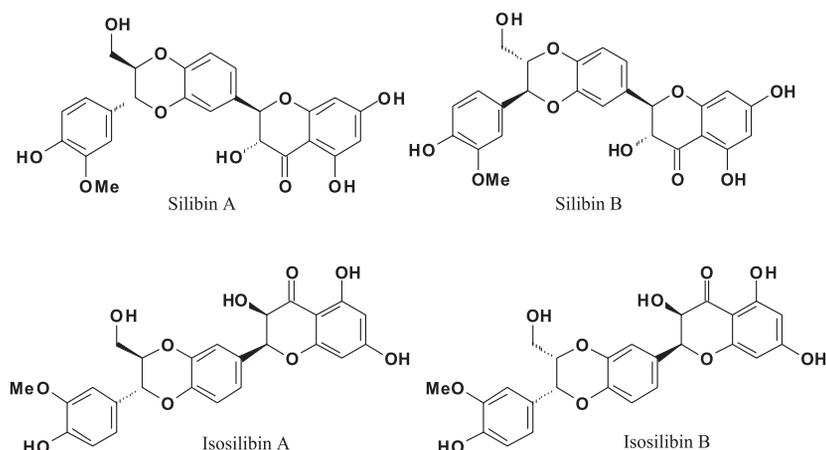


Figure 9. Silibins

spindle – a property which may find application in clinical oncology (41).

#### Quercetin and its glycosides

Flavonols and their glycosides are known to occur in variety of fruits and vegetables, with onions and apples being particularly rich sources. Serious interest in their biological activity started with A. Szent-Györgyi postulate that quercetin glycoside – rutin, is a vitamin (then named vitamin P), necessary for bioavailability of the anti-scorbutic factor – vitamin C (8, 13). The next serious assignment of flavonol glycosides to human health concerns related to venous and capillary vessel problems. A mixture of partially hydroxyethylated rutin became a topically applied drug for improvement of capillary blood vessels around 1950. This line of interest in flavonol glycosides application for treatment of venous insufficiency is successfully continued by diosmin, which is now applied orally.

Quercetin glycosides are common in food: 3-O-galactoside (mango, plums, berries); 3-O-glucoside (onions, beans, plums); 3-O-rhamnoside (pepper, cranberry, mango); 3-O-glucuronide (lettuce, chicory); 3,4'-O-diglucoside (onions); 3-O-rutinoside (plums, cherries, tomatoes, buckwheat) are not only well established plant constituents, but methods for their quantification are presently precise and widespread, rendering a new dimension to nutritional sciences (42–44). It is assumed that all these quercetin glycosidic derivatives undergo biotransformation to the aglycone, although their particular PK values and metabolic fates may differ considerably. Quercetin and rutin are now subjects of Natural Standard Monographs, which provide a

wealth of medicinal and clinical information connected mainly to their use as vasoprotectants and constituents of innumerable herbal remedies with dietary supplement status.

#### Resveratrol

Interest in biological effects of stilbenes, represented by resveratrol, stems from results of nutritional studies, which reached wide publicity in form of “French paradox” discussion, redefining Mediterranean diet and role of red wine consumption. Resveratrol, 3,5,4'-trihydroxy-trans-stilbene, is present in grape skin in minute amounts only and its level in wine can hardly have any health influence, but owing to the general public interest, its properties, both chemical and biological, have been thoroughly tested. Its reported involvement in delay of aging of some model organisms has evoked enormous interest and resulted in an avalanche of dietary supplements on western markets. Surprisingly, the mechanism by which resveratrol exerts antiaging metabolic benefits has been uncovered only very recently. It has been found that it works by inhibiting cAMP phosphodiesterases, thus elevating cAMP level and starting a chain of events that leads to activation of sirtuin-1, an enzyme which has histone deacetylase activity and is involved in chromatin silencing (45). There are seven human sirtuins, expressed in nucleus, cytoplasm and mitochondria. Since effects of Sirt1 modulation on age related disorders are already well established, new way of interest in resveratrol as a drug lead can be expected. It should be remembered, however, that resveratrol is very poorly bioavailable and rather unstable chemically.

Resveratrol, a polyphenol in red wine, has been reported as a calorie restriction mimetic with potential antiaging and antidiabetogenic properties. It is widely consumed as a nutritional supplement, but its mechanism of action remains a mystery. Here, we report that the metabolic effects of resveratrol result from competitive inhibition of cAMP-degrading phosphodiesterases, leading to elevated cAMP levels. The resulting activation of Epac1, a cAMP effector protein, increases intracellular  $\text{Ca}^{2+}$  levels and activates the  $\text{CamKK}\beta$ -AMPK pathway *via* phospholipase C and the ryanodine receptor  $\text{Ca}^{2+}$ -release channel. As a consequence, resveratrol increases  $\text{NAD}^+$  and the activity of Sirt1. Inhibiting PDE4 with rolipram reproduces all of the metabolic benefits of resveratrol, including prevention of diet-induced obesity and an increase in mitochondrial function, physical stamina, and glucose tolerance in mice. Therefore, administration of PDE4 inhibitors may also protect against and ameliorate the symptoms of metabolic diseases associated with aging. Despite resveratrol's well-documented health benefits, its mechanism of action remains controversial. In particular, the direct molecular target of resveratrol has been elusive. Park et al. now show that resveratrol directly inhibits cAMP-dependent phosphodiesterases, triggering a cascade of events that converge on the important energy-sensing metabolic regulators AMPK, Sirt1, and PGC-1 $\alpha$  (46, 47).

### Sylibin

Sylimarin is a complex of secondary metabolites isolated from the milk thistle plant (*Silybum marianum*) containing several flavonolignan constituents, with established efficacy in protection of the liver against natural and synthetic toxins (48). The complex, which have been used extensively for treatment of the liver ailments, including hepatitis, cirrhosis and jaundice, consists of 70–80% of flavonolignans, with up to 30% of polyphenolic fraction (oxidized and polymeric materials). The main components are silibin A and silibin B, which are diastereoisomers, reflecting the fact that enzymatic addition of coniferyl alcohol to taxifolin occurs with a low stereospecificity. Isosilibins constitute another isomeric pair of lower abundance, while silydianin and silychristin bear some skeletal modifications. Sylimarin complex also contain parent flavonol taxifolin and glycosides such as quercitrin and apigenin 7-O-D-glucoside. All these compounds are presently easy to identify and quantify on variety of HPLC columns, with gradient elution and MS detection (49, 50). On the other hand, preparative separation of sylimarin complex is very

difficult – isolation of silibins requires glycosylation, following column separation of the glycosides and their hydrolysis. Owing to success of such procedure, structural analysis and configurational assignments were possible, but there is still no source of pure individual silibins, which could be used for drug development process.

### CONCLUSIONS

The idea of plants as source of drugs, turned into practice in human medicine over millennia, is still viable today – in recent decades more than half of newly registered drugs have close relationship to structure of secondary metabolites (7, 8, 12, 17). Many plant phenolics have been recognized as promising drug candidates but progress in their clinical evaluation is sluggish. Meanwhile, they are extensively exploited as active ingredients of innumerable products on ill-regulated food supplement markets. It seems reasonable to postulate, that since biological functions of phenolics in plants are distinctly different from those expected to be performed in human body, some structural modifications are imminent. For a start – to enhance improvement of PK and BA parameters, phenolic functions should be protected from rapid mammal second phase metabolism, resulting in glucuronidation and sulfation. At the same time, oxidative metabolism, leading to formation of new phenolic functions, should be carefully evaluated as possible source of toxicity, in the entire context of systems biology. Presently, a variety of plant phenolics can be considered drug leads, but their majority are not yet fit as proper drug candidates. In summary, and in reference to the title question, it seems obvious that the first thing needed for successful development of SM, such as plant phenolic, is IP protection, preferably in form of patent. Although ca. 2 million patent applications are filed annually worldwide, our region is falling behind (134 580 applications were filed in 2009 at the European Patent Office) indicating dramatically poor prospects for future health-care. Natural products can not in principle be patented as novel structures, but their medicinal uses are amenable for protection, as exemplified for taxol, galantamine, camptothecin, etoposide, etc. Next, technically reliable and economically viable source of active ingredient of the future drug should be secured. More often than not, isolation from natural sources is too cumbersome and API manufacturing process, based on chemical synthesis, or biotech process is designed *de novo*, scaled up, validated and implemented. Suitable pharmaceutical formula-

tion must be elaborated, to supply therapeutic dose in a patient-friendly manner, rendering it bioavailable and efficacious. Successful completion of the last task should be evidenced by convincing results of clinical trials. In view of the above short review, it comes as a surprise, that only so few plant phenolics have attained drug registration level.

Plant phenolics, when considered under restrictive upper molecular weight limit, constitute relatively small group of compounds, which is dense with biological activity (e.g., practically all its members exhibit antioxidant properties) and for which basic biochemical knowledge is already abundant. Moreover, examples of remarkable selectivity in systemic biological activity and pharmacological action are relatively frequent in the group, which seems to have a right balance between molecular similarity and structural diversity. Therefore, the class in its entirety should be treated as a privileged medicinal chemistry library, not only for mining of new drugs, but also for testing contemporary chemoinformatic and bioinformatic tools, which are far from being perfect. The title question could be managed under uniform library format, combining chemical data (structural, analytical, synthetic) with consolidated information on validated biological targets, toxicology, GLP, PK and metabolic data, and reliable clinical trial results, when available. We believe that rational application of biochemoinformatic tools to the plant phenolics library can help in mining new candidates and propose new solutions in ADMET area for the old ones. There is a paramount problem of availability of the pharmaceutical quality materials for a drug development process. Although multiple natural sources are known for most of the plant phenolics and their glycosides, availability of compounds with acceptable chemical purity is rare and often chemical synthesis remains to be single practical solution. Since IP status of a drug candidate is crucial for attracting a sponsor, some spur of innovative research aimed at patentable pharmaceutical formulations is expected on the way to ethical drugs market. In any case, the need for clinical trials before registration will remain to be a critical economical and logistic hurdle on the drug development track. For plant phenolics, which are already part of our diet, such transition to the level of registered drug does not seem very likely, unless they are converted into new chemical entities for which more specific medical claims are possible. Despite of some critical shortcomings in the present status of plant phenolics as medicinally useful materials, there is a considerable progress in collecting crucial information which can

be used for computer assisted programs and procedures known as *in silico* structure driven drug discovery. More than 500 phenolics occurring in foods and beverages have been recently gathered in form of a database called Phenol-Explorer ([www.phenol-explorer.eu](http://www.phenol-explorer.eu)), which reports food content, pharmacokinetics and metabolism. There is no doubt that such library represents very high value for planned and ongoing dedicated medicinal chemistry projects.

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