

ANTIOXIDANT POTENTIAL OF SANSEVIERIA ROXBURGHIANA SCHULT. AND SCHULT. F

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ABSTRACT

In the present study the herb, *Sansevieria roxburghiana* was selected to evaluate its antioxidant properties of methanol, acetone and ethyl acetate extracts of leaves using DPPH and NO radical scavenging activity method which showed a significant percentage of inhibition in a dose dependent manner with BHT (butylated hydroxytoluene) as a standard reducing agent. The result showed that the different solvent extracts of *S. roxburghiana* exhibited good antioxidant effect and strong free radical scavenging effects on free radicals and oxidants (DPPH, NO).

Keywords: Antioxidant, scavenger, *Sansevieria roxburghiana*, Antioxidant potential, Reactive oxygen species.

INTRODUCTION

Sansevieria roxburghiana belongs to the family Dracnaceae, commonly referred to as bowstring hemp, piles root¹ and Jaang Mattai in Tamil (Vernacular). In recent years much attention has been devoted to natural antioxidant and their association with health benefits. Plants are potential sources of natural antioxidants and produce various antioxidative compounds that have therapeutic potentials. Antioxidant-based drug formulations are used for the prevention and treatment of many complex diseases. In living systems, oxidation is a basic part of the normal metabolic process, in which Reactive oxygen species (hydrogen peroxide and hypochlorous acid) and many free radicals (hydroxyl radical (OH) and superoxide anion) are generated^{2,3,4}. Rapid production of free radicals may cause alteration in the structure and function of cell constituents and membranes and can result in human neurologic and other disorders such as cancer, diabetes, inflammatory disease, asthma, cardiovascular, neurodegenerative diseases, and premature aging^{5,6}. Therefore, the prevention of the above conditions requires the presence of antioxidants or the free radical scavenging molecules in the body.

MATERIALS AND METHODS

Collection of plant samples

Healthy, disease free leaves of *Sansevieria roxburghiana* were collected, authenticated, washed and air dried leaves were cut into small pieces, pulverized in a domestic blender and used for the preparation of solvent extracts.

Solvent extracts

The pulverized leaves material was mixed with sufficient quantity of solvents viz., methanol, acetone, and ethyl acetate. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at -47.5°C. The dried extracts thus obtained was weighed and preserved at 4°C for future use.

Antioxidant potential of *S. roxburghiana*

The antioxidant activity of methanol, acetone and ethyl acetate leaf extracts of *S. roxburghiana* was determined by two *in vitro* methods. All the assays were carried out in triplicate.

DPPH radical scavenging capacity

DPPH radical scavenging capacity of leaf extracts of *S. roxburghiana* were determined according to the method described^{4,7}. The leaf extracts of different solvents were treated with different concentrations ranging from 100 µg/ml to 1000 µg/ml in 95% (v/v) ethanol. 1ml of freshly prepared DPPH solution was added in each of these test tubes and was shaken and incubated for 25 min at room

temperature. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Butylated Hydroxy Toluene (BHT) was used as standard. Control sample was prepared without any extract or BHT. 95% ethanol was used as blank. Percent scavenging of the DPPH free radical was measured using the following equation⁸.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Where:

Absorbance of control = Absorbance of DPPH radical+ethanol

Absorbance of sample = Absorbance of DPPH radical + sample extract /standard.

Assay of nitric oxide scavenging method

Nitric oxide scavenging capacity of leaf extracts of *S. roxburghiana* were carried out according to the procedure described⁹. Sodium nitroprusside was mixed with different concentrations of methanol, acetone and ethyl acetate leaf extracts of *S. roxburghiana* (100, 200, 300, 400, 500, 1000 µg/ml) dissolved in 95% ethanol and water and incubated at 25 °C for 180 min to which Griess reagent was added. The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine dichloride was read at 546 nm and referred to the absorbance of BHT, used as a positive control treated in the same way with Griess reagent. Control sample was prepared without any extract or BHT^{10,11}.

$$\text{Nitric Oxide scavenged (\%)} = \frac{(\text{Absorbance of control reaction} - \text{Absorbance in the presence of the samples}) \times 100}{\text{Absorbance of control reaction}}$$

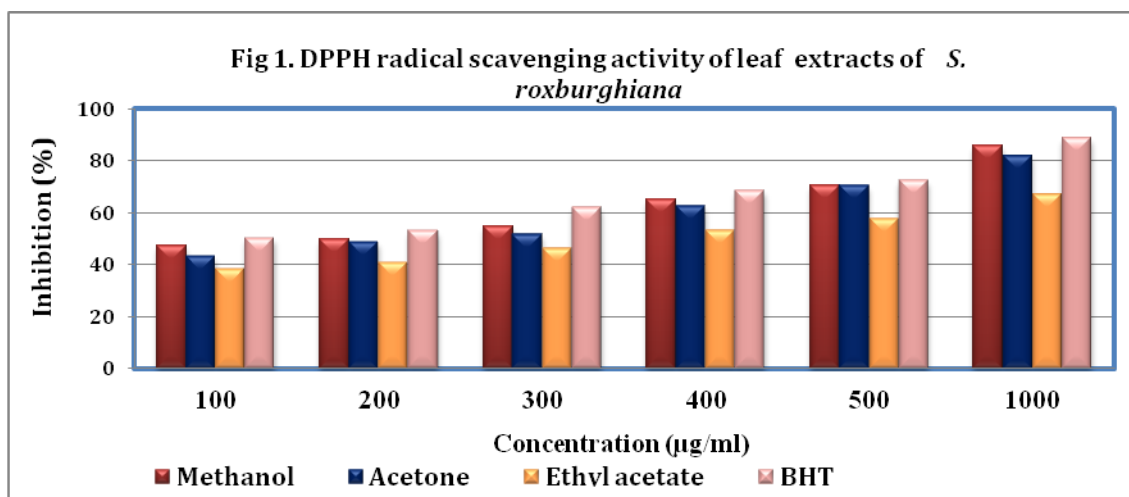
Statistical Analysis

The experimental data were expressed as mean ± SD. The significance of difference among the various treated groups and control group were analyzed by means of one-way ANOVA. The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The DPPH radical scavenging capacity of the different solvent extracts of *S. roxburghiana* leaves are presented in Fig. 1. The methanol leaf extract at 1000 µg/ml exhibited about 85.68 ± 3.47 DPPH activity, was found to be comparable to BHT, which showed a DPPH scavenging activity of 88.66 ± 1.32. At this concentration, acetone and ethyl acetate extracts also showed an appreciable DPPH scavenging activity of 81.87 ± 1.45 and 67.09 ± 4.34 respectively.



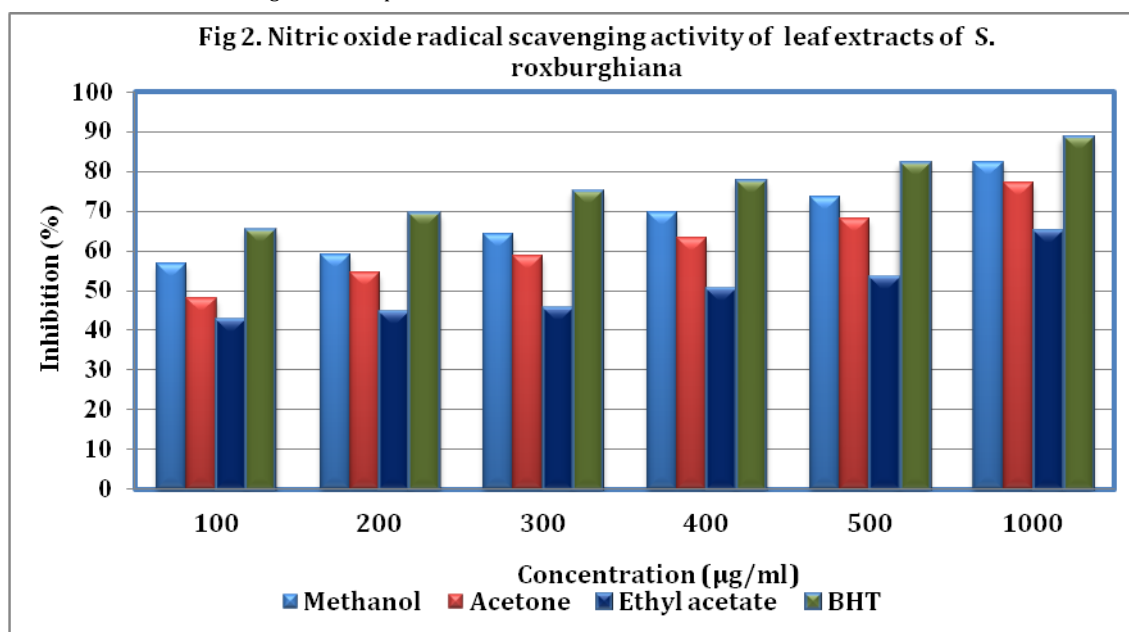
The free radical scavenging activity of methanol, acetone and ethyl acetate extracts of leaves of *S. roxburghiana* was evaluated based on its ability to scavenge the synthetic DPPH. This assay provided useful information on the reactivity of the compounds with stable free radicals, because of the odd number of electrons. DPPH shows a strong absorption band at 517 nm in visible spectrum (deep violet color)¹². As the electron is paired in the presence of free radical scavenging, the absorption vanishes and the resulting discoloration stoichiometrically coincides with respect to the number of electrons taken up. The bleaching of DPPH absorption is representative of the capacity of the methanol, acetone and ethyl acetate extracts to scavenge free radicals independently^{13, 14, 15}. It is shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples (100-1000 µg/ml) and standard (BHT) to a certain extent and hence are said to be strongly dependent on the extract concentration.

However, the antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of

chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, and radical scavenging^{16, 17}.

Nitric oxide radical scavenging activity

The dose dependent response of nitric oxide radical scavenging activity of leaf extracts of *S. roxburghiana* compared with BHT is presented in Fig. 2. It was observed that methanol leaf extract at 1000 µg/ml exhibited about 86.07 ± 2.18 of NO scavenging activity which was found to be comparable to BHT that showed a scavenging activity of 89.12 ± 2.39 . Nitric oxide scavenging activity method, showed significant percentage of inhibition in a dose dependent manner. At the same concentration acetone and ethyl acetate extracts also showed a remarkable NO scavenging activity of 80.54 ± 3.61 and 64.80 ± 3.18 respectively. Even though the scavenging activity of all extracts were lower than that of standard, still the result implies that radical scavenging activity of the extracts may be attributed to their strong proton donating ability.



Sodium nitroprusside (SNP) serves as a chief source of free radicals. The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine is used as a marker for nitric oxide scavenging activity^{18, 19}.

Suppression of released NO may be partially attributed to direct NO scavenging, as the extracts of *S. roxburghiana* decreased the amount of nitrite generated from the decomposition of SNP *in vitro*.

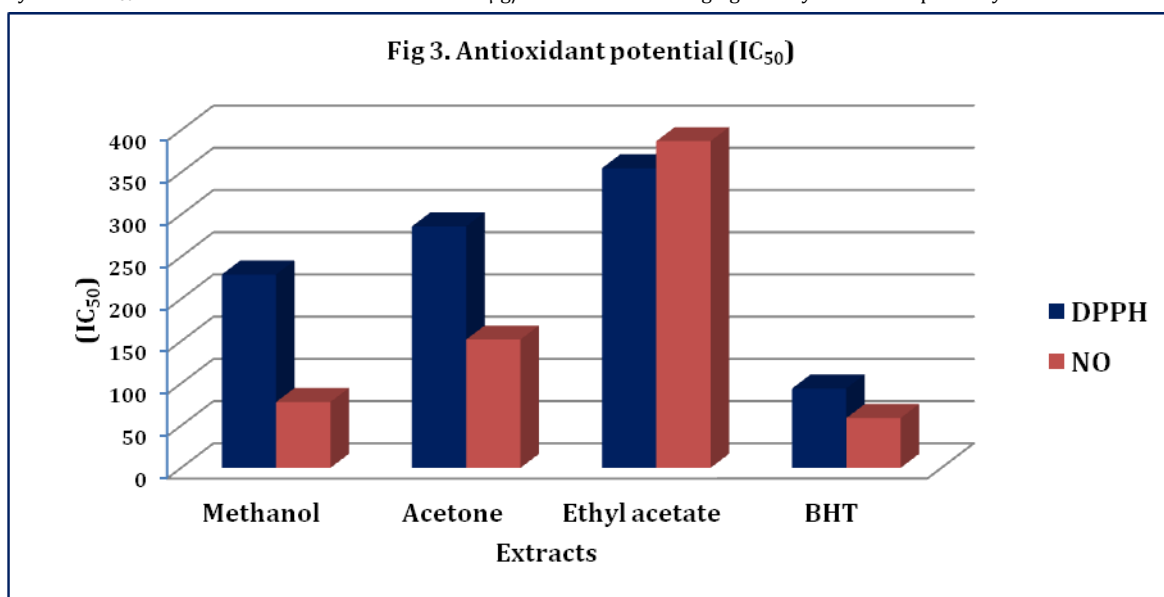
Methanol leaf extract seem to be a better antioxidant when compared to acetone and ethyl acetate extracts.

Antioxidant potential (IC₅₀)

Concentration of sample at which the inhibition percentage reaches 50% is the IC₅₀. The IC₅₀ values are shown in Fig 3. Lower the IC₅₀ higher the antioxidant potential. Methanol extract showed better antioxidant potential when compared with acetone and ethyl acetate

leaf extracts using DPPH and NO scavenging activity method. Methanol extract showed IC_{50} of $228.06 \pm 4.82 \mu\text{g/ml}$ and $77.81 \pm 4.32 \mu\text{g/ml}$ by DPPH and NO scavenging activity method respectively. While IC_{50} of acetone extract was $285.50 \pm 3.19 \mu\text{g/ml}$

and $152.03 \pm 2.87 \mu\text{g/ml}$ by DPPH and NO scavenging activity method respectively, whereas ethyl acetate extract showed IC_{50} of $355.74 \pm 2.56 \mu\text{g/ml}$ and $387.18 \pm 5.43 \mu\text{g/ml}$ by DPPH and NO scavenging activity method respectively.



The present study showed that the leaf extracts of *S. roxburghiana* had a strong antioxidative power on DPPH radicals and NO radicals and this could be attributed to the different phytochemical compounds present in these extracts²⁰. The role of flavonoids and phenolic compounds as antioxidants has been well established and there have been numerous reports on structure-activity relationships in the last decade^{21, 22}. The pronounced antioxidant potential of the methanol extracts could be attributed to the presence of phenols and flavonoids²⁰. Apart from phenols and flavonoids, methanol extract of leaves showed the strong presence of alkaloids, glycosides, phytosterol and steroids²⁰. Therefore, it is yet to be ascertained whether the antioxidant potential reported can be attributed to the flavonoids and phenols alone, or to any of the other phytochemicals, or to a synergistic effect of the compounds present^{23, 24}.

Literature survey shows a strong relationship between alkaloid content and antioxidant activity²⁵. As alkaloids also have a significant protective effect against H_2O_2 and they have the ability to scavenge hydroxyl radicals which contributes to their antioxidant effects²⁶. The present study on *S. roxburghiana* was comparable with the available literature on the related specie such as *Sansevieria hyacinthoides*²⁷.

The present study shows that DPPH and NO radical scavenging revealed the antioxidant potential of methanol extract to be more pronounced than acetone and ethyl acetate extracts and this could be attributed to the presence of high content of alkaloids, sterols, flavonoids and saponins.

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