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Research Article

FREE RADICAL SCAVENGING ACTIVITY OF NEEM TREE (*AZADIRACHTA INDICA* A. JUSS VAR., MELIACEAE) ROOT BARK EXTRACT

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ABSTRACT

Purpose: The aim of this study was to investigate the antioxidant activity of the root bark extract from the neem tree (*Azadirachta indica* A. Juss var., Meliaceae) using the 1, 1-diphenyl-2-picryl hydrazyl (DPPH) scavenging assay.

Method: The root bark was extracted by soxhlet extraction method using 80% ethanol and the resultant extract was subjected to free radical scavenging activity by DPPH assay and total antioxidant activity. Qualitative analysis and thin-layer chromatography of the extract was also investigated.

Result: The results showed that the root bark extract exhibited higher free radical scavenging effect on the DPPH assay with 50% scavenging activity at 27.3 µg/ml. The total antioxidant activity of this extract was found to be 0.58 mM of standard ascorbic acid.

Conclusion: The results suggested that root bark extract of the neem tree have strong antioxidant potential. Thin-layer chromatography showed spot corresponding to quercetin.

Keywords: *Azadirachta indica*, Neem tree, Root bark, Antioxidant activity, DPPH, Total antioxidant activity.

INTRODUCTION

The family of free radical generated from the oxygen is called reactive oxygen species (ROS) which cause damage to other molecules by extracting electrons from them in order to attain stability. ROS are various forms of activated oxygen which include free radicals such as super oxide anion radicals (O_2^-), hydroxyl radicals (OH^-), non free radicals (H_2O_2) and singlet oxygen[1]. The molecular basis of many diseases is known to involve oxidative stress caused by free radicals. Recently progressive research has been directed at natural antioxidants (AO) [2]. Medicinal plants are important sources of antioxidants [3]. The secondary metabolites like terpenoids and flavonoids from plants have been reported to be potent free radical scavengers [4].

Azadirachta indica Juss (AI) is a large ever green tree which belongs to the *Meliaceae* family. This plant is abundantly found in every part of India and has been used "sarvaroganivarini"[5, 6]. All parts of the plant have been used for medicinal purposes including fruits, seeds, leaves, roots and barks[7]. This tree has been screened for antioxidant activity and it was found that various parts like leaves, seeds, flowers, stem bark and root bark could promote high antioxidant activity[8-13].

The extracts from several parts of neem tree showed free radical scavenging activity against 1,1-diphenyl- 2-picrylhydrazyl (DPPH) and 2,2'-azinodi-(3-ethylbenzthiazoline sulphonate) (ABTS) radicals, especially the leaf, flower and stem bark extracts which showed strong activities. Reports suggest that neem tree can probably be used as a healthy food [14]. As there is no established *in vitro* report on AO activity of root bark of AI, present research has taken up to investigate the AO activity of the hydro alcoholic extract of root bark of neem tree.

MATERIALS AND METHODS

Plant material

The root bark of AI was collected from Hyderabad, India during the period of December 2010 to February 2011. The plant sample was authenticated at botany division, Osmania University and the voucher specimen was deposited at the same place (0125).

Chemicals

Rutin, DPPH, DMSO, ammonium molybdate

Preparation of extract

Shade dried root bark was coarsely powdered and 100g of plant material was subjected to soxhlet extraction by using 80% ethanol for three cycles, filtered and evaporated under reduced pressure to yield the hydro alcoholic extract (4.5% w/w).

DPPH Assay

Free radical scavenging activity of extract and the standard rutin solutions were determined based on their ability to react with the stable DPPH free radical [15].The DPPH free radical is reduced to a corresponding hydrazine when it reacts with hydrogen donors. The DPPH radical is purple in color and upon reaction with hydrogen donor changes to yellow color [16]. It is a discoloration assay, which is evaluated by the addition of the antioxidant to a DPPH solution and the decrease in absorbance was measured at 490nm.

Reagents

2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 µM)

22 mg of DPPH was accurately weighed and dissolved in 100 ml of methanol. From this stock solution, 18 ml was taken and diluted to 100 ml using methanol to obtain 100 µM DPPH solution.

Preparation of test solutions

42 mg each of the extracts was dissolved in distilled DMSO separately to obtain solutions of 42 mg/ml concentrations. Each of these solutions was serially diluted separately to obtain lower concentrations.

Preparation of standard solutions

10 mg each of rutin were weighed separately and dissolved in 0.95 ml of dimethyl sulfoxide (DMSO) to get 10.5 mg/ml concentrations. These solutions were serially diluted with DMSO to get lower concentrations.

Procedure

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used were 2000, 1000, 500, 250, 125, 62.5, 31.25 and 15.625, 7.812 µg/ml. The plates were incubated at 37° C for 30 min and the absorbance of each

solution was measured at 490 nm, using a microplate reader. The corresponding blank readings were also taken and percent inhibition was then calculated as follows[17]:

$$\% \text{ inhibition} = \frac{(A_{blank} - A_{extract})}{A_{blank}}$$

The IC_{50} value, the concentration of sample required for 50% scavenging of the DPPH free radical, was determined from the curve of percent scavenging plotted against the concentration. Each sample was tested in triplicate. The average of the IC_{50} value was then calculated.

Total Antioxidant activity[18]

The total antioxidant capacity was determined by phosphomolybdenum method and is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compounds and the formation of a green Mo (V) complex which has the maximal absorption at 695 nm.

Preparation of test and standard solutions

Weighed accurately 55 mg of each extract and the standard ascorbic acid were dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO.

Procedure

An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined in an Eppendorff tube with 1 ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min. The samples were cooled to room temperature, and the absorbance of each solution was measured at 695 nm using Shimadzu UV-1800 spectrophotometer. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.

Thin-layer chromatography (TLC)

Based on the antioxidant activity obtained from above assays we performed the qualitative analysis[19, 20] and TLC. TLC was performed on pre coated silica gel 60 using ethyl acetate: formic acid: glacial acetic acid: water (100: 11:11: 26) as mobile phase[21, 22].

RESULTS

Qualitative chemical analysis of root bark extract showed the presence of saponins, flavonoids and terpenoids.

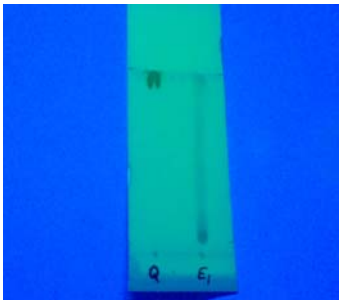

Fig. 1: Thin layer chromatogram of *Azadirachta indica* root bark extract obtained from soxhlet extraction.

Q: Standard quercetin, E_1: Soxhlet extract, Solvent system: ethyl acetate: formic acid: glacial acetic acid (100: 11: 11:26)

Table 1: Free radical scavenging and total antioxidant capacity of extract from the root bark of the *Azadirachta indica*

Sample	DPPH IC_{50} values ± SE µg/ml	Total Antioxidant capacity *
Extract	27.3 ± 0.23	0.58 ± 0.66 mM
Rutin	4.91 ± 0.09	-

* The total antioxidant capacity was expressed as mM equivalent of ascorbic acid per gram of dry weight

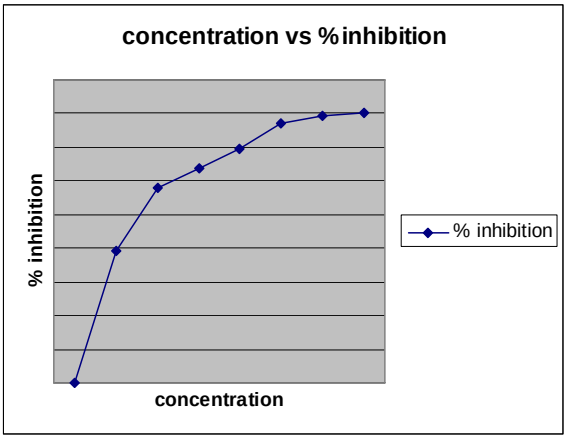

Fig. 2: DPPH radical scavenging activity of root bark extract of *Azadirachta indica*

The root bark hydro alcoholic extract of *Azadirachta indica* exhibited significant free radical scavenging activity as determined by DPPH assay and total antioxidant activity.

DISCUSSION

Root bark hydro alcoholic extract of *Azadirachta indica* exhibited significant antioxidant activity. Because previously there is no evidence of evaluation of antioxidant activity of root bark of *Azadirachta indica* by DPPH and total antioxidant activity, this part of the neem tree was selected for antioxidant activity.

Chemicals associated with root bark of *Azadirachta indica* are tricyclic diterpenoids like margocin, margocinin, margocilin, gedunin and poly-saccharides. The bark also yields an antitumor polysaccharide. Besides polysaccharides, several diterpenoids such as nimbinone, nimbolicin, margocin, nimbidiol, nimbione, etc. have been isolated from root bark. There has been no report regarding antioxidant components in root bark of *Azadirachta indica*. A phytochemical screening by chemical tests and TLC showed that the root bark hydro alcoholic extract presented flavonoid, quercetin. Therefore, flavonoids might play a role in the antioxidant activity of the neem tree root bark. Researchers have studied the bioavailability of some antioxidants including quercetin and rutin. However there are no data concerning extracts of root bark of neem.

CONCLUSION

In conclusion the hydro alcoholic extract of root bark of *Azadirachta indica* had antioxidant activity against the DPPH radical and also had significant total antioxidant capacity. TLC showed spot of quercetin flavonoid. Further investigations are required to isolate and identify the antioxidant components, both qualitative and quantitative and assess the mechanisms of the activity. Clinical trials including toxicity test are also needed.

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