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Study on natural antioxidants: sources, mechanisms of action and health benefits

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Abstract. The role of natural antioxidants in protecting the body from free radicals is well known, and the importance of these compounds in maintaining health and preventing disease is essential. The health benefits of these compounds are numerous, the most important of which are protecting cells by neutralizing free radicals and decreasing oxidative stress at the cellular level, improving the immune system, preventing chronic diseases and slowing down the ageing process. The paper presents aspects related to the mechanism of action of natural antioxidants, their sources, antioxidant capacity and the role of natural antioxidants in the prevention of certain diseases.
Keywords: *antioxidants, natural sources, mechanism of action, benefits*

1. Introduction

The natural antioxidants are compounds that help protect the body against oxidative stress and free radicals, which can damage cells and contribute to chronic diseases. The mechanism of action of natural antioxidants is based on the neutralization of free radicals by donating an electron, a process which helps to reduce oxidative stress in the body and thereby prevent chronic diseases such as cardiovascular disease, cancer, diabetes and/or neurodegenerative diseases. At the same time, the important role that natural antioxidants play in improving the immune system, maintaining cellular integrity and increasing resistance to disease by protecting immune cells from free radicals should also be emphasized [1].

Natural antioxidants are present in many plant foods, including fruits, vegetables, nuts, seeds and teas. Vitamin C found in citrus fruits, kiwi fruit, strawberries, broccoli and bell peppers are essential for the immune system and collagen synthesis. Vitamin E in nuts, seeds, vegetable oils and spinach protect cell membranes from oxidation. Beta-carotene from carrots, lycopene from tomatoes and lutein from spinach are other examples of natural antioxidants that help protect eyes and skin. Polyphenols are also an important category of natural antioxidants, with resveratrol from grapes and red wine, catechins from green tea and flavonoids from dark chocolate known to have anti-inflammatory and anti-cancer properties. Another example is selenium, an antioxidant mineral found in brazil nuts, fish and meat, which contributes to the normal functioning of the immune system [2].

Consuming these foods is associated with multiple health benefits. Their effects on the body are due to their synergistic action and bioavailability, which means that their beneficial effects are enhanced

when consumed together as part of a balanced diet. Natural antioxidants are safer and more tolerable than synthetic antioxidants. Studies show that the positive effects of a diet rich in natural antioxidants are more pronounced than those of synthetic supplements. Thus, it should be noted that excessive consumption of synthetic antioxidants can lead to toxicity and adverse effects. For example, excessive supplementation with synthetic vitamin E has been associated with an increased risk of bleeding. In contrast, natural antioxidants, consumed in the context of a balanced diet, have a minimal risk of side effects, are generally better tolerated and safer than synthetic supplements, reducing the risk of toxicity and side effects. They are an integral part of food and contribute to balanced nutrition and overall health, whereas synthetic antioxidants are often consumed in supplement form, which can lead to unbalanced intake. One of the benefits of synthetic antioxidants would be that they can be formulated at precise concentrations, allowing accurate dosing, which can be beneficial in specific clinical situations or for treating deficiencies. A diet rich in natural antioxidants promotes balanced nutrition and can help to maintain a healthy body weight, reducing the risk of obesity and related diseases. Regular consumption of antioxidant-rich foods is essential for protecting the body against oxidative damage and promoting a healthy and balanced life, with natural antioxidants generally preferred over synthetic antioxidants due to their synergism, superior bio-availability and safety [3,4].

2. Antioxidants types and mechanisms of action

During certain processes essential for maintaining the functionality of vital systems, the body releases large amounts of free radicals. These include oxidation processes of nutrients, immune system response processes against various antigens, synthesis of hormones, prostaglandins and neurotransmitters. These are all examples of processes that involve the formation of free radicals which when generated in excessive amounts can have destructive effects at the cellular level. The environment can also trigger the formation of free radicals through various forms of air pollution, as a result of excessive radiation or toxic waste. A very common habit of a large number of people is smoking, which also promotes the formation and accumulation of free radicals in the body. Because they are formed in most tissues and organs, free radicals contribute, through the oxidative stress they create, both to the modulation of physiological processes and to the triggering of pathological conditions [5].

Antioxidants are substances that help to protect the body against oxidative stress by neutralizing reactive oxygen species (ROS) and reactive nitrogen species (RNS), by this mechanism they prevent cell damage and contribute to overall health. The mechanism of action of antioxidants involves several defense strategies, which can be divided into: neutralizing free radicals, reducing oxidative reactions and regeneration of other antioxidants.

Reactive oxygen species (ROS) play an essential role in the body's functioning, but an imbalance can lead to cell damage and the development of pathologies. Adequate management of ROS through antioxidant mechanisms is vital for maintaining health as they can have, depending on their concentration and the activity of antioxidant enzymes, both beneficial and harmful effects on the body. ROS are highly reactive oxygen-containing molecules with a dual role in cells, they are formed during normal cellular metabolism and can occur as by-products during biological processes such as mitochondrial respiration. Examples of reactive oxygen species include [6,7]:

- superoxide anion (O_2^-) - formed by adding an electron to molecular oxygen. This is one of the most common ROS.
- hydrogen peroxide (H_2O_2) - less reactive, but can generate other, more dangerous species through further chemical reactions.
- hydroxyl radical (•OH) is highly reactive and can cause significant cell damage.
- singlet oxygen ($^1O_2$) - a highly reactive oxidant of cell membranes and other structures.

The main sources of reactive oxygen species are normal cellular processes, typically ROS are produced during cellular respiration in mitochondria. They occur as by-products in the electron transport chain. In inflammatory processes, during the immune response, phagocytic cells generate ROS to destroy invading microorganisms. Among external factors, exposure to UV radiation, smoking, pollution and toxic chemicals (such as heavy metals) can lead to increased ROS production. The

literature emphasizes in several studies the dual character of reactive oxygen species. Their beneficial roles are in: cell signaling, ROS are involved in signal transduction and regulation of cellular processes such as proliferation and apoptosis (programmed cell death), and in the immune response, immune system cells such as neutrophils and macrophages use ROS to eliminate pathogens in the process of phagocytosis [7].

The damaging effects of ROS materialize in oxidative stress. An imbalance between the production of ROS and the body's ability to neutralize them (via antioxidants) leads to oxidative stress, which can cause damage to DNA, proteins and lipids. Another damaging effect is cell ageing knowing the fact that prolonged exposure to ROS can accelerate cell and tissue ageing, leading to degeneration. Also excessive ROS are associated with a range of diseases including cancer, cardiovascular disease, diabetes and neurodegenerative diseases (e.g. Alzheimer's, Parkinson's). [5-7].

Reactive nitrogen species (RNS) - also play an important role in biological processes, similar to reactive oxygen species (ROS). Although they may have important physiological functions, excess RNS can lead to nitrosative stress and cause cell damage, contributing to various diseases. Examples of reactive nitrogen species (RNS) [8]:

- nitric oxide (NO) - a free radical involved in cell signaling and regulation of vascular tone;
- nitrogen dioxide (NO_2) - a reactive species formed from nitric oxide and other reactive molecules; it is more toxic and more reactive than NO.
- peroxynitrite ($ONOO^-$) formed from the interaction between nitric oxide (NO) and the superoxide anion (O_2^-); it is highly reactive and can cause significant cell damage.
- nitrosothiols (RSNOs) - nitric oxide derivatives bound to the thiol (SH) groups of proteins.

The main sources of reactive nitrogen species that can be mentioned are:

- *endogenous nitric oxide synthesis*. Nitric oxide is produced by enzymes called nitric oxide synthases (NOS), which are found in endothelial cells, neuronal cells and the immune system (iNOS).
- *interaction with ROS.* Reactive nitrogen species can arise from the reaction between nitric oxide and ROS, forming more toxic compounds such as peroxynitrite.
- *external factors:* pollution, smoking, toxic chemicals and radiation can stimulate the production of RNS in the body.

As with ROS, the role of RNS in the body is twofold. Among the beneficial effects is cell signaling, so nitric oxide (NO) is a key molecule in cell-to-cell communication, playing an essential role in regulating blood flow, relaxing blood vessels (vasodilation) and transmitting neuronal signals. Another beneficial effect is the immune response, as nitric oxide is produced by immune cells, such as macrophages, and is used to destroy pathogens, including bacteria and viruses. The regulation of mitochondrial function is also one of the beneficial effects of RNS, as NO controls energy production in mitochondria by regulating the activity of the electron transport chain. The harmful effects of RNS become evident in the following situations [8,9]:

- *nitrosative stress* - as with oxidative stress, excess RNS can lead to damage to proteins, lipids and DNA. This type of stress can contribute to neurodegenerative, Parkinson's, Alzheimer's, cardiovascular and inflammatory diseases.
- *inactivation of enzymes*. Peroxynitrite ($ONOO^-$) it can inactivate essential enzymes by altering the thiol groups in their structure, which can disrupt metabolic processes.
- *nitrosamine formation*. Under certain conditions, reactive nitrogen species can lead to the formation of nitrosamines, carcinogenic compounds present in processed foods and in the body.

To control and neutralize ROS and RNS, the body uses various mechanisms including enzymatic antioxidants such as superoxide dismutase (SOD), catalase, glutathione peroxidase and non-enzymatic antioxidants such as vitamin C, vitamin E, glutathione, coenzyme Q10, protecting cells against oxidative and nitrosative stress.

The main mechanisms of action of antioxidants can be categorized as follows [9-11]:

Neutralizing free radicals. Free radicals are unstable molecules containing an unpaired electron. They tend to capture electrons from other molecules, causing oxidative damage. Antioxidants work by donating an electron to free radicals, stabilizing them without becoming dangerous radicals themselves.

This process prevents chain reactions that can damage lipids, proteins and DNA. Examples of antioxidants that neutralize free radicals which can be mentioned are vitamin C - donates electrons to free radicals in the extracellular environment, or vitamin E which protects cell membranes from lipid peroxidation by neutralizing free radicals in the lipid layer.

The prevention of free radical formation. Certain antioxidants inhibit the formation of reactive species, thus preventing oxidative processes before they start. For example, antioxidant enzymes remove substances that can generate ROS, of these can be mentioned:

- superoxide dismutase (SOD) catalyzes the conversion of the superoxide anion (O_2^-) into hydrogen peroxide (H_2O_2), which is less harmful.
- catalase breaks down hydrogen peroxide (H_2O_2) into water and oxygen, thus preventing the formation of the hydroxyl radical, one of the most dangerous reactive species.
- glutathione peroxidase reduces hydrogen peroxide and other organic hydroperoxides to water, protecting cell lipids and DNA from oxidation.

The regeneration of other antioxidants. Some antioxidant molecules have the ability to regenerate other antioxidants after they have been oxidized. This mechanism helps maintain a continuous protective balance against oxidative stress. Vitamin C can regenerate oxidized vitamin E, restoring it to its active form and ready to continue protecting lipids in cell membranes. Glutathione plays a crucial role in the regeneration of other intracellular antioxidants, including reducing peroxides and oxidized vitamins [10,11].

The inhibition of pro-oxidant metals. Transition metals, such as iron and copper, can catalyze reactions that lead to the formation of the hydroxyl radical via the Fenton or Haber-Weiss reaction. Certain chelating antioxidants (metal binders) can bind these metals and prevent their participation in pro-oxidative reactions. Ferritin and transferrin bind iron and prevent the formation of the hydroxyl radical by the Fenton reaction and ceruloplasmin binds copper, inhibiting copper-mediated oxidative reactions [11].

Repaying oxidative damage. In addition to preventing the formation of free radicals and neutralizing them, antioxidants also help repair oxidative damage that has already occurred. The body uses enzymes and mechanisms to repair damaged proteins, lipids and DNA, can be mentioned: DNA repair enzymes that repair oxidative DNA damage, preventing mutations respectively cancer and heat shock proteins (HSPs) that help restore proteins damaged by oxidative stress or remove them from the cell.

In the specialized literature, several types of antioxidants are specified that can be classified according to the mechanism of action [8-11]:

- preventive antioxidants that inhibit the formation of reactive species for examples: superoxide dismutase, catalase;
- radical-scavenging antioxidants that neutralize free radicals by donating electrons (vitamin C, vitamin E, carotenoids);
- repairing antioxidants that repair already damaged molecules: DNA repair enzymes, HSP proteins.
- chelation antioxidants. These bind transition metals, preventing oxidative reactions (ferritin, transferrin).

3. Natural sources of natural antioxidants - health benefits

Among the most important natural antioxidants with health benefits can be mentioned:

- vitamin C (ascorbic acid) - found in citrus fruits, strawberries, kiwi fruit, bell peppers, broccoli;
- vitamin E (tocopherol) - found in nuts, seeds, vegetable oils (olive oil, sunflower oil) and green leafy vegetables;
- carotenoids - are plant pigments that include beta-carotene (carrots, sweet potatoes, spinach), lutein and zeaxanthin (in greens like spinach and kale);
- polyphenols - plant compounds that have powerful antioxidant properties. Found in berries, dark chocolate, green tea, red wine, olives;
- selenium - an essential trace element found in brazil nuts, fish, poultry and eggs;
- lycopene - a powerful antioxidant found in tomatoes, watermelon, pink grapefruit;

- flavonoids - present in green tea, cocoa, red wine, onions, apple, berries.

The antioxidant content of fruits varies significantly depending on the type of fruit, variety, growing methods and ripeness. The main antioxidants in fruits are phenolic compounds, flavonoids, vitamins (especially vitamin C and vitamin E), carotenoids and anthocyanins. Table 1 lists the most antioxidant-rich fruits and the predominant types of antioxidants they contain [12]:

Table 1. Examples of fruits high in antioxidants - ORAC score [12]

Type of fruit	Key antioxidants	ORAC score (units/100 g)	Benefits
Blueberries	anthocyanins, vitamin C, flavonoids	4.669	- brain support, cardiovascular health - protection against premature ageing
Raspberries	anthocyanins, vitamin C, ellagic acid	5.065	- protection against inflammation - have anticarcinogenic potential due to their high ellagic acid content
Strawberries	vitamin C, anthocyanins, flavonoids	4.302	- boost skin health and immunity by helping neutralize free radicals
Blackberries	anthocyanins, vitamin C, ellagic acid	5.347	- may prevent cardiovascular disease - improve memory and cognitive function.
Pomegolds	punicalagins, anthocyanins, vitamin C	2.860	- protects against inflammation - may have beneficial effects in heart health and cancer prevention
Red grapes	resveratrol, flavonoids, vitamin C	1.837	- resveratrol helps protect the heart and may have anti-aging effects.
Plums	vitamin C, carotenoids, anthocyanins	6.259	-they support digestion and can help fight constipation
Cherries	Anthocyanins, vitamin C, quercetin	3.365	-may reduce inflammation and have beneficial effects in preventing muscle soreness after strenuous exercise
Goji	Carotenoids (beta-carotene, zeaxanthin), vitamin C	25.000	-have potential in improving vision and immune health due to their high zeaxanthin content
Oranges	Vitamin C, flavonoids, carotenoids	2.103	-supports immune system and skin health, helping to prevent oxidative stress

**ORAC score - Oxygen Radical Absorbance Capacity*

In addition to the fruits shown in Table 1, other foods rich in antioxidants are: vegetables such as tomatoes, spinach, carrots, broccoli, red peppers, dried fruits and nuts, almonds, brazil nuts, peanuts, beverages such as green tea, black tea, red wine. Including a variety of antioxidant-rich foods in your daily diet is a great way to support long-term health.

Although the ORAC score can be a useful measure of antioxidant capacity in vitro, it does not necessarily reflect how antioxidants act in the body. Some antioxidant substances may have a low bioavailability or interact differently in the real biological context, therefore the ORAC score can only be used as a relative indicator of antioxidant potential. Figure 1 shows the antioxidant capacity according to the ORAC scale of some high antioxidant fruits.

Antioxidant-rich foods that have high ORAC scores are considered beneficial for health because [9, 11-13]:

- *Combat oxidative stress*. Antioxidants neutralize free radicals, preventing oxidative damage at the cellular level.

- *Reduce inflammation*. Antioxidants can help reduce chronic inflammation, which is involved in many degenerative diseases.
- *Improve cardiovascular health.* Eating antioxidant-rich foods can support heart health, reducing the risk of atherosclerosis and other cardiovascular diseases.
- *Delays cell ageing*. Antioxidants can help protect cells from damage associated with premature ageing. Eating antioxidant-rich foods such as berries, dried fruit and dark chocolate can help prevent oxidative stress and support overall health.

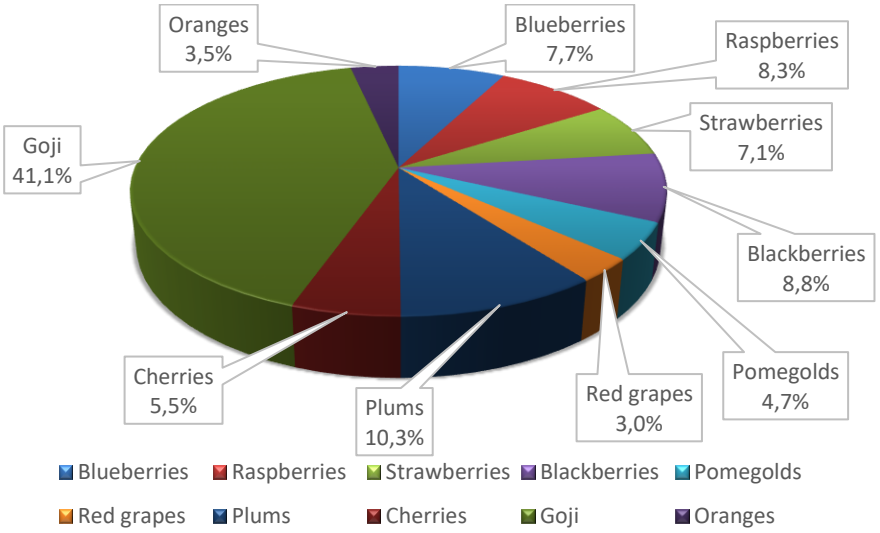

Figure 1. Fruits rich in antioxidants - according to the ORAC scale.

Some other examples of foods and their ORAC score (units per 100 g): clove powder (314.446), cinnamon powder (267.536), unprocessed sumac bran (312.400), tannin-rich sorghum bran (240.000), dried oregano powder (200.129), turmeric powder (159.277), freeze-dried acai berries (102.700), black sorghum bran (100.800). Figure 2 shows the antioxidant capacity of the healthiest foods according to the ORAC scale (units/100 g product) [12].

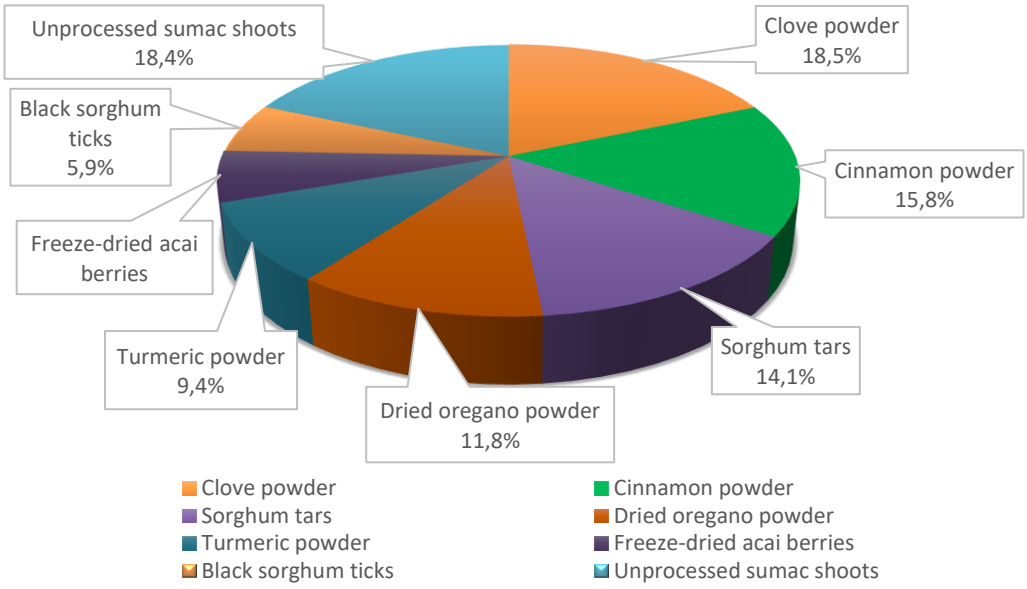

Figure 2. Antioxidant-rich foods - by ORAC score

4. Plants and herbs with antioxidant properties

The plants and herbs with antioxidant properties are essential for good health and their inclusion in the diet has long-term beneficial effects. Below are some examples of plants and herbs with powerful antioxidant properties.

Green tea (Camellia sinensis)

The main antioxidants in green tea are catechins (in particular - epigallocatechin gallate - EGCG). The ORAC score of green tea is approximately 1.253 ORAC units per 100 g (green tea in dry leaf form). Regular consumption of green tea reduces the risk of heart disease, improves brain function and helps with weight loss, is recognized for its anti-inflammatory effects and protection against certain types of cancer. EGCG is one of the most powerful catechins, with the ability to neutralize free radicals and prevent oxidative stress [14,15].

Rosemary (Rosmarinus officinalis)

The main antioxidants in rosemary are phenolic acids (especially carnosic acid and rosmarinic acid) which protect the brain from oxidative damage and have anti-inflammatory properties.

Turmeric (Curcuma longa)

The main antioxidant is curcumin. The ORAC score of turmeric is approximately 127.068 ORAC units per 100 g (turmeric powder). Main benefits: Protects against chronic inflammation and may reduce the risk of degenerative diseases such as Alzheimer's. Curcumin has antioxidant, anti-inflammatory and anti-carcinogenic properties, being effective in neutralizing free radicals and preventing oxidative damage at the cellular level.

Basil (Ocimum basilicum)

It contains as main antioxidants flavonoid compounds and volatile oils (such as eugenol) and has multiple benefits, namely it supports liver and skin health, having anti-inflammatory and antimicrobial effects.

Thyme (Thymus vulgaris)

The main antioxidants found in thyme are thymol and carvacrol. Thyme in various pharmaceutical forms, tincture, tea, etc. supports the immune system and can help fight respiratory infections.

Mint (Mentha spicata)

The main antioxidants in mint are flavonoids and phenolic acids. Peppermint tea improves digestion and can reduce oxidative stress in the digestive system.

Gingko Biloba

The main antioxidants in Gingko Biloba are flavonoids (ginkgoflavones) and terpenoids (ginkgolides and bilobalides). It is known to improve blood circulation, especially in the brain, and to protect neuronal cells against oxidative stress. It can help improve memory and reduce the risk of cognitive impairment. ORAC score approximately 6.000 ORAC units per 100 g (dried Ginkgo biloba leaves) [15,16].

The importance of these plants for health is essential, they provide cellular protection, contribute to improving general health and strengthening the immune system, help prevent degenerative diseases. Many of these herbs also have antimicrobial and anti-inflammatory effects, supporting overall health.

5. Conclusions

Antioxidants protect cells through a variety of mechanisms, including neutralizing free radicals, preventing their formation, regenerating other antioxidants, and inhibiting pro-oxidant metals. Through these mechanisms, they play an essential role in maintaining the redox balance of cells and preventing oxidative damage that can lead to premature aging, chronic diseases and cancer. Establishing the mechanism of free radical formation led to the creation of ways to prevent oxidative reactions in food, medicine, cosmetic products, etc.

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are important components of cell biology and play a dual role: beneficial in physiological regulation and harmful when produced in excess, contributing to various pathologies. Controlling the balance between the production of ROS and RNS and antioxidants is essential for maintaining cellular health. Knowing the role of free radicals in

triggering degenerative diseases (cancer, inflammatory diseases, cardiovascular diseases, etc.) and especially neurodegenerative diseases (Alzheimer's, Parkinson's, multiple sclerosis) created the conditions for the prevention and remedy of these diseases by means of antioxidant substances. They have the ability to limit the production of free radicals or to neutralize or stabilize radicals.
The beneficial health effects of natural antioxidants include: protecting cells, improving the immune system, preventing chronic diseases and slowing the aging process. Regular consumption of antioxidant-rich foods can reduce the risk of cardiovascular disease, cancer, and other aging-related conditions. Fruits rich in antioxidants contribute to the body's protection against oxidative stress, reducing the risk of chronic diseases and improving general health. Berries such as blueberries, raspberries and blackberries have among the highest antioxidant contents, followed by other fruits such as plums, pomegranates and red grapes. A number of plants and herbs are powerful sources of antioxidants that can help protect the body against oxidative stress and inflammation, particularly turmeric which stands out as one of the most powerful natural antioxidants.

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