EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF HYDROETHANOLIC LEAF EXTRACT OF *Sesbania bispinosa*

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Abstract: *Free radicals are produced as a result of a variety of metabolic processes, and their excessive generation causes a variety of disorders. As a result, excess free radicals must be neutralised The present study was aimed to investigate the in vitro free radical scavenging activity of leaf of Sesbania bispinosa . Standard methods were used to assess the free-radical scavenging activity of Sesbania bispinosa extracts. The plant Sesbania bispinosa has a high DPPH, Hydroxyl radical, hydrogen peroxide and Nitric oxide scavenging activity. The plant's resistance to free radicals was assessed using a reducing potential assay. It can be infered that the plant effectively scavenges free radicals. It is concluded that the hydroethanolic leaf extract of Sesbania bispinosa can be used as a possible source of antioxidants and as a therapeutic agent in free radical-induced disorders. According to the findings. Isolation and characterisation of the active antioxidants, which could serve as a potential supply of natural antioxidants, would require more research.*

Keywords: Free radicals; *Sesbania bispinosa*; Scavenging activity

1. INTRODUCTION

Free radicals are molecules or components of molecules with an unpaired electron in their atomic or molecular orbitals (1). Free radicals' biological reactivity and their contributions to oxidative stress have drawn a lot of interest and debate (2). Reactive oxygen species are produced when cells use oxygen, which is required for cell proliferation (ROS). The term "ROS" refers to a group of reactive oxygen species that are active in biological systems, including the superoxide anion radical (), hydrogen peroxide (H2O2), hydroxyl radical (), and others (3). Overproduction of reactive oxygen species (ROS) or free radicals causes oxidative stress, which damages proteins, DNA, and lipids. Oxidative stress is linked to chronic degenerative diseases like cancer (4), ischemic heart disease, inflammation, diabetes, ageing, atherosclerosis, immunosuppression, and neurodegenerative disorders (5).

Reactive oxygen species-induced damage can be alleviated using certain substances known as antioxidants, which are molecules capable of inhibiting oxidation of other molecules (6). The chemical process of oxidation involves the transfer of electrons from a substance to an oxidising agent. Free radicals can be produced during oxidation processes, and they can trigger cell-damaging chain reactions (7). Antioxidants stop these cascade processes by eliminating the free radical intermediates, and they block more oxidation reactions by becoming oxidised (8).

Antioxidants are chemicals found in plants that defend the body against injury from dangerous molecules known as free radicals (9). Antioxidants inhibit the oxidation process by dissipating free radicals (10). Natural antioxidants are particularly potent at preventing the damaging processes brought on by free radicals, either in the form of raw extracts or their chemical components (11). Numerous studies have focused on natural antioxidants, and many unprocessed extracts and pure natural components from plants have been found to possess antioxidant effects (12). As a result, plants and natural products may be a significant source of antioxidants that can scavenge free radicals and defend against diseases brought on by excessive oxidative stress.

Sesbania *bispinosa* (L.) Pers. is a soft wooded tree belonging to the family Paplionaceae. Antioxidant ability of the plant extract of *Sesbania bispinosa* was evaluated by using various *in-vitro* studies including DPPH (1, 1 diphenyl hydrazyl) radical scavenging activity, hydroxyl radical scavenging activity, hydrogen peroxide scavenging activity, Nitric oxide scavenging activity and total antioxidant assay. Various concentration of (100-500µg/ml) 50% ethanolic plant extract of *Sesbania bispinosa* and standard were analyzed for the evaluation of the antioxidant activity.

2. MATERIALS AND METHODS

2.1. Plant Collection and Authentication

In December 2014, the entire plant of *Sesbania bispinosa* was collected from the local regions (folklore shops) in Coimbatore district, Tamil Nadu, India. The plants were dried in the shade at room temperature. India's Nature Survey, Southern Regional Center, Coimbatore, India received and confirmed whole dried plants (No. BSI / SRC / 5/23/201415 / Tech1641).

2.2. DPPH Radical Scavenging Activity (13):

The radical scavenging activity of sample against DPPH was determined spectrophotometrically in a dark room by this method. The reaction mixture in a total volume of 3 ml contains 1 ml of DPPH, 0.5 ml of sample and made up to 3 ml with water. The tubes were incubated for 30 minutes at 37^0C. A blue color chromophore was formed, the absorbance of which was measured at 515 nm. Ascorbic acid was used as a standard for comparison.

2.3. Hydroxyl Radical Scavenging Assay (14):

The reaction mixture contained 0.1 ml of deoxy ribose (2.8 mM), 0.1 ml EDTA (0.1 mM). 0.1 ml H_20_2 (1 mM), 0.1 ml ascorbate (0.1 mM), 0.1 ml KH_2PO_4-KOH buffer, pH 7.4 (20 mM) and various concentration of plant extract in a final volume of 1 ml the reaction mixture and was incubated for 1 hour at 37^0C. Deoxyribose degradation was measured as TBARS and the percentage inhibition was calculated.

2.4. Hydrogen Peroxide Scavenging Assay (15):

The reaction mixture containing 0.5 ml of 4 mM H_2O_2 and 0.5 ml of different concentration (100 µg to 500 µg/ml) of various extracts were incubated at 37^0C for 10 minutes in dark. Control experiments without the test compound, but with equal volume of 4 Mm H_2O_2 was added and incubated in dark for 10 minutes.Absorbance was measured spectrophotometrically at 230 nm.

2.5. Nitric Oxide Radical Scavenging Activity (16):

The reaction mixture (3 ml) containing 2 ml of sodium nitropruside (10 mM), 0.5 ml of phosphate buffer saline (1 M) and 0.5 ml of different concentration (100 µg-500 µg/ml)of various extracts were incubated at 25^0C for 15 minutes. After incubation 0.5 ml of the Griss reagent was added. Control experiments without the test compound, but with equal volume of buffer were added. The absorbance of the chromophore formed during diazotitation coupling with naphthylethylenediamine was read at 540 nm.

2.6. Total Antioxidants Assay

2.6.1. Reducing Potential Assay (17):

1 ml of different concentration of extracts were mixed with 2.5 ml phosphate buffer (1 ml, 0.2, pH=6.6) and potassium ferricyanide $(K_3Fe(CN))_3$(1 ml, 10 %). The mixture was

incubated at 50^0C for 20 minutes. Trichloroacetic acid (1 ml, 10 %)was added to the mixture, which was then centrifuged for 10 minutes at 3000 rpm. 2.5 ml of the supernatant was taken. 2.5 ml distilled water and 0.5 ml of $FeCl_3$ (0.1 %) and the absorbance was measured at 700 nm using spectrometer.

2.7. Statistical analysis:

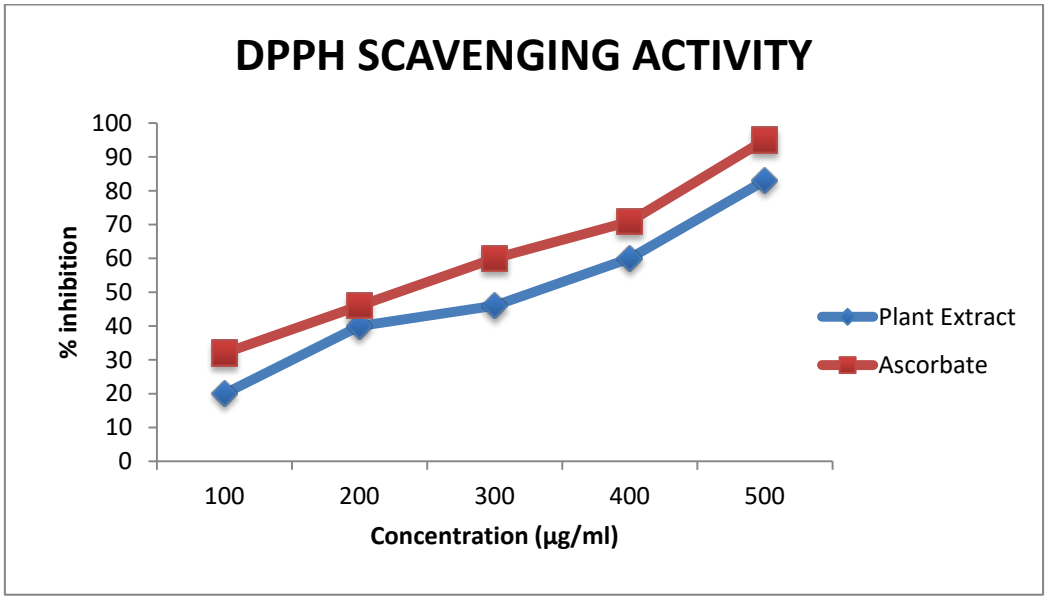
All the analyses were performed in triplicate and the results were statistically analyzed and expressed as mean (n=3) ± standard deviation.

3. RESULT AND DISCUSSION

3.1. DPPH Radicals Scavenging Assay

DPPH radical scavenging activity was shown by both standard ascorbate and *Sesbania bispinosa* extracts and was concentration dependent with an IC_{50} value of 254.48±1.68 and 302.40±1.10 µg /ml respectively The results of inhibition study are presented in Figure.1 Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain oxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation (18).

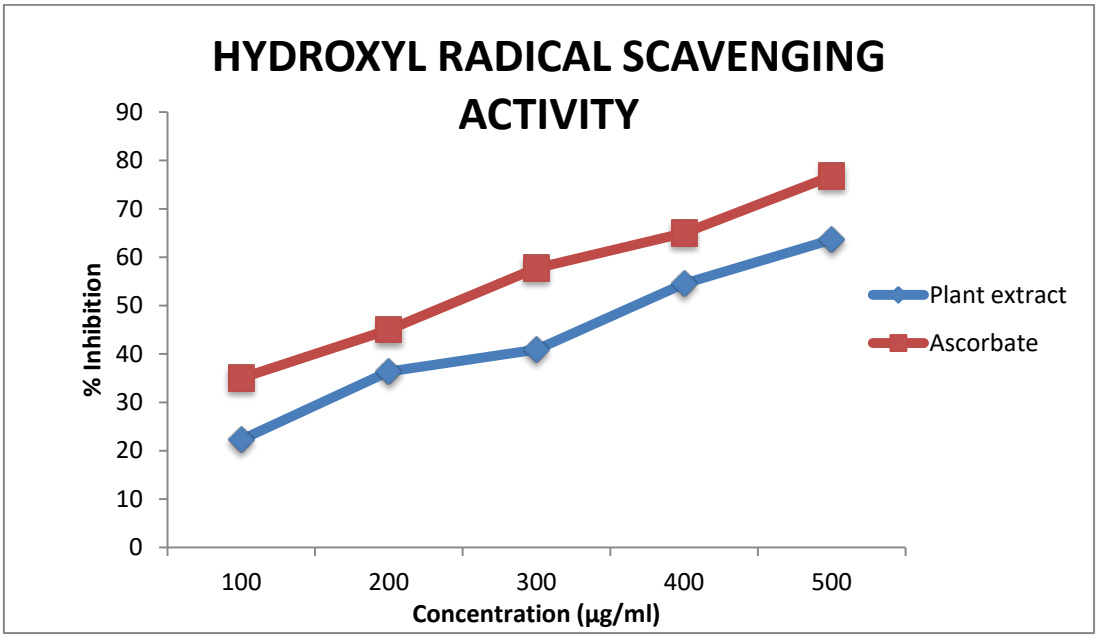
Figure 1: **DPPH radical scavenging activity plant extract of *Sesbania bispinosa***



3.2. Hydroxyl Radical Scavenging Activity:

In this study, the concentration of the studied plant extract capable of scavenging 50% of the hydroxyl radicals (IC50) was also determined. This study determined that 50% ethanolic plant extract of *Sesbania bispinosa* showed better antioxidant potential by hydroxyl (OH^-) radical scavenging method when compare to standard ascorbic acid and IC_{50} value found to be as 291.81±2.79 and 358.63±1.21µg/ml for ascorbic acid and *Sesbania bispinosa* extract respectively. So, we can say this plant is having antioxidant activity which was shown figure 2.

Figure 2: **Hydroxyl radical scavenging activity plant extract of *Sesbania bispinosa***

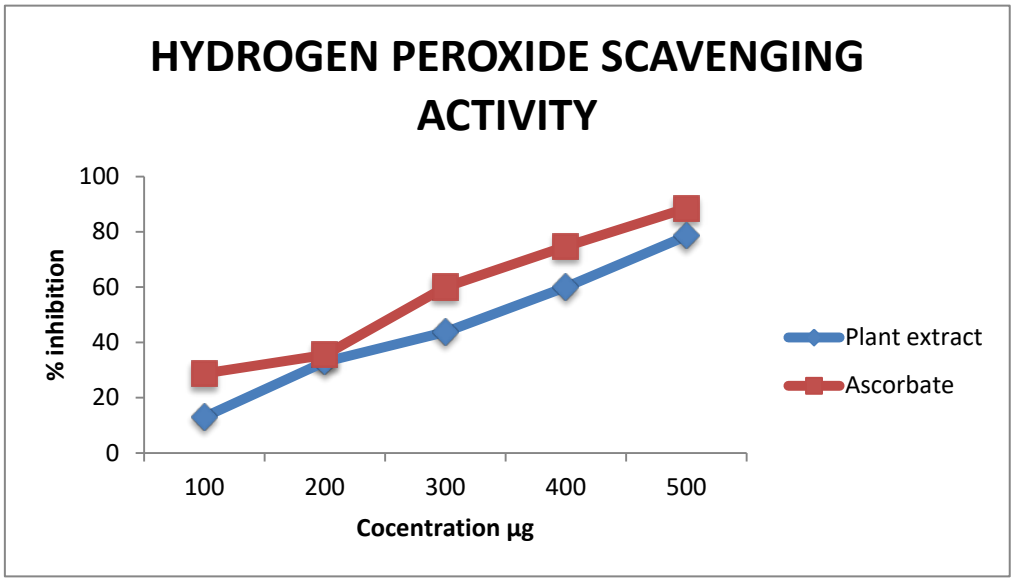


In the biological system, hydroxyl radical is a potent reactive oxygen species. It reacts with polyunsaturated fatty acid moieties of cell membrane phospholipids and causes damage to cell (19). By the reaction of H_2O_2 and the ferrous the hydrogen radicals were produced that would react with 2-deoxyribose. The reaction was terminated by adding TBA reagent that would give a red colour, if the malonaldehyde was formed as the result of the reaction between the radical and 2-deoxyribose. Hydroxyl radical scavenging capacity of an extract is directly proportional to its antioxidant activity which is indicated by the decreased degree of red colour (20).Direct interactions of hydroxyl radicals with DNA, resulting in DNA breakdown and therefore playing an important role in cancer formation (21).

3.3. Hydrogen Peroxide Scavenging Activity:

In this study, the extract of Sesbania bispinosa was compared to the IC50 values of ascorbic acid in scavenging hydrogen peroxide in a concentration-dependent manner. As demonstrated in figure-3, the scavenging action of hydrogen peroxide IC_{50} value found to be as 265.81±0.79 and 320.63±1.21µg/ml for ascorbic acid and *Sesbania bispinosa* extract respectively.

Figure 3: Hydrogen peroxide scavenging activity plant extract of *Sesbania bispinosa*



Hydrogen peroxide was a strong oxidising chemical that can directly inactivate a few enzymes by oxidising crucial thiol (-SH) groups (22). Hydrogen peroxide has the ability to quickly cross cell membranes, and once within the cell, it can likely combine with Fe2+ and potentially Cu2+ to create hydroxyl radicals, which could be the source of many of its damaging effects (23).

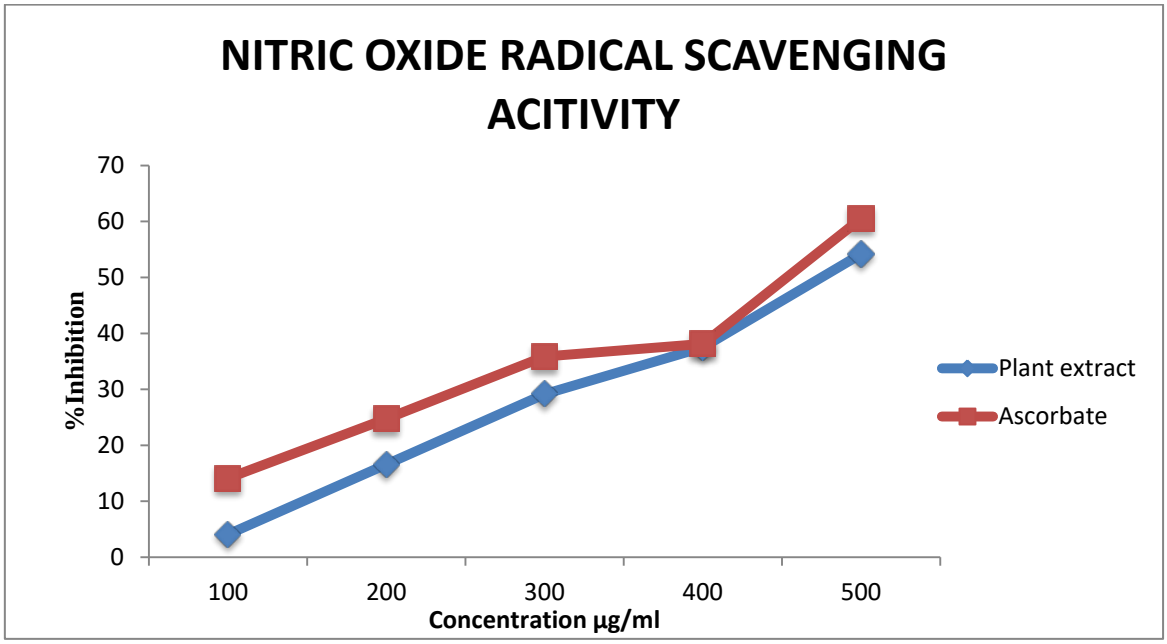
The extract's ability to scavenge hydrogen peroxide can be linked to active secondary metabolites called phenolics, which donate electrons to hydrogen peroxide, neutralising it and converting it to water (24). Ebrahimzdehet et al., 2010 reported a similar study employing medicinal plants (25).

3.4. Nitric Oxide Radical Scavenging Acitivity:

The scavenging effect of different concentrations of *Sesbania bispinosa* extract on hydrogen peroxide was concentrationdependent ((100-500 µg/ml) as shown in Figure-4. Result

displayed strong Nitric oxide radical scavenging activity (IC_{50} 354.90±1.10 µg/ml) whereas that of the standard, plant ectract exhibited 408.68±0.95 µg /ml.

Figure 4: Nitric oxide radical scavenging activity plant extract of *Sesbania bispinosa*



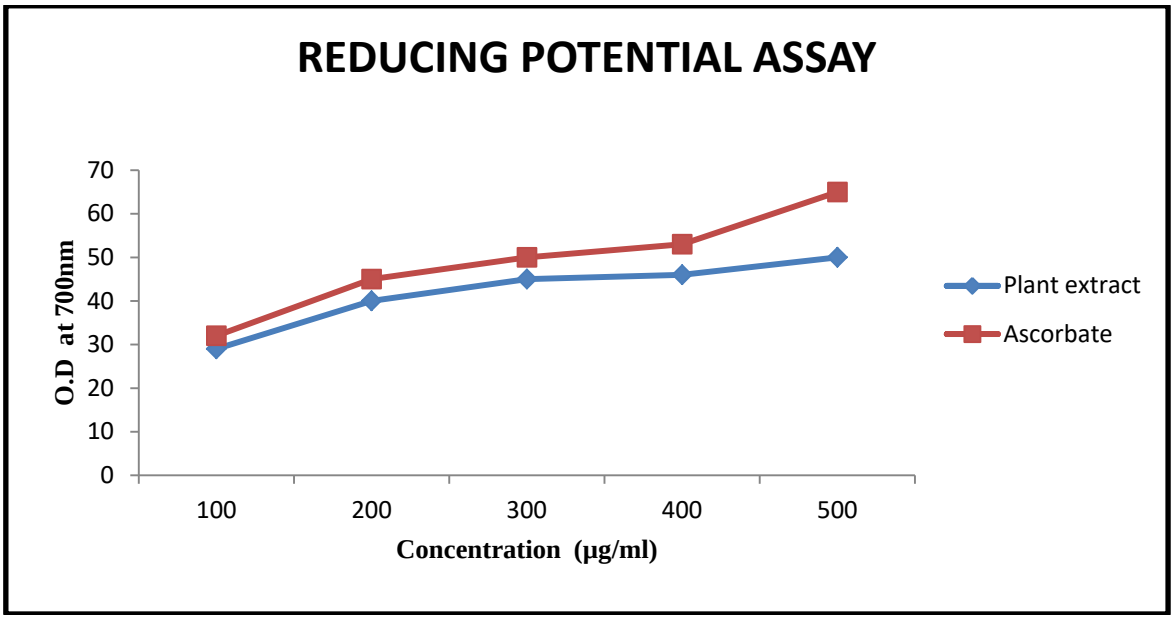
Nitric oxide (NO) was a reactive oxygen species that has been compared as a modulator of vascular endothelial cell responses (26). When interacted with superoxide (O2) in the epithelium, it can cause hypertension and DNA oxidative damage due to the creation of peroxynitrite anion ($ONOO^-$) which has the ability to mutilate supercoiled DNA structure (27) Nitric oxide is also a potent pleiotropic inhibitor of physiological processes like smooth muscle relaxation, neuronal signalling, platelet aggregation inhibition, and cell-mediated toxicity regulation. It plays an important role in neuromodulation and as a neurotransmitter in the central nervous system (28).

3.5. Total Antioxidant Assay:

3.5.1. Reducing Pontential Assay:

The data for the extract's reduction power was shown in the graph. The extract's reducing power grew as the concentration of the extract increased, and it had a moderate reducing power that was comparable to that of vitamin C. Figure 5 shows the dose response curves for the reducing powers of *Sesbania bispinosa* and ascorbate was 420.81±2.56 and 341.36±1.54 µg/ml respectively.

Figure 5: Reducing potential assay of plant extract of *Sesbania bispinosa*



Reducing power was linked to antioxidant activity and may be a useful indicator of antioxidant activity (29). The reducing power of a bioactive molecule depends upon the presence of reductants which can be ascribed by its hydrogen donating ability to convert free radicals into more stable metabolites (30).

The presence of e-donating chemicals caused Fe3+ (ferricyanide) to be reduced to Fe2+ in this experiment (ferrous) in dose dependent manner.Compounds having reducing power are electron donors that can reduce oxidised intermediates in lipid peroxidation processes, making them primary and secondary antioxidants (31).

4. CONCLUSION

From the current study, *Invitro* antioxidant activity of plant extract of *Sesbania bispinosa* were observed. It possesses a strong scavenging activity against DPPH, Hydroxyl radical and Nitric oxide. The capacity of the plant against free radicals was measured by reducing potential assay. It can be infered that the plant scavenges the free radicals efficiently. Thus, the *Sesbania bispinosa* extracts can be considered as new sources of natural antioxidants. Bioactive molecules present in the extracts can be further used as aprototype for development of new drugs and/or as a source of antioxidant pharmaceutical raw material.

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