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Antioxidant potential of Morinda Pubescence leaves

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Abstract: Antioxidants are nutrients as well as enzymes that can help to prevent or slow down oxidative damage of body. 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. *Morinda Pubescence* is a valuable medicinal plant. Its root, stem, bark, leaves, fruits, seeds and seed oil are applied in traditional medicines to cure various health complaints. The solvent extracts of leaves exhibit indicative to prominent antimicrobial activity. Antioxidant potential of various extracts is determined using spectrphotometric methods. Results of DPPH and Nitric Oxide assay confirm that extracts obtained from leaves of *M. pubescence* possess significant antioxidant property.
Key words: *Morinda Pubescence*, Antioxidant potential, DPPH, Nitric Oxide, UV Spectrophotometer.

Introduction

Antioxidants help our bodies repair and prevent damage caused by everyday living. Living cells produce different reactive oxygen species (ROS) such as superoxide, hydroxyl, peroxyl free radicals, singlet oxygen and hydrogen peroxide molecules. In living cells, ROS are continuously produced during normal physiologic events and removed by antioxidant defense mechanism[1-3]. Attack of ROS upon proteins produces carbonyls and other amino acid modifications that cause disorders of protein functions. ROS has also damage effects on the base and sugar units in DNA strand[4,5]. Consequently, accumulation of potentially harmful ROSs causes increase in stress, disease and aging periods and there is loss of haemostatic control and organ functions[6]. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxytoluene (BHT) have restricted use in food as they are under great consideration for toxicological reasons[7]. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in food. The role of antioxidants has attracted much interest with respect to their protective effect against free radical damage that may cause diseases including cancer. Many plant phenols, flavonols etc. other than antioxidant vitamins such as: C, E and carotenoids exert powerful antioxidant effects[7] .In recent years, the search for natural antioxidants, especially plant origin has greatly increased[8] .It is hard to prevent free radicals from forming in our

bodies, but we can reduce their destructive power by eating foods with plenty of antioxidants[9].
In Ayurvedic system of medicines *M. pubescence* has a great value[10]. Genus "Morinda" is known to elaborate a number of anthraquinones[11], both in a free condition and in the form of glycosides. The roots are used as cathartic[12-14] and febrifuge and applied externally to relieve pain in gout. Leaves are considered as tonic[15] and febrifuge. These are used in healing application for wounds and ulcers. The juice of leaves is externally applied in gout16.Fruits are used for spongy gums, throat complaints, dysentery, leucorrhoea and sapraemia17.The coloring
principle of plant root is present in the bark mainly as the glucoside [18-20]. Literature survey revealed the presence of anthraquinone [21,22].Taking into consideration all these facts an attempt is made to evaluate the antioxidant activity of the extracts of *M.Pubescence* leaves using DPPH assay and Nitric Oxide method.

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 Pune, Maharashtra; India. It was authenticated at Agharkar Research Institute, Pune Maharashtra, India. Its authentication No. is AHMA-21220

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 analysed to prevent any degradition. 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[23]: 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[24]

In this spectrophotometric method the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyethylenediamine 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 screening for their possible antioxidant activities. For this purpose, DPPH free radical scavenging activity and Nitric Oxide scavenging methods using UV- VIS spectrophotometer were employed. 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 [25].
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 [26]. Nitric oxide is generated from the decomposition of 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. IC50 values were calculated from plotted graphs of scavenging activity against the concentrations of samples. The values of IC50 for each standard and extract is explain by graph.

Conclusion

The flavonoids and phenolic compounds in plants have been reported to exert multiple biological effects including antioxidant, free radical scavenging abilities, anti-inflammatory, anticarcinogenic etc[27]. All extracts exhibit higher range of the radical scavenging activity. It means these extracts are rich in flavonoids as well as phenolic compounds which along with other polyphenolics in the plant material may be responsible for the antioxidant activities of these extracts. Above results strongly support the antioxidant potential of *M.Pubescence a*nd its importance as a rich source of natural antioxidants.

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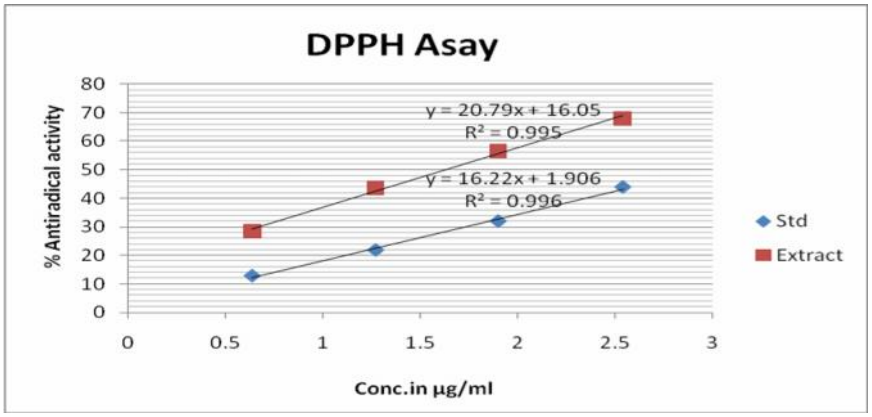

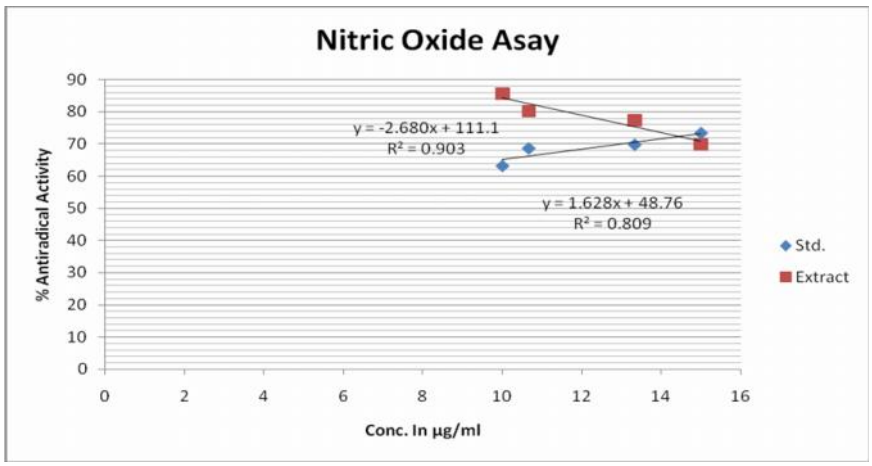

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