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# Determination on Antioxidant Activity of *Sansevieria cylindrica* Bojer ex Hook. Leaf Extract

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ABSTRACT

Antioxidant compounds work by preventing or detecting oxidative chain reactions caused by free radicals, stabilizing generated radicals and thus reducing the risk of oxidative damage in the body. They are sometimes called as free radical scavengers. Plants are naturally rich in antioxidants which contains bioactive compounds with high antioxidant activity. The work was carried out to verify whether different types of method shows the same sensitivity and/or capacity to determine the antioxidant activity of two different extract of *Sansevieria cylindrica* Bojer ex Hook. Their extracts were analyzed using 4 four different methods (i) DPPH (ii) Hydrogen peroxide (iii) Superoxide (iv) Nitric Oxide scavenging activities. These four methods could quantify the antioxidant capacity. Antioxidant evaluation methods must be fast, reproducible, require small amounts of the chemical compounds being analyzed, and not be affected by the physical characteristics of the compounds. The highest antioxidant activity was observed in both the extracts 13.6% (Ethanolic extract) and 13.9% (Chloroform extract) in 20$\mu$g/ml concentration of DPPH activity and the lowest antioxidant activity was also observed in ethanolic extract of Nitric oxide radical scavenging activity 68.7% in 100$\mu$g/ml concentration. Finally the application of DPPH free radicals are higher than the results of other three methods of free radical activity.

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## INTRODUCTION

Modern technology makes use of pharmacologically active compounds that are provided by medicinally important plants in the discovery of new therapeutics. According to WHO, about 80% of the world's population relies on medicinal plants for their medicinal properties [1]. There are many different bioactive components in plants and herbal dietary supplements that have physiological actions in the body that could be used to treat diseases with minimal side effects. Also, these herbal plants can be used by people from all ages and sexes.

A major source of bioactive constituents are antioxidants [2], polyphenols including tannins, and peptides. These molecules slow or prevent oxidation and protect the body against reactive free radicals and metals that are chelating. Phytochemical studies have suggested that the antioxidant abilities of plants are due to their phenolic content [3, 4]. In recent years, there has been increased interest in finding antioxidant phytochemicals in order to prevent degenerative diseases like atherosclerosis, heart attacks, aging, cancer and others [5, 6]. The process of producing free radicals by the

organism and removing them by it is in balance; this is referred to as oxidative balance. In case of disruption of this balance, reactive oxygen species increase. The condition is known as oxidative stress [7].

Increasing the amount of free radicals in an environment can deplete the antioxidant defense systems and affect the equilibrium status of antioxidant defenses. Free radicals have both a short half life and high reactivity, which can damage cellular components, including DNA, proteins, carbohydrates, nucleic acids, and membrane lipids, resulting in cell death and tissue damage [8]. Therefore, oxidative stress can cause several chronic diseases including cancer, diabetes, Alzheimer's disease, coronary heart diseases, and aging [9].

In humans, some studies have shown that dietary antioxidants may reduce the risk of these diseases, improve general health, and prevent death [10]. In this way, the plant might be used to produce potential antioxidant drugs for the treatment of diseases caused by oxidative stress. Foods, cosmetics, and pharmaceutical products now contain natural antioxidants, which are beneficial through their abundance and magnitude of activity, and they can adjust imbalances to a great extent [11, 12].

In the plant kingdom, Sansevieria cylindrica Bojer ex Hook. is a perennial plant belonging to the Asparagaceae family. It is native to the subtropical regions of the African continent and is cultivated in Egypt for ornamental purposes [13]. It is one of the most recommended plants for improving air quality and capable of capturing 107 types of toxins, including air pollution, cigarette smoke (nicotine) [14]. It is utilized as a traditional remedy, especially in remote rural areas where herbal forms of medicine are still commonly used as medicines since the whole plant is useful for treating cuts, sprains and broken bones, while the roots are useful in treating snakebites. The main goal of this study was to determine the antioxidant activity of *S. cylindrica* Bojer ex Hook.

## MATERIALS AND METHOD

### Collection of Plant Material and Preparation of Plant Extracts

A taxonomist from Rapinat Herbarium and Center for Molecular Systematics, St. Joseph's College, Trichy, Tamil Nadu, identified the plants collected from Holy Cross College Campus (Autonomous), Nagercoil. India. It was deposited at the Rapinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil Nadu, India. Plant materials were then washed separately and dried under shade for a few days. To store the dried materials for future use, the materials were ground into coarse powder by grinding machines and stored at room temperature. With two solvents, ethanol and chloroform, the sample could be extracted and then evaporated to dryness with a rotary evaporator. The resulting residue was then tested for antioxidant activity.

### Antioxidant Activity

Antioxidants protect cells from damage caused by reactive oxygen species. Recent studies have shown that natural antioxidants can prevent disease and promote health. Since phytochemicals have such complex structures, different methods can be used to evaluate their antioxidant properties [15]. DPPH [16], hydrogen peroxide [17], superoxide [18] and nitric oxide [19] were analysed and compared with standard rutin as antioxidants. In addition to being an antioxidant, rutin is also known as vitamin P, which can be found in vegetables, fruits, and herbs such as asparagus. Among its pharmacological actions, as being described by Patel et al. [17], rutin has antibacterial, antiprotozoal, antitumor, anti-inflammatory, antiallergic, antiviral, cytoprotective, hypolipidemic, antiplatelet, antispasmodic, and antihypertensive activities.

### DPPH Radical Scavenging Assay

For determining antioxidants' ability to scavenge free radicals, the DPPH method is commonly used. DPPH (1,1-diphenyl-2-picrylhydrazyl) is a highly stable free radical compound and has the ability to accept electrons or hydrogen radicals. Before measurement, the lyophilized fractions were sieved and powdered. In an Eppendorf tube, 10 mg of the powdered sample were added, followed by 1.7 mL of the DPPH reagent. At 0, 15, and 25 minutes, the mixture was vortexed for three minutes to increase the surface area available for the reaction between the insoluble matter and the DPPH reagent. After centrifugation at 9200 g for 2 minutes, the optically clear supernatant was measured with a Shimadzu 3100 UV-vis spectrophotometer at 517 nm. After mixing the insoluble matter with DPPH, all measurements were taken after 30 minutes. As a standard, Rutin was used and the results were expressed as a percentage. Based on the DPPH scavenging method relative to control, the following equation was used:

$$DPPH\ \ Scavenging\ \ Activity = ((Absorbance\ \ of\ \ Control - Absorbance\ of\ Sample)/Absorbance\ of\ Control) \times 100$$

This test is quick and easy, and provides reliable results. It requires only the use of a UV-Vis spectrophotometer to perform, so it has widely been

used for screening antioxidant properties.

**Hydrogen Peroxide Scavenging Activity**

With a little modification, we were able to show that extracts can scavenge hydrogen peroxide. Instead of using phosphate buffer saline of pH 7.4, the hydrogen peroxide solution was prepared by adding various concentrations of aqueous and ethanolic extracts (100–1000 g/ml) to a hydrogen peroxide solution (2 ml) [20]. Following a 10-minute incubation, the absorbance of hydrogen peroxide at 230 nm was measured against a blank solution containing only phosphate buffer. The blank samples for each concentration were taken separately. In the case of the control sample, take hydroperoxide solution and see the absorbance at 230 nm. Based on the formula, determine the percentage inhibition activity.

$$H_2O_2\ Scavenging\ Activity = [(A_0 - A_1)/A_0] \times 100$$

where $A_0$ is the absorbance of the control and $A_1$ is the absorbance of extract.

**Superoxide Radical Scavenging Activity**

By nitroblue tetrazolium reduction, superoxide scavenging was determined. One ml of the nitroblue tetrazolium solution (l M NBT in 100 mM phosphate buffer, pH 7.4), one ml of the NADH solution (l M NADH in 100 mM phosphate buffer, pH 7.4), and 0.1 ml of mixed fractions and ascorbic acid (50 mM phosphate buffer, pH 7.4) are mixed together in the reaction mixture. In order to start the reaction, 100 milliliters of PMS solution (60 milliliters of PMS in 100 milliliters of phosphate buffer, pH 7.4) were added to the mixture. Incandescent visible light was evenly emitted into the tubes for 15 minutes, and optical density was measured before and after illumination at 530 nm. Using absorbance values from control and experimental tubes, we evaluated the percentage inhibition of superoxide generation. The ability of each compound to scavenge the superoxide radical was calculated with the following formula:

$$\%\ Scavenging = (1 - Ae/Ao) \times 100$$

where Ao is the absorbance without sample, and Ae is absorbance with sample.

**Nitric Oxide Radical Scavenging Activity**

In response to sodium nitroprusside dissolved in phosphate buffer saline, the radical nitric oxide is inhibited. Cos et al., 1998 evaluated this method: For 0.5ml concentration of extract, 2.0 ml of sodium nitroprusside PBS (0.7425 g/250 ml, pH 7.4) was added, and the mixture was incubated for 150 minutes at room temperature.

Pour 0.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, and 0.1% naphthyl ethylenediamine dihydrochloride) into the mixture and let it stand at 25 °C for five minutes. By scavenging NO, oxygen is consumed, which produces a chromophore with a light pink-to-deep purple hue at 546 nm. Positive and negative controls were performed in the same manner as the sample, except for adding only the solvent to the negative control, and adding rutin to the positive control. Ruthin served as a standard. A decrease in absorbance was calculated by the equation below.

$$\%\ Inhibition = A0 - A1/A0 \times 100$$

where $A_0$ is the absorbance of the control (without sample) and $A_1$ is the absorbance of the extract.

**RESULTS AND DISCUSSION**

Figure 1 and Figure 2 illustrate the antioxidant activity as measured by $H_2O_2$, DPPH, SOD and NO. In comparison with standard rutin, the two solvent extracts showed much-reduced scavenging activity. According to Mustafa et al. [21], antioxidant properties are significantly associated with the presence of phenolic compounds and flavonoids. Furthermore, plants containing gums, tannins, and phenols are known to provide a high level of antioxidants [22].

Both extracts showed the greatest level of DPPH scavenging activity at concentrations of 20$\mu$g/ml: 13.6% in ethanol and 13.9% in chloroform. The lowest antioxidant activity was also determined in the ethanolic extract of Nitric oxide radical scavenger activity, 68.7% in 100$\mu$g/ml concentration. Although the antioxidant potential of the extracts were lower than that of rutin, the study showed that both extracts had free radical scavengers and/or inhibitors, which were possibly acting as primary antioxidants [23].

Among the extracts that inhibit superoxide radical scavenging activity, ethanolic extract exhibits the highest inhibition activity of 15% in 20$\mu$g/ml concentration, whereas chloroform extract exhibits the lowest inhibition activity of 41.3% in 100$\mu$g/ml concentration. A possible explanation could be that the extract contains bioactive compounds such as phenolics and flavonoids that can scavenge superoxides and may prevent the oxidation of the major biomolecules [23].

With regard to scavenging Nitric oxide radicals, the highest antioxidant activity was detected in chloroform extract with inhibition activity of 35.1% in 20$\mu$g/ml concentration, whereas the lowest antioxidant activity was detected in ethanol extract with inhibition activity of 68.7% in 100$\mu$g/ml concentra-

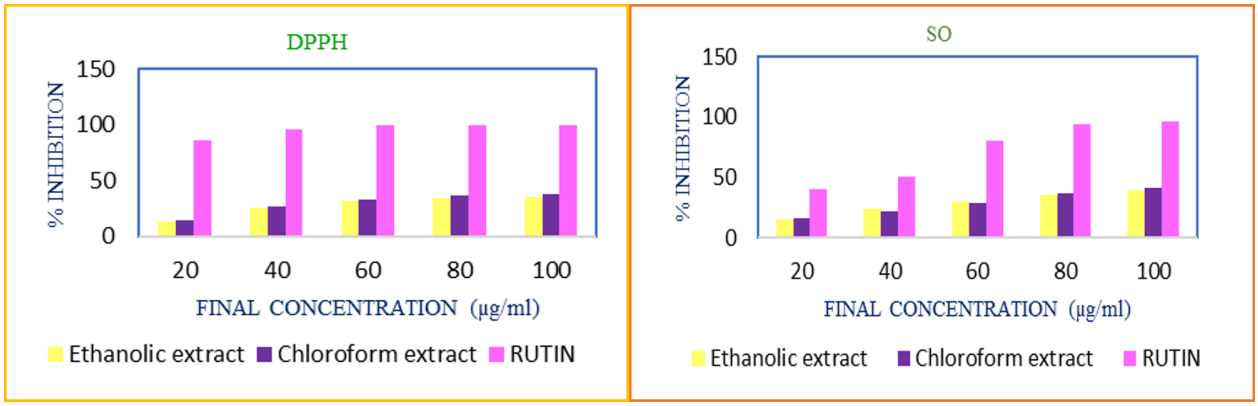


**Figure 1: Antioxidant Activity of *S. cylindrica* Bojer ex Hook. extracts – DPPH and SO radical scavenging activity**

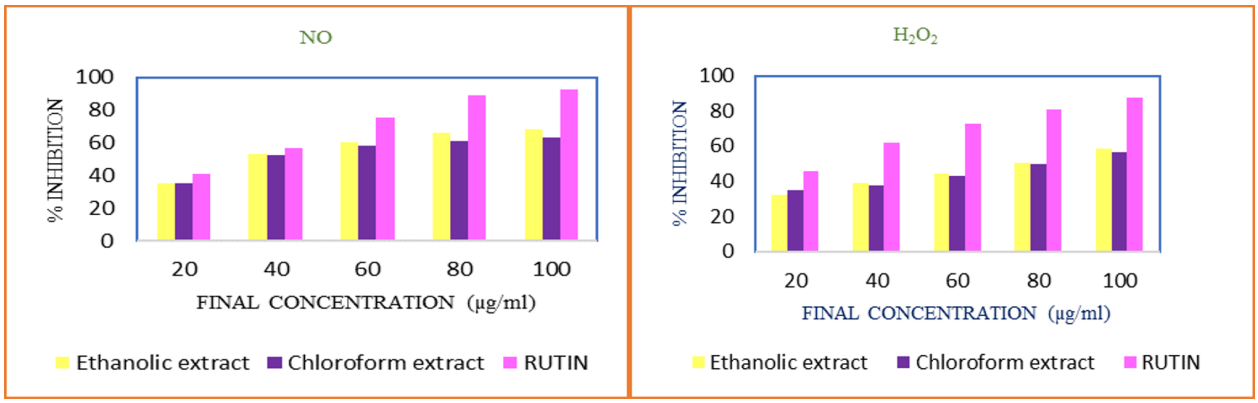


**Figure 2: Antioxidant Activity of *S. cylindrica* Bojer ex Hook. extracts – NO & $H_2O_2$ radical scavenging activity**

tion. Also, the scavenging process helps to interrupt the chain of reactions initiated by excessive NO production that harm human health.

When it comes to hydrogen peroxide oxide radical scavenging activity, ethanolic extract had the highest antioxidant activity, with an inhibition activity of 32.1% at a 20g/ml concentration, and ethanol extract had the lowest antioxidant activity at 58.3% at a 100g/ml concentration respectively. Since hydrogen peroxide can give rise to hydroxyl radical inside the cell, it may sometimes be toxic to cells, even though it is not very reactive [24]. Increased concentration of the fractions resulted in increased percentage scavenging activity. Rutin, used as positive controls for comparison, was relatively more effective than *S.cylindrica* Bojer ex Hook at scavenging for free radicals. Taking into consideration the results of the present study, when comparing the antioxidant activity of four different methods, it can be seen the results obtained from the application of DPPH free radicals are greater than the results from other free radicals. A possible explanation for this maximum free radical scavenging activity is the presence of polyphenolic compounds in sufficient levels [25].

## CONCLUSION

Traditionally, *S. cylindrica* Bojer ex Hook. is used as a treatment for various diseases. Extracts from the leaves of this plant had antioxidant potential which may be useful for nutritional and medicinal functions. This plant may serve as an effective free radical inhibitor or scavenger which is useful in pharmaceutical products. Thus, the plant appears to be a promising material for further studies leading to the ability to develop a relatively inexpensive and efficient way of developing drugs and discovery.

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### Conflict of Interest

The authors declare that they have no conflict of

interest.

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