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Antioxidant potential of Artemisia pallens roots

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Abstract: Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Dietary antioxidants include selenium, vitamin A and the related carotenoids, vitamin C, and vitamin E, plus various phytochemicals such as lycopene,lutein, and quercetin. They are believed to play a role in preventing the development of such chronic diseases as cancer, heart disease, stroke, Alzheimer's disease, Rheumatoid arthritis and cataracts. *Artemisia pallens* is a valuable medicinal plant. Essential oils from *Artemisia* are of botanical and pharmaceutical interest. Its root, stem, bark, leaves, fruits, seeds and seed oil are applied in traditional medicines to cure various health complaints. Antioxidant potential of various extracts is determined using spectrphotometric methods. Results of DPPH and Nitric Oxide assay confirm that extracts obtained from roots of *A. pallens* possess significant antioxidant property.
Key words: *Artemisia pallens*, Antioxidant potential, DPPH, Nitric Oxide, UV Spectrophotometer.

Introduction

An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation reactions can produce free radicals and start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death of the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, an inhibit other oxidation reactions, where they get oxidized and behaves as antioxidants. They are often reducing agents such as thiols, ascorbic acid, or polyphenols.[1] The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants like phenolics, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases[2]. Dietary antioxidants, such as carotenoids, might help to prevent and fight several human diseases[3].

A. pallens , an aromatic medicinal herb, is cultivated for its fragrant leaves and flowers, which are used in floral decorations, religious offerings and for the extraction of an essential oil - Davana oil. This oil is mainly used in the flavouring of cakes, pastries, tobacco, and also some costly beverages[4]. Plants are accredited with anthelmintic, tonic, and antipyretic properties. They are also considered as good fodder[5]. Earlier, the phenolic content of microwave-assisted ethanol extract was reported by Kanimozhi P. *et al*[6]. Taking into consideration all

these facts an attempts were made to evaluate the antioxidant activity of the extracts of *A. pallens* roots using DPPH and Nitric Oxide assays.

Material And Methods

1,1-diphenyl-2-picrylhydrazyl (DPPH), sulphanilamide, naphthyl ethylenediamine dihydrochloride were obtained from Sigma Chemicals Co., USA. All other chemicals and reagents were of analytical grade. UV Spectrophotometer (UV-VIS1700Pharma Spectrophotometer Schimadzu) was used to measure the absorbance at various concentrations of the extracts under study.

Plant material: The plant material was collected from Jejuri, Maharashtra state, India. It was authenticated at Botanical Survey of India, Pune. Its authentication No. is BSI/WC/Tech/2008/1059.

Preparation of extracts: Air shade dried and pulverized material was used. The extracts of known volume of ethanol and distilled water were prepared. The freshly prepared extracts were analyzed to prevent any degradation. Solvents were recovered under reduced pressure to obtain crude extracts. Exactly weighed amounts of dried extracts were dissolved in known volume of ethanol and various aliquots of each extract were prepared and used for the DPPH and Nitric Oxide assays.

DPPH radical scavenging activity[7] 1, 1-diphenyl -2-picryl-hydrazyl (DPPH) is converted to 1, 1-diphenyl -2-picryl hydrazine when it reacts with antioxidants. A change in color from purple to yellow is observed. Aliquots of extract solutions were taken and a total volume of 3ml was made using methanol. 0.15ml of freshly prepared DPPH solution (98µg/ml) was added, stirred and left to stand at room temperature (27^0C) for 30 minutes in dark. The control contains only DPPH solution in methanol while methanol served as the blank (negative control).The reduction capability of DPPH radicals was determined by the decrease in its absorbance. Absorbance was noted at 517nm by using UV-VIS spectrophotometer.

Nitric Oxide scavenging activity[8] In this spectrphotometric method the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylene diamine dihydrochloride was measured. Sodium nitroprusside (SNP-5mM) in phosphate-buffer saline was mixed with an equivalent amount of methanol to get the control. Methanol served as blank. Methanol was added to test solutions at different concentrations to make up a volume of 3ml and incubated at room temperature (270C) for 90 minutes. This incubated solution (1.5 ml) was added to 1.5 ml of Greiss Reagent (1% sulphanilamide, 2% phosphoric acid and 0.1% naphthyethylenediamine dihydrochloride). Absorbance at 546nm was noted using UV –VIS spectrophotometer. In both methods the capacity of scavenging free radicals was calculated as follows:

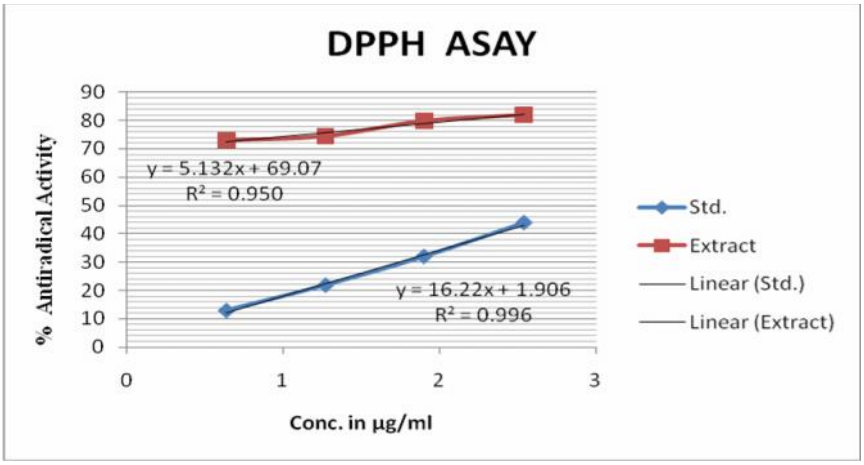
Scavenging activity (%) = {(Control Abs.—Sample Abs.)/Control Abs} × 100

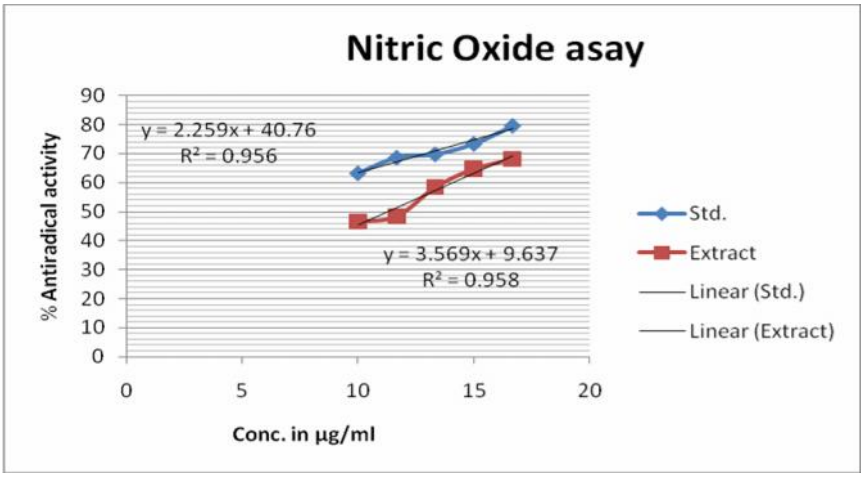
Ascorbic acid was used as the reference compound (positive control) with concentrations 20 to 500µg/ml for both the above spectroscopic methods of evaluating the radical scavenging activity.

Results And Discussion

Freshly prepared extracts of the dried plant material were subjected to screen for their possible antioxidant activities. DPPH free radical scavenging activity and Nitric Oxide scavenging methods were used. UV- VIS spectrophotometer was used for the experiments. DPPH radical scavenging test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to form a stable diamagnetic molecule. DPPH radical is reduced to the corresponding hydrazine, a color change of the solution from violet to yellow is observed and that is monitored spectrophotometrically. More reduction of DPPH radical is related to the high scavenging activity of the particular extract[9]. Nitric Oxide (NO) is a diffusible free radical that plays many effective roles in diverse biological systems including neuronal messenger, vasodilatation, antimicrobial and antitumor activities.[10] Nitric oxide is generated from the decomposition of Sodium nitroprusside (SNP) and measured by

Greiss Reagent.SNP in aqueous solution at physiological pH spontaneously generates NO, which interacts with oxygen to produce nitrite ions that can be measured by the use of Greiss reagent. A significant decrease in the NO radical is due to the scavenging activity of the extracts. At the range of concentration under study, ascorbic acid exhibited 90.16% inhibition; acetone extract exhibited higher radical scavenging activity than all other extracts by DPPH assay and by Nitric oxide method but it is lower than ascorbic acid. IC_{50} values were calculated from plotted graphs of scavenging activity against the concentrations of samples. The values of IC_{50} for each standard and extract is explain by graph.





Conclusion

In DPPH scavenging assay the IC_{50} value of the standard was 3.024µg/ml and IC_{50} value of extract was 3.013 µg/ml.In Nitric Oxide scavenging assay the IC_{50} value of the standard was 13.11µg/ml and IC_{50} value of extract was 12.66 µg/ml. The *A. pallens* roots show significant antioxidant activity against the standard.

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