

Literature Review

Antioxidant Potential of *Epigallocatechin-3-gallate*, Ascorbic Acid, and Sodium Ascorbate in Solution and Gel Forms by *2,2-diphenyl-1-picrylhydrazyl* (DPPH) Assay

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ABSTRACT

Background: Free radicals are molecules without any electron pairs, unstable, and highly reactive. Antioxidants are needed to reduce free radicals. Antioxidants provide various benefits in dentistry as a preventive agent for caries, healing, bone formation, mouthwash, preventive and therapeutic cancer, and reduction of periodontal disease progressions. Additionally, research on antioxidants is still undergone due to the existence of free radical residues on bleached teeth. *Epigallocatechin-3-gallate* (EGCG), ascorbic acid (AA), and sodium ascorbate (SA) are ingredients that have antioxidant properties. Antioxidants can be in two forms solution and gel. Solutions have a higher substance releasing power than gel. Gel is extremely adhesive, so it does not flow easily. Meanwhile, solutions are rather unstable because it flows easily. Antioxidant activities were evaluated with *2,2-diphenyl-1-picrylhydrazyl* (DPPH) Assay method. **Purpose:** Analyzing through literature reviews the potential antioxidants of EGCG, AA, and SA in solution and gel forms by DPPH Assay. **Reviews:** There were eight journal articles used in this review. The first article described antioxidant solutions in which EGCG was higher than sodium ascorbate. The second article showed that antioxidant gel EGCG was lower than sodium ascorbate. The third journal explained that ascorbic acid was higher than sodium ascorbate both in solution and gel forms. The other articles provided some information about the antioxidant activity percentages of EGCG, SA, and AA in the forms of gel and solutions by DPPH assay. **Conclusion:** EGCG has a higher antioxidant activity than SA, but it is lower when compared to AA in both solution and gel forms by DPPH assay.

Keywords: EGCG; ascorbic acid; sodium ascorbate; DPPH; antioxidant

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INTRODUCTION

Antioxidants are stable molecules that can donate electrons to free radicals and prevent the formation of free radical (peroxide) reactions. Antioxidants have abilities to scavenge and break down radical chains and inhibit oxidation reactions by scavenging peroxy and other radicals.¹ In dentistry, antioxidants provide various benefits to prevent caries, reduce progressions of oral problems such as periodontitis and gingivitis, heal and form alveolar bones, and act as preventive and therapeutic agents in carcinogenesis.² Additionally, antioxidants can be used as a mouthwash to prevent halitosis.³ Applying antioxidants after internal bleaching procedures can prevent interference polymerization of composite resin to restore bond strength.⁴

Preparations in gel forms are semi-solids that contain lots of water. Gel has a large concentration and molecular weight making it difficult to spread and penetrate into

tissue. The gel has high adhesion, so it does not flow easily. The greater the viscosity of a substance is, the smaller the diffusion coefficient gets.⁵ On the other hand, preparations in solution forms refer to a homogenous mixture with a faster initial action of drugs because they are absorbed quickly and easy to use. However, solutions possess unstable properties.⁶ They have better penetration than gel. The application of antioxidants in gel form can reduce the diffusion capacity of materials.⁷

AA as an antioxidant is a good electron donor.⁸ It is stable in dry states and oxidized easily in solution forms. Ascorbic acid has a short duration of action and a high acidic pH, so it can affect tooth structures and cause accumulations of *Streptococcus mutans*.⁹ EGCG is the most active catechin and enormous in number. EGCG is a safe material and able to dissolve in water.¹⁰ EGCG as an antioxidant can scavenge radicals and metal ion chelates such as iron and copper.¹¹ It has 20 times greater antioxidant properties than sodium ascorbate. Moreover, EGCG is a

natural material, non-cytotoxic, easy to obtain, affordable and durable. However, it is very unstable in the open air.⁹ EGCG as an antioxidant is stable at pH 6-8, so it does not either affect tooth structures or cause irritation.¹² SA also is able to act as a high antioxidant with low toxicity and remove free radicals from bleaching agents. Sodium ascorbate has a pH about 5.6-7 and is water soluble, so it can be removed easily.¹³

DPPH assay is a fast and easy method to evaluate substances that have antioxidant properties by spectrophotometry. DPPH free radical method as an antioxidant assay is based on electron transfers which will produce a purple solution in ethanol. Those free radicals are stable at room temperature and can be reduced due to the presence of antioxidant molecules, giving a pale yellow in ethanol solution.¹⁴

REVIEWS

Free radicals can be defined as a species of molecules which does not have electron pairs in an atomic orbital. Most of the radicals are unstable and very reactive. There are various types of oxygen that play a role in several disease states, including hydroxyl radicals, superoxide anion, hydrogen peroxide, singlet oxygen, and hypochlorite. Free radicals can attack significant macromolecules in the body, damage cells, and disrupt homeostasis. The targets of radicals include a variety of molecules in the body, including lipids, nucleic acid, and proteins.¹⁵

Antioxidants are stable molecules that can donate electrons to free radicals and neutralize them by reducing their destructive ability.¹⁶ Antioxidants have low molecular weight, so they can react with free radicals and break chain reactions before molecular damage occurs. Antioxidants act as radical scavengers, hydrogen and electron donors, peroxide decomposers, singlet oxygen traps, enzyme inhibitors, and metal ion chelating agents. The mechanism principle of antioxidants is by donating electrons to free radicals. Meanwhile, the second mechanism involves eliminating ROS by quenching the chain-initiating catalyst.¹⁵

EGCG is the most active catechins and enormous in number. Catechins are chemical antioxidants that have an ability to damage free radicals. EGCG is a safe material and able to dissolve in water. Various studies have shown that the catechins in green tea can be metabolized rapidly and associated with its antioxidant properties.¹⁰ The flavonols in green tea leaves, especially EGCG, provide advantages as antioxidant agents. Polyphenols prevent formations of free radicals and neutralize their presence by exchanging electrons through the tri-hydroxyl and di-hydroxyl groups in ring B. Several studies have shown that the administration of the antioxidant EGCG can restore bond strength in enamel that has been bleached. In addition, the catechins in green tea can remove free radicals from tooth structures, and the application of EGCG can be an alternative antioxidant for adhesive restoration after bleaching treatment.⁴

Antioxidant activities of EGCG are due to its chemical structure and polyphenolic properties. Each molecule of the materials has four rings, namely ring A, B, C and D. The tri-hydroxyl and di-hydroxyl groups in ring B have a role towards the antioxidant properties of EGCG. Polyphenols inhibit the formation of excessive ROS, increase the degree of polymerization, and raise bond strength composite resin on enamel bleaching.⁹ EGCG has 20 times greater antioxidant properties than sodium ascorbate and several advantages because it is a natural material, non-cytotoxic, easy to obtain, affordable, and durable.⁹

EGCG as an antioxidant plays a role in scavenging radicals and oxidants and also has abilities to chelate metal ion. The chelate contributes to antioxidant activities by preventing redox-active metal change in the form of free radicals.¹¹ Besides, the antioxidant activity mechanism of the flavonoid group itself is based on the transfers of a hydrogen atom and a single electron. The reaction between EGCG and free radicals is the transfer mechanism of EGCG hydrogen atoms. One hydrogen atom of flavonoid (FI-OH) will be transferred to a free radical (R \cdot), and a phenoxyl radical (FI-O \cdot), which is a more stable form than (R \cdot), will be formed.¹⁷

Ascorbic acid has an important role of protection against oxidative stress in various tissue. Oxidative stress refers to a condition where there is an imbalance between the production of ROS and the defense mechanism of antioxidants including enzymatic and non-enzymatic ones. ROS, free radicals, and peroxides are produced in cells when there is incomplete oxygen metabolism in the mitochondrial respiratory chain. Ascorbic acid can be oxidized in the extracellular environment which converts metal ions into dehydroascorbic acid entering cells via glucose transporter. Ascorbic acid can become a reducing agent and neutralize ROS such as hydrogen peroxide.¹⁸

Ascorbic acid in its antioxidant functions can capture oxygen and nitrogen free radicals generated during normal cellular metabolism. The antioxidant mechanism of ascorbic acid is based on the atomic donor of hydrogen. Additionally, ascorbic acid is an excellent electron donor due to the low standard 1-electron reduction potential, i.e. 282 mV, generation of relatively stable semi-dehydroascorbic acid, and easiness to convert DHA acid (dehydroascorbic) into ascorbic acid. The fast transfer reaction of hydrogen atom makes ascorbic acid a good antioxidant. In determining the function of antioxidants and pro-oxidants, ascorbic acid can be categorized according to several factors, including redox potential in cells, presence or absence of transition metals, and the concentration of ascorbic acid.⁸

Ascorbic acid is the most unstable of all vitamins, and it can get damaged easily during storing and processing phases. The rate of breakdown increases because the work of metals, especially iron or copper, becomes an efficient catalyst for the breakdown of ascorbate. Ascorbic acid is stable in dry conditions. However, in solution forms, it is easily oxidized to dehydroascorbic acid, especially under the influences of oxygen, light, and pH. Ascorbic acid is stable in pH below 4. It is also an ingredient that is usually

used as an antioxidant because it has an ability to donate hydrogen ion and reduce side effects of free radicals. When ascorbic acid (AscH) reacts with reactive free radicals (ROO \cdot), it will form ascorbate radicals (Asc \cdot) in a stable form and ROOH.¹⁹

Sodium ascorbate is a sodium salt of ascorbic acid (vitamin C) which has the chemical formula C₆H₇NaO₆. Sodium ascorbate is a compound dissolving in water and has an ability to act as a strong antioxidant with low toxicity and reduce reactive free radicals in organic systems. Moreover, it can remove free radical residues from bleaching agents. Sodium ascorbate neutralizes free radicals by providing one electron derived from ascorbate (Asc) and producing water and ascorbyl free radicals (AFR). The AFR pair form one molecule of dehydroascorbic acid (DHAA) and one ascorbate. DHAA can be reduced back to ascorbate. DHAA breaks down to form diketogulonic acid, threonic acid, and oxalic acid which are an ascorbate. Sodium ascorbate is water soluble, so it can be removed easily.¹³

When sodium ascorbate is in a gel form, the effectiveness of the gel material is much less than in liquid form. Sodium ascorbate 10% has a pH about 7.4-7.7 as a weak alkali. The more alkaline it is, the greater the dissociation constant (pKa) gets. A large of pKa results in less release of H⁺ ions.²⁰ Sodium ascorbate interacts with free radicals to become more stable, reduce their reactivity, and prevent or reduce cellular damage caused by free radicals.

DPPH is a method used to measure the ability of antioxidant towards free radical. This test can be used to measure a compound ability to act as free radical scavengers or hydrogen donors and applied to assess antioxidant activity.²¹ DPPH assay method is based on the reduction of DPPH as a stable free radical. DPPH free radicals have a maximum absorption at a wavelength of 517 nm with a purple colour. When antioxidants react by DPPH, which is a stable free radical, they will pair up because they get a hydrogen donor and are reduced to DPPHH so that they can reduce the absorbance of DPPH. The formation of DPPH-H from a radical results in a colour change from purple to yellow. When the DPPH solution has been mixed with a compound that can donate hydrogen atoms, it can cause a reduced form, Diphenylpicrylhydrazine, which is a non-radical form with a change from purple to pale yellow.²²

DISCUSSION

This literature review describes antioxidant activities of EGCG, AA, and SA in reducing free radicals. In dentistry, antioxidants provide many benefits such as halitosis mouthwash, caries prevention, therapeutic and preventive carcinogenesis, and others. DPPH method is used to measure the antioxidant activity percentage of a substance. The greater percentage value is, the better the antioxidant activities of substance get.

Antioxidants in the forms of gel have abilities to penetrate and release power lower than a solution because gel has large molecular weight and can reduce the

diffusability of a substance. De Carvalho *et al.*, found that EGCG in gel forms had antioxidant activity percentages of 90.58%, 91.87%, 91.47% at concentration 10%, 20% and 30%.⁷ Meanwhile, Garcia *et al.*, demonstrated that the percentage of antioxidant ascorbic acid was 95.33%, while sodium ascorbate gel was 94.16% at 20% concentration and 76.04% at 10% concentration.^{14,7} From this review, the highest antioxidant gel form was found in AA, EGCG, and SA.

A material in a solution form has faster release and can be absorbed easily even though it has unstable properties since it flows with little effort. Three studies proved EGCG in solution forms had antioxidant activity percentages of 56.57%, 77.2%, 73.6 and 93.8%.^{23,24,25} Whereas, other studies found that ascorbic acid had 73%, 89.95%, 90.31%, 95.65%, 81.57%, 84.01% and 87.28%.^{26,27,14,28} Meanwhile, Bansal *et al.*, and Garcia *et al.*, revealed that sodium ascorbate in solution forms had antioxidant activity percentages of 9.10% and 51.79%.^{23,14} In solution preparations, the highest to the lowest percentages of antioxidants were found in ascorbic acid, EGCG, and sodium ascorbate.

From some of the reviews above, the results proved that the highest to the lowest antioxidant activity percentages of the three ingredients both in gel and solution forms could be obtained from ascorbic acid, EGCG, and sodium ascorbate. Antioxidant EGCG and ascorbic acid are higher in solution forms, while sodium ascorbate is higher in gel forms. Sodium ascorbate in gel forms contains a thickening agent (natrosol gel) which has a function to improve the abilities to spread and penetrate into tissue so that good adhesion and penetration could be obtained.¹⁴

Ascorbic acid has good antioxidant properties, but its pH is about 1.8, so it is not recommended for clinical use because it can affect tooth structures and lead to the accumulation of *Streptococcus mutans*. Besides, ascorbic acid has a short duration of action.¹⁴ Ascorbic acid can restore bond strength on bleached enamel.¹⁴ Oladimeji also explains that ascorbic acid can inhibit oxidation reactions which can produce free radicals causing tissue damage. Ascorbic acid is also used as a standard of comparison with other antioxidants with higher activities than vitamin E.²⁸ Similar to the research of Maesaroh *et al.*, a study by Kaur *et al.* demonstrated an in-vitro test of ascorbic acid solutions by using DPPH assay. The higher the concentrations are, the higher antioxidant activities get.^{26,27}

In this case, EGCG may be used as an alternative antioxidant to replace ascorbic acid because EGCG is natural and non-cytotoxic substance. Moreover, it has high antioxidant activities with a long duration of action and has pH about 6-8, so it does not affect tooth structures or inflict any irritation.¹² EGCG is a catechin which can be found a lot in green tea.¹⁰ Antioxidant activities of EGCG are related to its chemical structures and polyphenolic properties. Each molecule of the materials has four rings: ring A, B, C and D. The tri-hydroxyl and di-hydroxyl groups in ring B have a role in the antioxidant properties of EGCG.²⁹ EGCG has 20 times greater antioxidant properties than sodium ascorbate.⁹

On the other hand, sodium ascorbate as an antioxidant is also a biocompatible, neutral, and non-toxic material.³⁰ Sodium ascorbate, which is the sodium salt of ascorbic acid, is a compound that dissolves in water and has the abilities to act as a strong antioxidant with low toxicity. Additionally, sodium ascorbate can reduce receptive free radicals in organic systems and remove their residues from bleaching agents.¹³

Antioxidants that have the ability to inhibit oxidation and harmful free radicals provide various benefit, including EGCG. As an antioxidant, it has a radical scavenger effect used as caries prevention. Vitamin C can change the progression of oral problems such as periodontitis and gingivitis by suppressing the antioxidant ability of GCF and plasma. Besides, antioxidants can also increase bond strength of orthodontic brackets. Healing and bone formation also have potential as a preventive and therapeutic agent in carcinogenesis.² In addition, antioxidants can be used as a mouthwash to prevent halitosis.² Moreover, antioxidant can restore bond strength composite resin and teeth after bleaching procedures.¹⁴

ACKNOWLEDGEMENT

We gratefully thank the anonymous referees for their useful suggestions.

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