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Research Paper

Anti-Oxidant Activity of Methanolic Extract of Azadirachta Indica Shoots

Vijaya Lakshmi Nandikatti*, S. Srihitha, S. Asha, T. Venkatesh Reddy

Hindu College of Pharmacy, Guntur, Andhra Pradesh.

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ABSTRACT

Azadirachta indica (neem) is a well-known medicinal plant widely used in traditional medicine for its diverse therapeutic properties. The present study focuses on the phytochemical evaluation and antioxidant activity of methanolic extract of neem shoots (young leaves and tender twigs). Fresh shoots were collected, shade-dried, and extracted using methanol through maceration. Preliminary phytochemical screening revealed the presence of important bioactive constituents such as carbohydrates, flavonoids, phenols, tannins, alkaloids, steroids, glycosides, and saponins. The percentage yield of the extract was found to be 2%.The antioxidant activity was evaluated using the hydrogen peroxide (H_2O_2) scavenging assay method. The results demonstrated a concentration-dependent increase in antioxidant activity, with percentage inhibition ranging from 16.12% to 56.45% for the extract, compared to 9.67% to 59.67% for the standard ascorbic acid. The decrease in absorbance values with increasing concentration indicates effective scavenging of hydrogen peroxide radicals.The IC_{50} values were determined to be approximately 420 µg/ml for the neem extract and 425 µg/ml for ascorbic acid, indicating comparable antioxidant potential. The decrease in absorbance with increasing concentration confirmed effective scavenging of hydrogen peroxide radicals.

INTRODUCTION

Medicinal plants remain a major source of bioactive compounds for traditional and modern therapeutics, and neem occupies a prominent position among them because of its broad ethnomedicinal and pharmacological importance . Different parts of the neem plant exhibit various therapeutic aactivities. Among these, neem shoots are especially valued in traditional practice for oral hygiene, wound care, skin ailments, digestive support, and immune enhancement . In addition to traditional use, the reviewed material attributes several biological activities to neem shoots,

***Corresponding Author:** Vijaya Lakshmi Nandikatti
Address: *Hindu College of Pharmacy, Guntur, Andhra Pradesh.*
Email ✉: nandikatti2014@gmail.com

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supporting the need for systematic phytochemical and antioxidant evaluation.

Plant Profile

Botanical identity

Azadirachta indica belongs to the family Meliaceae. The taxonomic classification provided in the here is: Kingdom Plantae, Division Magnoliophyta, Order Rutales, Suborder Rutinae, Family Meliaceae, Genus Azadirachta, and Species indica . The plant is known by several vernacular names including Nimbolide, Neem, Vepa, Vembu, Arya veppu, and Nimbin.

Neem Shoot

Fig No.1

Neem shoots consist of the tender growing parts of the plant, including young twigs and emerging leaves. These shoots are traditionally used as chewing sticks for oral hygiene and are reported to help in gum infections, tooth decay, and bad breath because of their antiseptic and astringent properties . The same source also notes their traditional use in digestive disorders, blood puri cation, skin disease management, wound healing, and immunomodulation. Because shoots represent actively growing tissue, they are likely to contain appreciable levels of secondary metabolites responsible for both protective and therapeutic biological ects .

Biological Activity

Azadirachta indica shoots possess several pharmacological activities such as antibacterial, antioxidant, anti-inflammatory, hypoglycemic, antiangiogenic, and antimalarial effects due to the presence of bioactive compounds like nimbidin, azadirachtin, and quercetin [7,9,10,11].

II.MATERIALS & METHODS:

Collection of plant materials:

Fresh neem shoots were collected during morning times in the month of february and march.

Preparation of Extract:

The collected shoots are made free from digested materials and was allowed for maceration by socking in 100ml methanol (100gm of neem shoot).After 48 hours the extract was collected by sqeez ing and allowed for air dry . The weight of concentrated extract was noted and calculated the percentage yeild. The extract was stored for further use.

Preliminary phytochemical tests:

The extract was allowed for phytochemical screening and the tested for Carbohydrates, Proteins,Aminoacids, Phenols, tannins, Steriods Alkaloids, flavanoids, Glycosides, Saponins, Resin and Terpinoids.The result were tabulated in table No.1.

III. Evaluation of Antioxidant activity:

There are many ways for evaluating the antioxidant activity. Among them Hydrogen peroxide scavenging assay method is selected, because of its accuracy and potency.

Hydrogen Peroxide Free Radical Scavenging Activity method:

Antioxidant activity was done by following the Hydrogen peroxide free radical scavenging activity assay method. In this method the scavenging activity of natural antioxidants found in plant extracts against hydrogen peroxide (H2O2) has been widely tested by detecting the

decrement of H2O2 in an incubation system containing H2 O2 and the scavenger, using the classical UV method at 230 nm.[4]A solution of hydrogen peroxide 20mm it was prepared in phosphate buffer saline (PBS PH 7.4). Different concentrations of the extract (100 to 500µg/ml) in Methanol (1ml) were added to 0.6ml of hydrogen peroxide solution in PBS. After 10 minutes, the absorbance of samples and standard (Ascorbic acid) was measured at 230nm against a blank solution.

Principle:

Hydrogen peroxide reacts with specific chromogenic substrates in the presence of the enzyme peroxidase to form a coloured compound. Alternatively, H_2O_2 can be measured by its direct absorption of ultraviolet light at around 230nm, where a decrease in absorbance indicates its decomposition or scavenging. Thus,the assay is widely used to evaluate antioxidant activity, enzyme functions, and oxidative stress by correlating absorbance with hydrogen peroxide concentration.

Procedure:

Prepare a phosphate buffer (pH ~7.4). Prepare a hydrogen peroxide (H_2O_2) solution in the buffer. Take different test samples (e.g., plant extract) in test tubes. Add a fixed volume of H_2O_2 solution to each test sample. Prepare a control (H_2O_2 + buffer, without sample). Mix the solutions properly. Incubate the mixtures for about 10 minutes at room temperature. Measure the absorbance at 230nm using a U.V- spectrophotometer. The absorbencies were tabulated in table number 2. The percentage scavenging activity was Calculated by using following formula.

Where: A0 = Absorbance of control.

A1 = Absorbance of Shoots extract .

The result was tabulated in table No.2. The graphs were plotted by taking the concentration on X-axis and absorbance on Y-axis and concentration vs percentage and presented in figure no-2. The concentration required to inhibit 50% of hydrogen peroxide radicals (IC50) may be calculated from the plotted graph of percentage scavenging versus concentration. Lower IC50 values indicate stronger antioxidant activity.

RESULTS AND DISCUSSION:

1.PERCENTAGE YEILD:

The weight of methanolic extract of Azadirachta indica shoots was 2 grams and the percentage yield is 2%.

2.Phytochemical screening:

The Qualitative analysis of methanolic extract of Azadirachta indica shoots shows the presence of phytochemicals like Carbohydrates, Flavanoids, Steroids, Saponins and Phenols.

Table 1: phytochemicals present in the methanolic extract of Azadirachta indica shoots.

Phytoconstituents	Shoot constituents
CARBOHYDRATES	+-+
PROTEINS &AMINOACIDS	++
PHENOLS &TANNINS	+++
STEROIDS	+
ALKALOIDS	+ +++
FLAVANOIDS	++
GLYCOSIDES	+
SAPONINS	+
TERPINOIDS	+

3.ANTIOXIDANT ACTIVITY

The evolution of anti-oxidant activity of methanolic extract of Azadirachta indica shoots was done and by observing the absorbance of test solutions and its percentage inhibition, it shown potent activity against Hydrogen peroxide.

Table 2: -Absorbance and % inhibition of methanolic extract of Azadirachta indica shoots and Ascorbic acid.

S.NO	CONCENTRATION	STANDARD		CONTROL		IC 50 VALUE			
		ABSORBANCE	PERCENTAGE (%)	ABSORBANCE	PERCENTAGE (%)	ABSORBANCE		%INHIBITION	
1	100	0.52	16.12%	0.56	9.67%	ASC	AIF	ASC	AIF
2	200	0.45	27.41%	0.49	20.96%	436	423	50	50
3	300	0.38	39.70%	0.41	33.87%				
4	400	0.32	48.38%	0.33	46.77%				
5	500	0.27	56.45%	0.25	59.67%				

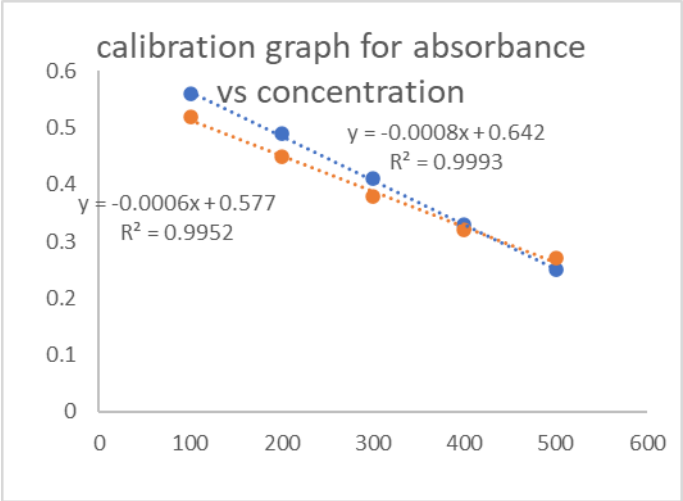

Fig. 1: Calibration graph for absorbance Vs concentration of methanolic extract of Azadirachta indica shoots (Orange) and Ascorbic acid (Blue).

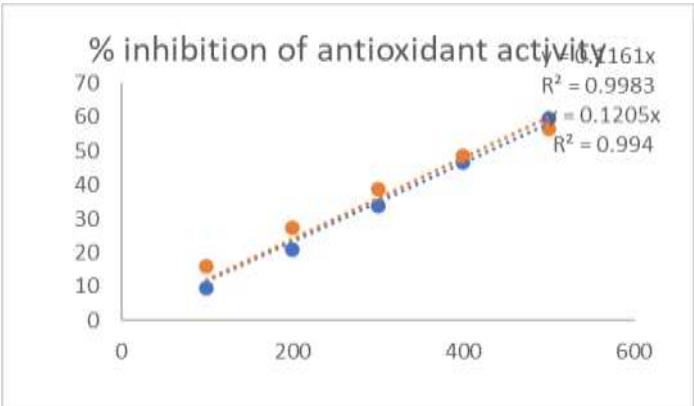

Fig. 2: Calibration graph for %inhibition Vs concentration of methanolic extract of Azadirachta indica shoots (Orange) and Ascorbic acid (Blue).

The absorbance values decreased with increasing concentration, confirming effective scavenging of hydrogen peroxide radicals. The IC_{50} values were calculated to be approximately 420 µg/ml for neem extract and 425 µg/ml for ascorbic acid, indicating that the antioxidant activity of neem shoots is comparable to the standard.

The observed antioxidant activity may be attributed to the presence of phenolic and flavonoid compounds, which are known to donate electrons and neutralize free radicals. These findings suggest that neem shoots are a potent natural source of antioxidants and may be useful in preventing oxidative stress-related diseases.

CONCLUSION

From our study, we found that neem shoots show good antioxidant activity. The results from different tests like DPPH and hydrogen peroxide scavenging indicate that the activity increases as the concentration increases. This means neem shoots contain natural compounds like flavonoids and phenolics which help in reducing free radical When compared with ascorbic acid, neem shoots showed strong antioxidant potential, and in some concentrations, even higher activity. Therefore, neem shoots can be considered as a natural and effective source of antioxidants and may be useful in preventing diseases caused by oxidative stress. Overall, our project proves that neem shoots have significant antioxidant properties and can be used in pharmaceutical and health-related applications.

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