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Evaluation of Herbal Products for Antioxidant Activity Using Superoxide Dismutase

Vaishali Amit Adsare [1], Rukhsana Mahiboob Pinjari [2]

[1] Research Scholar, Sunrise University, Alwar, Rajasthan

[2] Professor, Sunrise University, Alwar, Rajasthan

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Address for Correspondence: Vaishali Amit Adsare

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Abstract

Superoxide dismutase (SOD) is an important antioxidant enzyme that plays a crucial role in protecting cells from oxidative stress by catalyzing the dismutation of superoxide radicals (O2•−) into oxygen (O2) and hydrogen peroxide (H2O2). There are several methods to assay SOD activity, with the most common being the spectrophotometric method based on the inhibition of pyrogallol autoxidation. This research work was aimed to determine antioxidant potential of polyherbal formulation using superoxide dismutase.

Keywords: Herbs, polyherbal formulation, antioxidant activity, superoxide dismutase

Introduction

Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infection and degenerative disease. Since dietary antioxidants have been shown to be protective against chronic diseases. Some degradation processes of aerobic living organisms are mediated by reactive oxygen species, such as superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide radical and hydroxyl radical. Particularly $O_2^{\bullet}$ has been considered as a causative species to induce inflammation (Trenam et al., 2018). Nutritional antioxidant deficiency also leads to oxidative stress, which signifies the identification of natural antioxidative agents present in the diet consumed by the human population. There are certain naturally occurring antioxidants that can give protection to liver from hepatotoxins. Modern research is now directed towards natural antioxidants originated from plants due to safe therapeutics (Sharma & Gupta, 2008).

The purpose of the present study was to investigate & quantify the total phenolic and flavonoid content of Polyherbal formulation and herbomineral formulation and to evaluate its free radical scavenging activity by adopting different in vitro assay methods.

Materials and method

The The bark of Pterocarpus marsupium (Asana), Cinnamomum zeylanicum (Tvak), rhizome of Curcuma longa (Haridra), fruit pericarp of Terminalia chebula (Haritaki) fruit pericarp & Root of Plumbago zeylanica (Chitraka), India.

The samples were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered material was mixed then extracted using solvent extraction in the ratio 1:10 using Soxhlet apparatus. After extracting all colouring material the solvent was removed by evaporating on water bath which give rise to a solid mass of the extract. Then the concentrated extract was stored at 4ºC until use.

The ethanolic extract of the Polyherbal formulation and herbo mineral formulation was subjected to Superoxide dismutase (SOD) Spectrophotometric Assay of

Superoxide Dismutase (SOD) (Fridovich I 1975)

Principle: The assay is based on the ability of SOD to inhibit the autoxidation of pyrogallol in the presence of superoxide radicals generated by xanthine/xanthine oxidase system. SOD specifically scavenges superoxide radicals, thereby decreasing the rate of pyrogallol oxidation, which can be monitored spectrophotometrically.

Materials Needed:

- Phosphate buffer (pH 7.4)
- Pyrogallol solution (10 mM)
- Xanthine solution (2 mM) in buffer
- Xanthine oxidase solution (0.025 U/mL) in buffer
- Sample extract or purified SOD enzyme
- Control SOD (bovine erythrocyte SOD) for calibration curve
- EDTA solution (1 mM) (optional, to chelate metal ions)
- Assay cuvettes or microplates
- Spectrophotometer (UV-visible)

Procedure:

Preparation of Reaction Mixture:

Prepare a reaction mixture containing phosphate buffer (final concentration 50 mM, pH 7.4), pyrogallol solution (final concentration 1 mM), xanthine solution (final concentration 0.05 mM), and xanthine oxidase solution (final concentration 0.025 U/mL). Optionally, EDTA (final concentration 1 mM) can be added to chelate metal ions that could interfere with the assay.

Sample Preparation:

If using a crude extract, clarify by centrifugation and adjust protein concentration. For purified enzyme, dilute to appropriate concentration in buffer.

Assay Procedure:

Prepare assay cuvettes or microplate wells containing appropriate volumes of reaction mixture and sample (or control SOD).

Incubate the reaction mixture at 25°C for 5 minutes to allow for stabilization.

Initiation of Reaction:

Start the reaction by adding xanthine oxidase solution to each cuvette or well. Immediately mix the contents thoroughly.

Measurement:

Monitor the change in absorbance at 420 nm continuously for 3-5 minutes using a spectrophotometer. Record the initial linear rate of increase in absorbance due to pyrogallol autoxidation.

Calculation of SOD Activity:

Calculate the inhibition percentage using the formula:

$$\% \text{ inhibition} = (A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$$

where A control is the rate of autoxidation without SOD (only buffer and pyrogallol), and A sample is the rate of autoxidation in the presence of sample or standard SOD.

Use a calibration curve of known concentrations of control SOD to determine the specific activity of the sample (in units/mg protein or units/mL).

Results and discussion

Free radicals bear high chemical reactivity due to their unpaired electrons. Reactive oxygen species (ROS) comprise oxygen free radicals or oxygen free- radical-generating agents such as superoxide anion ($O_2^{-\bullet}$), hydroxy radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2) (Valko et al., 2006). Metabolic processes are usually associated with the generation of free radicals especially oxygen-derived radicals with the potency of oxidizing and damaging surrounding bio molecules (Valko et al, 2007).

The level of antioxidant enzymes assessed in ethanolic extract of Polyherbal formulation and herbomineral formulation. The highest activity of SOD was noted in the ethanolic herbomineral (8.4 ± 0.58) compared to the Polyherbal formulation extract (3.0 ± 0.16). SOD is a family of metallo enzymes catalyse the decomposition of $O_2^{\bullet}$ to O_2 and H_2O_2. It prevents the formation of $OH^{\bullet}$ and hence been

implicated as an essential defense against the potential toxicity of oxygen. The ROS scavenging activity of SOD is effective only when it is followed by the actions of CAT and GPx, because the dismutase activity of SOD generates H_2O_2, which needs to be further scavenged by CAT and GPx (Lee et al., 2003). This enzyme is present in all aerobic organisms and in all subcellular compartments susceptible to oxidative stress (Bowler et al., 1992). The result of SOD clearly shows that the plant possess significant amount of SOD, which could exert a beneficial action against pathological alteration caused by the presence of O_2 and $^{\bullet}OH$, hence it can be able to counteract the toxicity of oxygen.

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