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Antioxidants: A few key points

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ABSTRACT

An antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate. Antioxidants that scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be of major importance in preventing the onset and/or the progression of oxidative pathologies and provide protection to foods. Antioxidant secondary metabolites from in vitro cultures include three classes: Polyphenols (Anthocyanins, Flavonoids, Flavone, Lithospermic B),Isoprenoids (carotenoidLycopene, monoterpene, Xantophylls), Other structures:Tocopherols (vit E), Betalanins: Kinobeon A ,Vitamin B6 ,Ascorbate , Reduced glutathione, Superoxide dismutase(SOD),Catalase ,Selenium ,Manganese. In this paper, structure and the list of some medicinal plants with these compounds were reviewed.

Keywords: Medicinal plant, free radical, polyphenol, tocopherol, Superoxide dismutas

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INTRODUCTION

Oxygen is absolutely necessary for the life processes, in particular cell respiration. However, the metabolism of oxygen may generate reactive elements called free radicals, in particular the superoxide ion (O2–) and the hydroxyl ion (OH–). There is a growing body of evidence suggesting that free radicals play an important role in the development of tissue damage and pathological events in living organisms . Free radicals damage DNA, essential cellular proteins and membrane lipids (lipid peroxidation), which may lead to cell death. [1]. It means that we named free radical to an unbound compound (i.e free) haveing one or more unpaired electrons.

Oxidative stress is mediated by reactive oxygen species (ROS) which are generated during the normal and aberrant cellular metabolism that utilizes molecular oxygen. The imbalance between production of ROS like O2, H2O2, OH, ROO and the capacity of the normal detoxification systems in favour of the oxidants leads to oxidative stress, which itself leads to cellular damage caused by the interaction of ROS with cellular constituents. Oxidative stress is involved in many acute and chronic diseases including cancer, cardiovascular troubles and neurodegenerative diseases. The balance between antioxidations and oxidations is believed to be critical in maintaining a healthy biological system [2]. Lipid peroxidation of fats and fatty acids in foods results not only in their spoilage but is also a source of peroxyl and hydroxyl radicals that are associated with carcinogenesis, mutagenesis and aging [3]. Therefore, antioxidants that scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) may be of major importance in preventing the onset and/or the progression of oxidative pathologies and provide protection to foods. It has been reported that there is an inverse relationship between the antioxidative status occurrence of human diseases .

The use of plant cell and tissue culture methodology as a means of producing medicinal metabolites has a long history.

Cultured plant cells synthesize, accumulate and sometimes exude many classes of metabolites. Medicinal compounds are of particular interest and much effort has been devoted to obtaining some of the most active and precious therapeutics. Numerous alkaloids, saponins, cardenolides, anthraquinones, polyphenols and terpenes have been reported from in vitro cultures and reviewed several times [4]. Many health-related properties including anticancer, antiviral, antimicrobial, anti-inflammatory activities, antioxidant properties, effects on capillary fragility, and an ability to inhibit human platelet aggregation have been ascribed to phenolics [5]. The etiology of several degenerative and aging-related diseases has been attributed to oxidative stress, and numerous studies have been undertaken to search for the most effective antioxidants [6].

What do we call an antioxidant?
An antioxidant is a compound that inhibits or delays the oxidation of substrates even if the compound is present in a significantly lower concentration than the oxidized substrate. The scavenging of reactive oxygen species (ROS) is one of possible mechanism of action.Others include the prevention of ROS formation by metal binding or enzyme inhibition. Chain breaking antioxidants prevent damage by interfering with the free radical propagation cascades. The antioxidant compounds can be recycled in the cell or are irreversibly damaged, but their oxidation products are less harmful or can be further converted to harmless substances [7]. At the cellular and organism level the antioxidant protection is provided by numerous enzymes and endogenous small molecular weight antioxidants such as ascorbic acid, uric acid, glutathione, tocopherols and several others. Many compounds contain antioxidant activity in addition to their specialized physiological function, and their importance as antioxidants *in vivo* is sometimes ambiguous [8]. The antioxidant activity of plant secondary metabolites has been widely established in, *in vitro* systems and involves several of the above mentioned mechanisms of action.

Chemical classes of antioxidant secondary metabolites from *in vitro* cultures:
1. Polyphenols
It is ironic from a tissue culturist point of view, Polyphenols in plants derive mainly from the shikimic acid pathway through aromatic carboxylic acids cinnamic or benzoic. Most of them possess antioxidant properties, albeit to varying degrees. The physiological benefits of the plant phenolics have been attributed to their potential role in inhibiting lipid peroxidation, modulating cell signal transduction pathways and inducing apoptosis [9].From pharmacological and therapeutic points of view, the antioxidant properties of polyphenols, such as free radical scavenging and inhibition of lipid peroxidation, are the most crucial . In addition, they have been proved to be efficient in the treatment of various cancerous lesions of the stomach, colon, rectum esophagus and liver [10]. Tropical plants are generally tolerant of high levels of environmental stress induced by ultraviolet radiation thereby explaining the high levels of phenolic compounds in endemic plant species [11]. Several of these endemics have already been reported in the traditional pharmacopoeia against a wide range of disorders including bronchitis, diabetes, asthma and inflammatory diseases . Some endemic plant species of the family Rubiaceae; such as *Fernelia buxifolia*, *Myonima nitens*, *Myonima obovata* and of the family of *Myrtaceae* ; *Eugenia elliptica*, *Eugenia orbiculata*, *Eugenia tinifolia*, *Monimiastrum acutisepalum*, *Syzygium commersonii*, *Syzygium venosum*, *Syzygium glomeratum* (traditional use of migraine treatment) have antioxidant activities beacuse of their phenolic compound and proantocyanidine and flavonoid contents. Thus the endemic plants from the *Rubiaceae* and *Myrtaceae* family could be potential sources of dietary polyphenolic antioxidant compounds that may have potential benefits in health and disease management.

Some medicinal plants like *Thapsia garganica* of *Apiaceae* family, *Artemisia campestris* and *Anthemis arvensis* and *Artemisia herba halba* of *Asteraceae* family, *Artemisia arboresens* of Asteraceae (Compositaes) and *Oudneya africana* of Brassicaceae (Cruciferaceae) and *Juniperus oxycedrus* of *Cupressaceae* and *Globularia alypum* of Globulariaceae and *Teucrium polium* of Lamiaceae (Labiatae) and *Ruta montana* of Rutaceae and *Thymelaea hirsute* of Thymeleaceae are commonly used in Algerian popular medicine. Among these plants the highest total phenolic levels have been detected in ‘‘*Anthemis arvensis* ’’, ‘‘*Artemisia campestris* ’’ and ‘‘*Globularia alypum* ’’, and the lowest in ‘‘*Artemisia arboresens* ’’ and ‘‘*Ruta montana*’’. It has been noted that amount of total phenolic compounds in *Aseteraceae* varieties is higher than the other families [10].

The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, and singlet oxygen quenchers. They also may have a metallic chelating potential.The traditional uses of some plants in Iranian society and their total phenolic contents, which varied between 2.15 and 20.3 mg of GAE/g dw. The highest concentration of total phenolics was observed in leaves of *M. officinalis*, followed by leaves of *L. officinalis*, flowers of *S. lavandulifolium* and roots of *V. officinalis*. The flowers of *A. kurdica* had the lowest phenolics concentration. This plants in iranian society had traditional uses and they use flowers of *A. Kurdica* for inflammation and cough [12] and roots of *V. officinalis* for depression [13],also flowers of *S. Lavandulifolium* [14] and leaves of *L. officinalis and M.officinalis* for their sedative and antiviral effects [13].The high polyphenol compounds were found in epidermis of *Aloe vera*. Only 20 of about 360 Aloe species demonstrated antioxidant properties and only 2 of them are widely used, i.e. *Aloe barbadensis Mill.*, also called *Aloe vera* L. *Aloes arborescens Mill* [15].The interest in the Mediterranean diet originated from findings about low cardiovascular diseases mortality in southern Europe, particularly for coronary heart disease is high. Furthermore, a recent study shows that epidemiological data collected in the past decades also suggest a lower incidence of certain other diseases, including for example cancer, in Mediterranean populations. Latter observations have been linked to health-beneficial effects of the Mediterranean diet mediated by specific food components, in particular polyphenols and unsaturated fatty acids [16]. Some of the local Mediteranean wild plants foods have high polyphenols concentration i.e. *Cichorium intybus* L. and *Sonchus oleraceus* L. from *Asteraceae* family and *Papaver rhoeas* L. from *Papaveraceae* [17].

Anthocyanins

Many anthocyanin like flavonoid polyphenols frequently studied for in vitro production are anthocyanins. Pigments are useful products because their accumulation can easily be visually evaluated. Moreover, their beneficial health promoting properties as well as their growing importance as bioactive and natural food colorants additionally increases their attractiveness for plant biotechnology.Analogously to other polyphenols, more hydroxylated compounds express larger free radical scavenging capacity, whereas methylation of the glycosylation can also reduce the ability to scavenge free radicals,but on the other hand enhances the stability of the molecule.In several species listed below the level of anthocyanins in vitro equals or exceeds the natural sources and their metabolic profile is more useful. Thus, even if anthocyanins are not so expensive, the ability to obtain products with useful structural and functional properties may become economically feasible [18]. Anthocyanins are obtained from in vitro cultures of *Prunus sp*. [19], *Daucus carota*[20], *Glehnia litoralis* [21], *V. pahalae* [22],*Ipomaea batatas*, *Fragaria ananassa* [23], *Vitis Vinifera* , *Rudbeckia hirta* [24] and *Ajuga reptans* [25] and others.Interestingly, two species of *Catharanthus roseus* and *Camptotheca acuminata*, used medicinally due to their anticancer alkaloids, produce significant amounts of anthocyanins more than 200 µg/g fresh weight in cell cultures [26]. In *C. roseus*, p-coumaroyl glucosides of highly methoxylated malvidin or hirsutidin predominated, unlike in field grown flowers. In *C. acuminata* a rare cyanidin galactoside accounted for over 95% of all anthocyanins. In *Ajuga reptans* flower-derived cell cultures the anthocyanin composition was altered from delphinidin-based in flowers towards stronger antioxidants - di-acylated cyanidin glycosides [25]. This observed simplification and alteration of the metabolic profile, when compared to natural conditions, is typical for *in-vitro* produced anthocyanins. In the extensively studied *I. batatas*, in the tissue cultures induced from storage roots the accumulation of anthocyanins can even exceed the tubers by several times.Other examples of recently reported antioxidant activities of *in-vitro* cultured, polyphenol-rich plants including *Curcuma longa* mass propagation where significant and clone dependent DPPH scavenging was noted [27].

Flavonoids

The most powerful of the flavonoids are the flavanols and their oligomeric forms called condensed tannins or proanthocyanidins, flavones and flavonols and anthocyanins. Some isoflavones are also known as antioxidants.Among other groups of antioxidant polyphenols there are phenolic acids (caffeic acid derivatives), lignans, hydrolysable tannins (gallotannins and ellagitannins), stilbenes, and xanthones. Their physiological functions in plants are versatile and have been reviewed recently [27].Phenolic acids occur in plants in free form as glycosides and can be integrated into larger molecules in an ester form. They are common as depsides the intermolecular ester of two or more units composed of the same or different phenolic acids such as caffeic, coumaric, ferulic, gallic, and syringic. Flavonoids are a group of natural benzo-c-pyran derivatives and are ubiquitous in photosynthesising cells.They occur as aglycones, glycosides and methylated derivatives. Depending on the degree of oxidation of the central pyran ring, they can be subdivided into several classes of flavonoids and flavonoid-related compounds flavones, flavonols, flavanones, isoflavones, flavans, flavanols, and anthocyanins.

The pyran ring can be opened (chalcones) and recyclized into a furan ring (aurones).Several flavonoids have been reported to quench active oxygen species and inhibit *in-vitro* oxidation of low-density lipoproteins and therefore reduce thrombotic tendency. Flavonoids may inhibit cycloxygenase and thereby modulate metabolism of arachidonic acid and attenuate inflammation. Natural flavonoids may offer an alternative to protect lipids from oxidation in foods. Some of these flavonoids have been shown to inhibit lipid oxidation in meats, fish oil and lard.

Flavonoids are one of the largest groups of secondary metabolites based on a common structure and subclasses of flavonoid include flavonols, flavones (apigenin and luteolin), isoflavonoids,flavanons, flavanols(kaempferol & myricetin & quercetin), proanthocyanidins (catechins), and anthocyanidins . Chemically they possess a tricyclic phenylbenzopyran (with the exception of chalcones) structure but biosynthetically come from phenylalanine and malonyl-CoA in the phenylpropanoid pathway.

Flavone:(apigenin & luteolin):
Antioxidant properties of flavones depend on the hydroxylation pattern.
Flavone C-glycosides are of particular interest because of their limited occurrence in plants and they have important therapeutic properties, including the prevention of cardiovascular disorders related to oxidative stress. In tissue cultures of *Passiflora quadrangularis* several C-glycosylated flavones such as isoorientin, orientin, vitexin and isovitexin have been induced in varied amounts, and their antioxidant activity determined by DPPH assay[29]. Also we can see flavonoids in the shoot cultures of *Artemisia judaica* and in callus of *Stevia rebaudiana* , flavonols and flavones are in *Petroselinum sativum* too.

Oligomeric proanthocyanidins (also known as condensed tannins) are considered to be the most powerful antioxidants of all the flavonoids owing to their structure (procyanidin B1). They are responsible for the antioxidant activity of wines, teas and fruits as well as of popular nutraceuticals made from grape seeds or pine bark. The thorough genomic and metabolic engineering studies have been recently initiated, too [30].

Rosmarinic acid (RA):
Special attention should be paid to rosmarinic acid (RA), a depside composed of two caffeic acid molecules, found in many *Lamiaceae* and *Boraginaceae* species.

The antioxidant properties of rosmarinic acid are also well established. Several plants and various techniques have been used for in vitro production of this compound. Rosmarinic acid can accumulate in cell cultures to amounts greater than those in intact plants. The suspension cultures producing rosmarinic acid were generated from *Anchusa officinalis* [31], *Eritrichum sericeum*[31], *Lithospermum erythrorrhizon* (*Boraginaceae*) (Yamamoto *et al*., 2000), and *Coleus blumei* (Petersen *et al*., 1993), *Lavandula vera* (Georgiev *et al*., 2006a,b), *Ocimum basilicum* (Kintzios *et al*., 2004), *Salvia officinalis* (Hippolyte *et al*.,1992), *Zataria multiflora* (*Lamiaceae*) (Mohagheghzadeh *et al*., 2004), *Anthoceros agrestis* (Vogelsang *et al*., 2006), *Hyssopus officinalis* (Murakami *et al*., 1998), *Lavandula officinalis* (Kovacheva *et al*., 2006), *Salvia officinalis* (Grzegorczyk *et al*., 2007). An interesting example is also the production of rosmarinic acid of over 5% of cell dry mass in *Anthoceros agrestis* (Vogelsang *et al*., 2006). An extremely high level of RA production was reported by Hippolyte *et al*. (1992) in *S. officinalis* reaching 6.4 mg/l of suspension culture, although the result has not appeared in any other reference. Depending on the species, the yield of rosmarinic acid from suspension cultures can exceed 10% of cell mass and 6 g/l of medium. Another system used for rosmarinic acid production are transformed roots of *Salvia miltiorrhiza* (Chen *et al*., 2001) and *S. officinalis* (Grzegorczyk *et al*., 2007).In the second species, the accumulation was much higher reaching 4.5% of dry weight and its antioxidant activity has been confirmed with several assays. Extracts from rosmarinic acid accumulating lavender cells also have superior radical scavenging properties (Kovacheva *et al*.,2006). Even larger amounts (over 7% dw) of rosmarinic acid accumulated in *Agrobacterium* transformed callus of *C. blumei* (Bauer *et al*., 2004). Razzaque and Ellis (1977) report a yield of 8–11% of rosmarinic acid in *C. blumei* cell cultures.

Lithospermic B:
A closely related antioxidant is lithospermic acid B, a tetrameric depside, accumulating especially in the aforementioned hairy root cultures of *H. officinalis* and elicited *S. miltiorrhiza* as well as in *Lithospermum erythrorhizon* suspension cultures.

2. Isoprenoids:
_ carotenoids (especially β-carotene):
_Lycopene:
_monoterpene:(e.g γ-terpinene and limonene):
_Xantophylls:

Isoprenoids are produced in plant cells from isopentynyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These basic units are synthesized via cytoplasmic mevalonate or plastidic deoxyxylulosephosphate pathways. Both β-carotene and lycopene are recognized as dietary chemopreventive agents, protecting against cancerogenesis.

Crocin derived from saffron (stigmata of *Crocus sativus*) has been confirmed as a powerful antioxidant[33], stronger than α-tocopherol, and being a glycoside of an apocarotenoid acid, crocetin, it is water soluble. Whereas other carotenoids are readily available from abundant natural sources such as carrots or tomatoes as well as by means of microbial and fungal biotechnology.

Abietane diterpenes are present in many *Lamiaceae medicinal* plants as well as in some gymnosperms.Carnosic acid and carnosol, rosmanol and ferruginol are responsible for the antioxidant activity of sage (*Salvia sp*.) and rosemary

(*Rosmarinus officinalis*) along with the phenolic rosmarinic and salvianolic acids, but the organ distribution differs between the two mentioned chemical classes. However, the published tissue culture studies in *Lamiaceae* aimed at enhancing the rosmarinic acid production rather than at abietane diterpenoids. The tissue cultures as a source of abietane diterpenes have been established for *R. officinalis* , *S. officinalis* and *Salviasclarea*[34]. Light plays an important role in stimulating the diterpene biosynthesis in vitro even though in natural conditions they can only be found in the underground parts of plants.Volatile terpenoids responsible for fragrant properties of plant organs serve as a multipurpose chemical defense weapon against fungal and bacterial pathogens as well as a means of long distance chemical communication e.g. attracting pollinators.The antioxidant properties of several monoterpenes, such as γ-terpinene and limonene have been reported. This suggests that this group of compounds may receive more attention although at present they are underestimated as antioxidants.

Xantophyll:

Plants that have Xanthophylls are *Marsdenia glabra* (of Family *Asclepiadaceae*), *Mentha arvensis* (of Family *Labiatae*), *Ocimum americanum* (of Family *Labiatae*), *Ocimum basilicum*, *O. gratissimum*, *Orthosiphon grandiflorus* (of Family *Labiatae*), *Oenanthe Stolonifera*, *Centella asiatica* (of Family *Umbelliferae*) *Mentha arvensis* contained the high amount of total xanthophylls among these plants.all of this plants culture in Thailand as edible plants and they have had traditionaly used in Thailand for e.g used from leaves of *Mentha arvensis* for treated of antipyretic, Relieve headache and from leaves of *Marsdenia glabra* for Digestive tonic, restorative, antipyretic and from *Ocimum americanum* for Carminative, relieve cold and from *O. Gratissimum* for Expectorant, carminative, relieve stomach ache and flatulence and *Orthosiphon grandiflorus* for Uretic and from *Oenanthe stolonifera* for Digestive tonic, expectorant, relieve flatulence, and from *Centella asiatica* for Poultice for wound, scar or ulcer [35].

3. Other structures:

Tocopherols(vit E),Betalanins,Kinobeon A,Vitamin B6,Ascorbate,Reduced glutathione,Superoxide dismutase(SOD),Catalase,Selenium,Manganese

Tocopherol(Vit E):

Tocopherols, widely used in human nutrition as vitamin E and in food conservation, are powerful free radical scavengers and protect plant cells against oxidative damage in a lipophilic environment [36].Vitamin E protects lipids from the cell membrane bilayer from attack by free radicals. Plants are the primary source of natural tocopherols isolated from vegetable oils or maize embryos.Their biosynthesis integrates the aromatic aminoacids (shikimic acid) and plastidic isoprenoid deoxyxylulose phosphate (pentose) pathways. The first pathways create the aromatic (phenolic) head while the second gives rise to the hydrophobic tail of a tocochromanol skeleton [37]. Further, tocopherols were also known to protect lipids and other membrane components by physically quenching and chemically reacting with O2 in chloroplasts, thus protecting the structure and function of PSII. α-Tocopherol is the most active form of vitamin E, while the extraction from plant oils usually yields a mixture of β, γ, δ and α-tocopherols and tocotrienols. α-Tocopherols (vitamin E) are lipophilic antioxidants synthesized by all plants[38]. α-Tocopherol, found in green parts of plants, scavenges lipid peroxy radicals through the concerted action of other antioxidants. α-Tocopherols interact with the polyunsaturated acyl groups of lipids, stabilize membranes, and scavenge and quench various reactive oxygen species (ROS) and lipid soluble by products of oxidative stress. Singlet oxygen quenching by tocopherols is highly efficient, and it is estimated that a single α-tocopherol molecule can neutralize up to 120 singlet oxygen molecules in vitro before being degraded. Because of their chromanol ring structure, tocopherols are capable of donating a single electron to form the resonance stabilized tocopheroxyl radical. α-tocopherols scavenge lipid peroxy radicals and yield a tocopheroxyl radical that can be recycled back to the corresponding α-tocopherol by reacting with ascorbate or other antioxidants. α-Tocopherols scavenge and quench various ROS and Lipid oxidation products, stabilize membranes, and modulate signal transduction. Antioxidants including α-tocopherol and ascorbic acid have been reported to increase following triazole treatment in tomato, and these may have a role in protecting membranes from oxidative damage, thus contributing to chilling tolerance . Triazole increases the levels of antioxidants and antioxidant enzymes in wheat.Therefore there is a demand for alternative sources of pure α-tocopherol from plant tissues. Various culture systems of *Helianthus annuus* (sunflower) have been established for this purpose. *Gymnema inodorum*, also contained the highest amount of vitamin E this plant used in Thailand as Antihyperglycemia (the part of use are their leaves).An attempt has been also made to select the most efficient genotype of hazel (*Corylus avellana*) for the production of tocopherols in bioreactors [39].

Betalain:

The betalains are one of the most important natural colorants and are also one of the earliest natural colorants developed for use in food systems. Betalains are water-soluble nitrogen-containing pigments, which are synthesised from the amino acid tyrosine into two structural groups: the red-violet betacyanins and the yellow-orange betaxanthins. Betalamic acid, whose structure is presented in Fig.5a, is the chromophore common to all betalain pigments. The nature of the betalamic acid addition residue determines the pigment classification as betacyanin or betaxanthin.Betalains are food colourant and Colour is one of the most important attributes of foods but many naturally coloured foods, such as fruit products, are submitted to colour losses during processing, requiring the use of colourants to restore their colour. Nature produces a variety of compounds adequate for food colouring, such as the water-soluble anthocyanins, betalains, and carminic acid, as well as the oil soluble carotenoids and chlorophylls [40]. This pigments functionally replace anthocyanins in 13 taxons grouped in *Caryophyllales* (previously Centrospermae) order, such as the families: *Chenopodiaceae, Amaranthaceae, Portulacaceae, Cactaceae, Phytolaccaceae* and others. These nitrogen containing compounds although widely used as non-toxic food colorants, remain understudied in terms of their antioxidant potential [41].

The major advantages of betalains as dietary antioxidants are their bioavailability, which is greater than most flavonoids, and their superior stability in comparison to anthocyanins .Red beetroot (*Beta vulgaris*) is a rich natural source of betalains (mainly betanin) and is also an object of metabolic in vitro studies on their biosynthesis.

The production of beetroot pigments has been performed using a variety of techniques such as cell suspensions and transformed roots in bioreactors and show beet roots possessed high antiradical effect and antioxidant activity, representing a new class of dietary cationized antioxidants.Red beet betalains from hairy roots have been shown to be efficient free radical scavengers.

Indole glucosinolates are a tryptophane containing group from the diverse class of thioglycoside compounds typical to the *Brassicaceae*.In the family of *Amaranthaceae* (except *Amaranthus tricolor*) there are a great number of different betalains in this family there are red-violet betacyanins and yellow betaxanthins.The few edible known sources of betalains are red and yellow beetroot(Beta vulgaris L. ssp. vulgaris), coloured Swiss chard (*Beta vulgaris* L. ssp. Cicla), grain or leafy amaranth (*Amaranthus sp*.) and cactus fruits, such as those of Opuntia and Hylocereus genera [42]. Fruit pulps of Hylocereus cacti contain high concentrations of betacyanins (0.23–0.39%), both nonacylated and acylated, and (in contrast with beetroots) contain no detectable betaxanthins. On the other hand, Opuntia fruits cover a broad colouring range, from bright yellow to red-violet, depending both on the betacyanin⁄betaxathin ratio and their absolute concentrations[43].Stintzing *etal*.,(2005)reported broad ranges of betacyanin(0.001–0.059%)and betaxanthin(0.003–0.055%)contents in different Opuntia clones[40]. Preliminary data revealed that there were marked differences in antioxidant activity among several betalains (betanin, amaranthine, and vulgaxanthin I and II).

Antioxidant activity of the tested betalains decreased in the following order:
simple gomphrenins > acylated gomphrenins > dopaminebetaxanthin > (S)tryptophan-betaxanthin > 3-methoxytyramine-betaxanthin > betanin/isobetanin
> celosianins > iresinins > amaranthine/isoamaranthine [44].

Kinobeon A:
A red-colored compound isolated from safflower (*Carthamus tinctorius)* cell cultures uniquely combines the quinoid aromatic character with several conjugated double bonds [45] and has not been found in natural safflowers, other plants, animals or microorganisms we can found it under specific conditions. Safflower is a valuable plant used as an edible fat, as a Chinese medicine, in cosmetics, and in foodstuffs as a colorant. It has therefore been demonstrated as being a remarkably strong antioxidant, Kinobeon A efficiently quenches various reactive oxygen species, inhibits lipid peroxidation and supports the survival of mammalian cells during oxidative stress.

Vitamin B6:
Vitamin B6, the collective name given to pyridoxine, pyridoxamine, pyridoxal and their phosphorylated derivatives, is an essential cofactor for numerous enzymatic reactions. It is most notable for its contribution to amino acid biosynthesis where it serves as a cofactor for enzymes involved in decarboxylation, transamination, deamination, racemization and trans-sulfuration reactions. Therefore Vitamin B6, an essential cofactor in enzymatic reactions, has only recently been linked to cellular oxidative stress. The vitamin B6 pathway is poorly characterized in plants.Vitamin B6 biosynthesis has been thoroughly characterized in Escherichia coli and involves a de novo pathway that produces pyridoxine 50-phosphate as well as a salvage pathway that interconverts between the different vitamers.Recently documented that plants, fungi, archaebacteria, and most eubacteria use a distinct de novo biosynthetic pathwayinvolving two genes, PDX1and PDX2, that have no homology to the E. coli de novo biosynthetic genes pdxA and pdxJ.All three vitamers showed significant quenching abilities at the concentrations tested. Pyridoxal showed the strongest quenching activity.

Ascorbate: (vitamin C)
Ascorbate is one of the most extensively studied antioxidants and has been detected in

the majority of plant cell types, organelles and apoplast. Ascorbate is synthesized in the mitochondria and is transported to the other cell components through a proton-electrochemical gradient or through facilitated diffusion. Further, ascorbic acid also has been implicated in regulation of cell elongation.Two functions of vitamin C are: Quenches O2 in cytosol & Recycles vitamin E after it captures free radicals.

In the ascorbate-glutathione cycle, two molecules of ascorbic acid are utilized by APX to reduce H_2O_2 to water with concomitant generation of monodehydroascorbate. Monodehydro-ascorbate is a radical with a short life time and can disproportionate into dehydroascorbate and ascorbic acid. The electron donor is usually NADPH and catalyzed by monodehydroascorbate reductase or ferredoxin in water–water cycle in the chloroplasts. In plant cells, the most important reducing substrate for H_2O_2 removal is ascorbic acid. A direct protective role for ascorbic acid has also been demonstrated in rice. The partial protection against damage from flooding conditions in rice was provided by the prior addition of ascorbic acid. Ascorbic acid (AA) is an important antioxidant, which reacts not only with H_2O_2, but also with O_2, OH and lipid hydroperoxidases. AA is water soluble and also has an additional role in protecting or regenerating oxidized carotenoids or tocopherols [46]. Ascorbate (vitamin C) occurs in all plant tissues, usually being higher in photosynthetic cells and meristems (and some fruits)[38].

In a search beatwean edible plants of Thailand, some plants have high level of Vit C: *Macropanax dispermus* (Family *Araliaceae*), *Dregea volubilis* (Family *Asclepiadaceae*), *Gymnema inodorum* (Family *Asclepiadaceae*), *Marsdenia glabra* (Family *Asclepiadaceae*), *Telosma minor* (Family *Asclepiadaceae*), *Cucurbita moschata* (Family *Cucurbitaceae*), *Acacia pennata* (Family *Leguminosae*), *Caesalpinia mimosoides* (Family *Leguminosae), Cassia siamea* (Family *Leguminosae*), *Leucaena leucocephala* (Family *Leguminosae*). These plants have traditionaly used: *Macropanax dispermus* used for treatment of Digestive tonic, *Dregea volubilis* used), *Gymnema inodorum* used for Antihyperglycemia, *Marsdenia glabra* used for Digestive tonic, restorative, antipyretic, *Telosma minor* used for Restorative, detoxicant, *Cucurbita moschata* used for Carminative, expectorant, *Acacia pennata* used for Carminative, relieve flatulence, *Caesalpinia mimosoides* used for Digestive tonic, *Cassia siamea* used for Laxative, haemagogic, uretic and *Leucaena leucocephala* used for Relieve diarrhea[35].

Phenols and vitaminC determining as only the hydrophilic antioxidant components.

Reduced glutathione:

Glutathione is a tripeptide (α-glutamyl cysteinylglycine), which has been detected virtually in all cell compartments such as cytosol, chloroplasts, endoplasmic reticulum, vacuoles, and mitochondria.This reactivity along with the relative stability and high water solubility of GSH makes it an ideal biochemical to protect plants against stress including oxidative stress,heavy metals and certain exogenous and endogenous organic chemicals.Glutathione takes part in the control of H_2O_2 levels. The change in the ratio of its reduced (GSH) to oxidized (GSSG) form during the degradation of H_2O_2 is important in certain redox signaling pathways. It has been suggested that the GSH/GSSG ratio, indicative of the cellular redox balance, may be involved in ROS perception. Reduced glutathione (GSH) acts as an antioxidant and is involved directly in the reduction of most active oxygen radicals generated due to stress[38].

Catalase (CAT):

Among the antioxidant enzymes, catalases (Hydrogen peroxide oxidoreductase) are ubiquitous heme enzymes that are found in aerobic organisms, ranging from bacteria to higher plants and animals. Functionally, catalases are related to peroxidases; both promote H_2O_2 oxidation by mechanisms that involve ferryl intermediates . However, catalases differ from peroxidases, with the exception of chloroperoxidase and myeloperoxidase, in that they have the ability to utilize H_2O_2 as both an electron acceptor and donor that yields a disproportionate reaction. Due to this catalytic activity, the catalases are believed to be involved in the protective destruction of H_2O_2 that is generated in respiring cells. This enzyme catalyzes the decomposition of H_2O_2 to O_2 and H_2O and thus provides protection against the toxic effects of the oxygen radical.a crude extract of *Garnoderma lucidum,*a medicinally potent mushroom, profoundly increased the catalase gene expression and enzyme activities in mouse livers . These results suggest that methyllinoleate that is produced by *G.lucidum* stimulates the catalase expression at the transcription level [47].

Superoxide dismutase (SOD):

The determinant role of superoxide dismutase (SOD) in the antioxidant defense systems has been known since 1968. The powerful natural antioxidant enzyme superoxide dismutase (SOD) acts at the very source of the chain reaction resulting in reactive types of oxygen and therefore constitutes the first and one of the main links of the defense process against free radicals. It is well known that superoxide ion (O_2^-) is the starting point in the chain production of free radicals. At this early stage, superoxide dismutase inactivates the superoxide ion by transforming it into hydrogen peroxide (H_2O_2). The latter is then quickly catabolised by catalase and peroxidases into dioxygen (O_2) and water (H_2O).

SOD therefore seems to be the key enzyme in the natural defense against free radicals. [1]

Manganese:
Manganese is an element of great importance in the life cycle of plants and animals. For example, it plays an essential role as an activator of various enzymatic systems such as isoenzymes of superoxide dismutase [48]. Also manganese is an activator of several manganes metalloenzymes, including arginase, pyruvate carboxylase, glutamine synthetase and one form of superoxide dismutase (SOD). Therefor it is necessary for normal antioxidant defenses. In animals, manganese protects heart mitochondrial lipids against peroxidation. Also manganes is often considered to be one of the least toxic of trace element when consumed and also in animals, excess of manganese may inhibit iron absorption and result in iron-deficiency anemia.

Selenium:
The effects of a selenium-supplemented diet on the antioxidant enzyme activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) in many animals. Selenium is an essential constituent of antioxidant enzymes especially glutathione peroxidase and some other selenoproteins that participate in various physiological activities and protects the cell against the deleterious effects of free radicals. Current evidence indicates that selenium is involved in a wide array of physiological functions and pathological conditions which include normal thyroid functioning, enhancing immune function, arcinogenesis, cardiovascular diseases, male reproduction and in the prevention of pre-eclampsia etc. Selenium (Se), an essential trace element, has evolved from its toxic properties after a series of researches over the past several decades. Deficiency of selenium produced experimentally in animals resulted in abnormalities such as defective growth,Hepatic necrosis, myocardial degeneration and muscular dystrophy in sheep, cattle, chickens and horses. In humans, it is well recognized that selenium plays a crucial role in various physiological processes and its altered level has a direct impact on health leading to the development of disease.Selenium, an essential component of the antioxidant enzyme GSH-Px, functions as an antioxidant scavenging H_2O_2 and by reducing lipid hydro peroxides to their subsequent less reactive end products.

Although previous studies have reported about the toxic manifestations of excess selenium intake and existence of selenium deficiency in some pathophysiological conditions, the emerging evidence reflects the importance of selenium as an effective medical therapy in a wide array of conditions in association with other antioxidants such as vitamin E. Plant foods are the major sources of selenium in most countries throughout the world. The amount of selenium present in the plant material depends upon the concentration of selenium in the soil of that region as it varies by region. Low amount of selenium and dietary selenium deficiency has been reported in selenium deficient regions of China and Russia. Animals that eat grains or plants that were grown in selenium rich soil have higher levels of selenium in their muscle.

Methods used for assessment of antioxidant properties:
In most cases the antioxidant power of many secondary metabolites is so well established, that real time monitoring of activity during the culture seems to be redundant. On the other hand, the enormous variability among antioxidants, and their complex structure–activity relationships suggest that antioxidant and any other biological activities should be paid more attention during research. There are a number of simple, slightly more complex, and quite sophisticated methods for antioxidant testing [27].

The antioxidant testing can reveal various mechanisms of action, depending on features of the particular assay. Simple methods include free radical scavenging with use of colored, artificial stable free radicals such as 2,2 & prime;-azinobis-(3-ethylbenzothiazoline- 6-sulfonate) (ABTS used in the TEAC assay — Trolox equivalent antioxidant capacity) and DPPH & z.rad; (1,1-diphenyl-2-picrylhydrazyl free radical) [49], as well as transition metal reduction that can be monitored by colorimetry. The metal-based methods include the reduction of ferric ions: FRAP- (ferric reducing ability of plasma) and ferric thiocyanate assays, or molybdenum ion, phosphomolybdenum (P-Mo) assay .

Several assays based on a substrate degradation inhibition are available which can be used to find out whether the tested compound can really protect biomolecules from oxidative damage. Polyunsaturated lipids, proteins, components of nucleic acids, cellular membranes, microsomal fractions from different organs can serve as model systems to be protected.

The degradation products are monitored by spectrophotometry (thiobarbituric reactive substances — TBARS, carotene bleaching), fluorescence (ORAC assay — for oxygen radical absorption capacity) or chromatography [27].

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