E-ISSN: 2583-9667
Indexed Journal
Peer Reviewed Journal
https://multiresearchjournal.theviews.in

Received: 01-06-2023
Accepted: 10-07-2023

INTERNATIONAL JOURNAL OF ADVANCE RESEARCH IN MULTIDISCIPLINARY

Volume 1; Issue 1; 2023; Page No. 914-917

Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications

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DOI: https://doi.org/10.5281/zenodo.14823339

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Abstract

Oxidative stress has been identified as the root cause of the development and progression of several diseases. Supplementation of exogenous antioxidants or boosting endogenous antioxidant defenses of the body is a promising way of combating the undesirable effects of reactive oxygen species (ROS) induced oxidative damage. Plants have an innate ability to biosynthesize a wide range of non-enzymatic antioxidants capable of attenuating ROS- induced oxidative damage. Several *in vitro* methods have been used to screen plants for their antioxidant potential, and in most of these assays they revealed potent antioxidant activity. However, prior to confirming their *in vivo* therapeutic efficacy, plant antioxidants have to pass through several physio pharmacological processes. Consequently, the findings of *in vitro* and *in vivo* antioxidant potential assessment studies are not always the same. Nevertheless, the results of *in vitro* assays have been irrelevantly extrapolated to the therapeutic application of plant antioxidants without undertaking sufficient *in vivo* studies. Therefore, we have briefly reviewed the physiology and redox biology of both plants and humans to improve our understanding of plant antioxidants as therapeutic entities. The applications and limitations of antioxidant activity measurement assays were also highlighted to identify the precise path to be followed for future research in the area of plant antioxidants.

Keywords: Antioxidant activity, pharmacology, plants, prooxidants, secondary metabolites

Introduction

Antioxidants significantly delay or prevent oxidation of oxidizable substrates when present at lower concentrations than the substrate 1. Antioxidants can be synthesized *in vivo* (e.g., reduced glutathione (GSH), superoxide dismutase (SOD), etc.) or taken as dietary antioxidants 1,2. Plants have long been a source of exogenous (i.e., dietary) antioxidants. It is believed that two-thirds of the world's plant species have medicinal importance, and almost all of these have excellent antioxidant potential 3. The interest in the exogenous plant antioxidants was first evoked by the discovery and subsequent isolation of ascorbic acid from plants 4. Since then, the antioxidant potential of plants has received a great deal of attention because increased oxidative stress has been identified as a major causative factor in the development and progression of several life threatening diseases, including neurodegenerative and cardiovascular disease. In addition, supplementation with exogenous antioxidants or boosting of endogenous antioxidant defenses of the body has been found to be a promising method of countering the undesirable effects of oxidative stress 5.

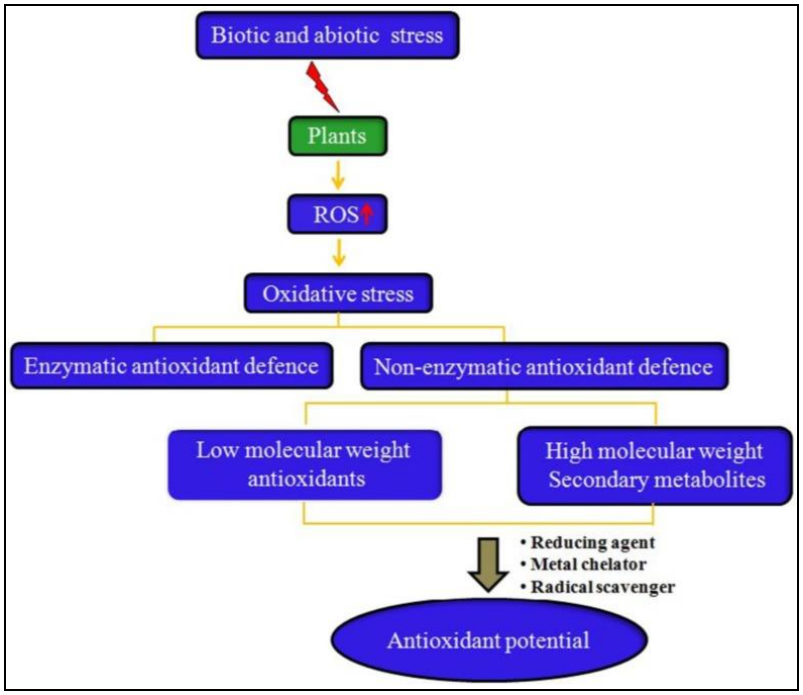
There are currently approximately 19 *in vitro* and 10 *in vivo* methods of assessing antioxidant activity that are commonly applied for evaluation of the antioxidant activity of plant samples 6. In most of these *in vitro* assays plant samples showed potent antioxidant activity. This is likely due to their innate ability to synthesize non-enzymatic antioxidants such as ascorbic acid and glutathione, as well as secondary metabolites such as phenolic compounds.

Despite many plants being reported to have antioxidant potential by *in vitro* assays, only a few of these antioxidant activities have been confirmed or investigated *in vivo* 7. *In vitro* assays are generally used to confirm the antioxidant activity of plant samples within particular reaction systems; accordingly, the relevance of the findings of these assays to *in vivo* systems is uncertain 8. Moreover, several phytochemicals have been found to possess antioxidant activity within *in vitro* assays. However, only a few of these have been shown to be therapeutically useful under *in vivo*

conditions due to their interference with physio pharmacological processes such as absorption, distribution, metabolism, storage and excretion. Nevertheless, phytochemicals are being screened for their *in vitro* antioxidant activity, and the results of these studies are then directly extrapolated to their therapeutic usefulness. This malpractice may raise fundamental questions about the significance of plants as exogenous sources of antioxidants and their therapeutic efficacies. Accordingly, in the present article, we briefly reviewed the physiology and redox biology of both plants and humans. In addition, the applications and limitations of antioxidant activity measurement assays are discussed 6,7. The information provided herein will enable correct interpretation of the findings of plant antioxidant potential assessment studies based on both *in vitro* and *in vivo* assays.

Why do all plants have antioxidant potential?

Chloroplasts and mitochondria are the two main powerhouses and sites of reactive oxygen species (ROS) generation within plant cells. These materials are also involved in maintenance of a fine balance between energy linked functions and control of ROS production. Peroxisomes, single membrane-bound subcellular organelles, are a third important site of production of ROS such as hydrogen peroxide (H2O2), superoxide (O2● -) and nitric oxide (NO●) within plant cells. Peroxisomes contain basic enzymatic constituents such as catalase (CAT), as well as hydrogen peroxide (H2O2)-producing flavin oxidases 9. Within the plant cell, ROS generation occurs at photosystem I and II (PS I and PS II) of the chloroplasts, membrane and matrix of the peroxisome, and complex I, ubiquinone and complex III of the mitochondrial electron transport chain (ETC) 10. Under normal physiological conditions, there is electron slippage from PS I and PS II of the chloroplasts, membrane of mitochondrial ETC and peroxisome. These electrons later react with molecular oxygen to produce superoxide radical (O2-●). The superoxide radical is subsequently converted to hydroperoxyl radical (HO2●) and finally to H2O2 11-13. Similar to ROS, reactive nitrogen species (RNS) such as the nitric oxide radical (NO•) and peroxinitrite (ONOO-) are also formed in various compartments of the cell including the chloroplasts, mitochondria and peroxisomes 14. The third type of free radical, reactive sulfur species (RSS), are reportedly formed from thiols by reaction with ROS 15. The overall process of free radicals generation is summarized in Fig. 1. These free radicals are constantly produced in the subcellular organelles of living cells. Most of the time, the production of free radicals is genetically planned, since they function as signaling molecules 12,16. However, overproduction of free radicals can also sometimes damage biomolecules such as DNA, proteins and lipids.



There may be two main reasons for the synthesis and accumulation of these non-enzymatic antioxidants by plants. First, the genetic make-up of plants imparts them with an innate ability to synthesize a wide variety of phytochemicals to perform their normal physiological functions and/or protect themselves from microbial pathogens and animal herbivores. Another reason for the synthesis of reductant phytochemicals could be the natural tendency of plants to respond to environmental stress conditions.

Plants synthesize low molecular weight antioxidants such as glutathione and ascorbate within the chloroplast stroma and cytosol using NADPH as the ultimate electron donor 11. These low molecular weight antioxidants function as redox buffers that interact with numerous cellular components and influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death 17. In addition, these antioxidants may influence gene expression associated with biotic and abiotic stress responses to maximize defense. Vitamin C (ascorbic acid/ascorbate) is generated during aerobic metabolism, after which it reacts rapidly with O2-●, singlet oxygen and ozone (chemically), and H2O2 (enzymatically) through

ascorbate peroxidase to neutralize their toxic effects. Vitamin C also helps regenerate antioxidant pigments, carotenoids (carotenes and xanthophylls), and vitamin E. Glutathione is a redoxactive molecule that can be present in a reduced form (GSH) or an oxidized disulfide form (GSSG) and plays important roles in biosynthetic pathways, detoxification, antioxidant biochemistry and redox homeostasis 18,19. GSSG is reduced to GSH by the enzyme glutathione reductase, which requires NADPH as the reducing power. GSH acts as an anti-oxidant by quenching reactive oxygen species and is involved in the ascorbate-glutathione cycle, which eliminates damaging peroxides 20. Plants also produce tocopherols (vitamin E) that act as important liposoluble redox buffer systems. Vitamin E, which is generally synthesized in chloroplasts and protoplastids, is located in the membranes of cells. This compound is a major singlet oxygen scavenger that provides protection against lipid peroxidation 17,21.

Plants also synthesize and accumulate a range of low and high molecular weight secondary metabolites that play important roles in ROS metabolism and avoidance of uncontrolled oxidation of essential biomolecules. These metabolites are also important to adaptation of plants to environmental fluctuations 22. Secondary metabolites provide passive and active resistance. In passive resistance, metabolites are continuously available, despite the presence of stressors, whereas in active resistance, metabolites are produced in response to specific stressors 23. These metabolites are synthesized through basic pathways, such as the glycolysis or shikimic acid pathways, which further branch out based on cell type, developmental stage and environmental cues. Secondary metabolites are generally derived from primary metabolites such as amino acids and carbohydrates via methylation, hydroxylation and glycosylation 24.

Higher plants survive in constantly fluctuating environments, due to their highly regulated and flexible metabolism 25. Under normal physiological conditions, the increase in free radical production is relatively small and housekeeping antioxidant capacity is sufficient to maintain redox homeostasis 26. The metabolic pathways of plants are sensitive to abiotic and biotic stress conditions such as high light intensity, heat, drought, anoxic conditions and pathogen attack, and it has been reported that there is an approximately 3 to 10 fold increase in free radicals production under stress conditions 14,25,27.

The ratio of GSH to GSSG has been shown to decrease due to the oxidation of reduced glutathione during detoxification of reactive oxygen species (ROS) in response to abiotic stresses 28. Moreover, plants increase the activity of GSH biosynthetic enzymes and glutathione levels in response to both abiotic and biotic stresses 29. Similar to glutathione, biosynthesis and recycling of ascorbic acid has been found to increase in response to various abiotic stresses within mutant and transgenic plant species 30,31. Vitamin E deficiency has also been shown to retard growth and change responses to abiotic stress conditions. In addition, increased vitamin E content has been shown to diminish detrimental effects of environmental stress in plants.

Some secondary antioxidant metabolites occur constitutively, while others are formed in response to biotic and abiotic stress conditions 33, 34. The accumulation of phenolic compounds along with enhancement of phenylopropanoid metabolism has been observed under different environmental stress conditions 35. In plants, phenolics can act as antioxidants by donating electrons to guaiacol-type peroxidases for the detoxification of H2O2 produced under stress conditions 36. Phenolics also provide protection against UV radiation through their potent radical scavenging ability. In addition, they function as enzyme inhibitors and feeding deterrents for herbivores while providing resistance against pathogens 37. Synthesis of flavonoids is known to be induced by UV stress, heavy metals toxicity, or low temperature and low nutrient conditions, which might attributed to their UV-absorbing, radical scavenging and metal cheating ability 35, 38,39. UV-B radiation was found to affect the production of various high molecular secondary metabolites such as tannins and lignin 40. Moreover, plants growing in tropical and high-altitude conditions have been shown to contain a higher proportion of flavonoids than those growing in temperate conditions owing to overexposure to light or UV radiation 41. Biotic stress like wounding has been found to induce phenolic metabolism such as increased synthesis of phenolic compounds 42. Tannins are reportedly useful for plant leaf defense against insect herbivores 43. Similar to phenolics, an increase in total indole alkaloid content in the shoots and roots of Catharanthus roseus has been observed under drought-induced stress 44. Alkaloids generally provide protection to plants against microbial or herbivore attack and UV-radiation 45,46. It has also been reported that monoterpenes and isoprenes are emitted at higher rates under high temperature 47.

Strategy for plant antioxidant potential measurement

In vitro antioxidant potential assessment methods do not provide exact therapeutic implications of plant antioxidants. Moreover, the antioxidant potential of plants or their phytochemicals is influenced by several factors under *in vivo* conditions, including gut absorption, metabolism, bioavailability, and presence of co-antioxidants and transition metal ions. Consequently the results of *in vivo* antioxidant assessment studies of plant antioxidants are not consistent 62. Hence, there is a need to develop an expansive study strategy that will include a set of *in vitro* and *in vivo* experiments to provide more accurate therapeutic values to plant antioxidants.

One commonly suggested strategy is that both *in vitro* and *in vivo* antioxidant assessment studies be conducted simultaneously to confer therapeutic antioxidant potential to plants or their components. Holst and Williamson 99 proposed that *in vitro* plant antioxidant assessment studies be driven by *in vivo* results, and not vice versa. They further suggested that once a phytochemical is shown to exert an effect *in vivo*, their mechanisms can be tested *in vitro* to avoid disappointments when testing *in vitro* concepts *in vivo*. It is believed that the proposed antioxidant activity assessment studies would be more suitable for investigation of antioxidant activity of flavonoids and lignans, as these phytochemicals are generally metabolized to low molecular antioxidants in the body. Most ingested flavonoids have been shown to be extensively degraded to various phenolic acids, which could have radical scavenging ability 100. Similarly, the lignan secoisolariciresinol diglucoside is

metabolized to more powerful antioxidants such as secoisolariciresinol, enterodiol and enterolactone within the body 101.

Conclusions

Over the past few decades, significant scientific information has been accumulated regarding plant redox biology and its antioxidant defense. However, this information has not been collectively discussed together with human redox biology and exogenous antioxidants metabolism, which is essential in understanding the therapeutic utility of plant antioxidants. Because of their high oxygen exposure physiology, plants may have more sites of ROS generation; therefore, they could evolve more efficient non-enzymatic antioxidant systems than humans. Plants synthesize and accumulate several non-enzymatic antioxidants such as ascorbic acid, glutathione and phenolics. Some of these antioxidants occur constitutively, while others are formed in response to abiotic and biotic stress conditions. Almost all plants or their phytochemicals exhibit some antioxidant activity under *in vitro* assays conditions. However, for *in vivo* studies, plant antioxidants have to pass through several physio pharmacological processes including absorption, distribution, metabolism, storage and excretion. Consequently, the antioxidant potential of plants or their phytochemicals is influenced by several factors *in vivo*, including gut absorption, metabolism, bioavailability, and the presence or absence of co-antioxidants and transition metal ions. Therefore, the results of the *in vitro* antioxidant potential assessment studies are often contradictory to those of *in vivo* studies. Nevertheless, without undertaking sufficient *in vivo* studies, the results of *in vitro* assays have been irrelevantly linked to the therapeutic applications of plant antioxidants. Hence, we proposed disease pathophysiology targeting combined *in vitro* and *in vivo* antioxidant activity to attribute more precise therapeutic value to individual or combined plant antioxidant entities.

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