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Research Article

Anti –Oxidant Activity of Methanolic Extract of *Azadirachta Indica* Flowers

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ABSTRACT

Azadirachta indica, commonly known as Neem, is a fast-growing, evergreen tree indigenous to India. All parts of the plant, including leaves, bark, seeds, roots, and flowers, are widely used in traditional medicine [1]. This study investigates the antioxidant potential of Neem flowers using a hydrogen peroxide (H_2O_2) scavenging assay. Extraction was collected by maceration, using the Methanol. Phytochemical screening was done by following schematic procedure and the Antioxidant activity was assessed based on the H2O2 (Hydrogen Peroxide) Free Radical Scavenging Activity Assay method, involving spectrophotometric measurement at 230 nm. The extract demonstrated the highest antioxidant activity, reaching 182.34% at 100 µg /ml, with an IC_{50} value of 67 µg /ml, compared to the ascorbic acid standard with an IC_{50} of 78.84 µg/ml. [5] Phytochemical screening confirmed the presence of flavonoids, phenols, and carbohydrates. The study reinforces the growing interest in plant-based therapies due to their safety, affordability, and minimal side effects when compared to conventional synthetic drugs. [2]

INTRODUCTION

Azadirachta indica (AI) is popularly known as "Neem", a fast-growing evergreen tree found commonly in India, Africa and America, with great medicinal value. It is one of the most versatile medicinal plants in that almost all of its parts like leaves, bark, flower, seed, and root etc., have long been used in Iranian, Indian, and Chinese traditional medicine [1].The plant product or natural products show an important role in diseases prevention and treatment like cancer.It is having pharmacological activities Anti-bacterial, anti-fungal, Anti-viral, Anti- infectious, Anti-cancer, Anti-Diabetic, Anti-Inflammatory, Hepatoprotective, Immuno-modulator, Anti-Hyper tensive, Anti-hyper lipidemic, Neuroprotection and maintain skin and hair health.[2][3] Oxygen free radicals interact with cells

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and damage them. The food ingredients and number of natural products that we are using in our daily life makes us free from these free radicals and maintaining healthy. *Azadirachta indica*, as mentioned above having different potent pharmacological activities including anti oxidant activity. The evalution of antioxidant activity was done by number of invitro methods. Among them, evalution of antioxidant activity by Hydrogen peroxide (H_2O_2) scavenging activity is the best and accurate when comparing with other. Hydrogen peroxide scavenging activity is physiological relevance, simple and cost-effective, reduces hydroxyl radical formation risk, ideal for natural product screening, flexible testing conditions, better mimic of oxidative stress, applicable in biological and industrial research, comparative accuracy over artificial radical assays thus we prefer this method.

MATERIALS AND METHODS:

Collection of plant materials and extraction:

The flowers of *Azadirachta indica* were collected from botanical garden of Hindu College Of Pharmacy, Guntur, A.P., in the month of March, during early mornings. The fresh Neem flowers (AIF) (250gms) were allowed for Maceration in Methanol (250ml) for 72hrs. Then the extract was concentrated by Rota- evaporator and crude extract wascollected and stored for further use.

Phyto chemical screening:

The methanolic extract was subjected for phytochemical screening by following the schematic procedure and performed the identification test for natural components like Carbohydrates, Amino acids, Proteins, Glycosides, Alkaloids, Flavonoids, Steroids, Saponins and Phenols. The result was presented in Table 1.

Anti-Oxidant activity:

H_2O_2 (Hydrogen Peroxide) Free Radical Scavenging Activity Assay 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 (H_2O_2) has been widely tested by detecting the decrement of H_2O_2 in an incubation system containing H_2O_2 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 (10 to 50µg/ml) in ethanol (1ml) were added to 2ml 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. The percentage of H_2O_2 scavenging activity of the AIF methanolic extract and the standard was calculated by using following equation.

H_2O_2 Scavenging activity (%) = [(Ac control – At)/At] X100

Where, AC = Absorbance of the control and AT= Absorbance of the tested compound or standards. The absorbance and the percentage inhibition values of both test and standard are mentioned in Table 2 and calibrated graph were ploted by taking concentration on X-axis and absorbance (Or) Percentage inhibition on Y-axis, mentioned in Figure 1 and 2.

RESULTS AND DISCUSSIONS:

Yield: The weight of methanolic extract of *Azadirachta indica* flowers was 5gram and the percent yield is 2%.

Phyto chemical screening:

The Qualitative analysis of methanolic extract of *Azadirachta indica* flowers shows the presence of phyto chemicals like Carbohydrates, Flavonoids, Saponins and Phenols.

Table 1: Phytochemicals present in the methanolic extract of *Azadirachta indica* flowers.

S. No	Phytoconstituents	Flower constituents
1.	Steroids	--
2.	Flavonoids	++
3.	Glycosides	--
4.	Phenols	++
5.	Saponins	-+
6.	Alkaloids	--
7.	Amino acids	--
8.	Carbohydrates	++
9.	Proteins	--
10.	Tannins	--

Where **(+)** indicates – presence, (-) indicates – absence of Phyto consistent.

Anti-Oxidant activity:

The evolution of anti-oxidant activity of methanolic extract of *Azadirachta indica* flowers 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* flowers and Ascorbic acid

S.NO	Concentration (µg/ml)	Absorbance		Inhibitory activity (in %)		IC 50 value			
		ASC	AIF extract	ASC	AIF extract	Absorbance		%Inhibition	
1.	10	00.640	00.690	18.75	10.14	ASC	AIF	ASC	AIF
2.	20	00.550	00.620	38.18	22.58	67.5	73.3	10.7	19.7
3.	30	00.530	00.580	43.39	31.03				
4.	40	00.470	00.540	61.70	40.74				
5.	50	00.410	00.460	85.36	65.21				

As the concentration of the extract increases there is decrease in the absorbance value, indicating the activity was dose dependent. This was supported by constructing calibrated graphs, showing the linearity.

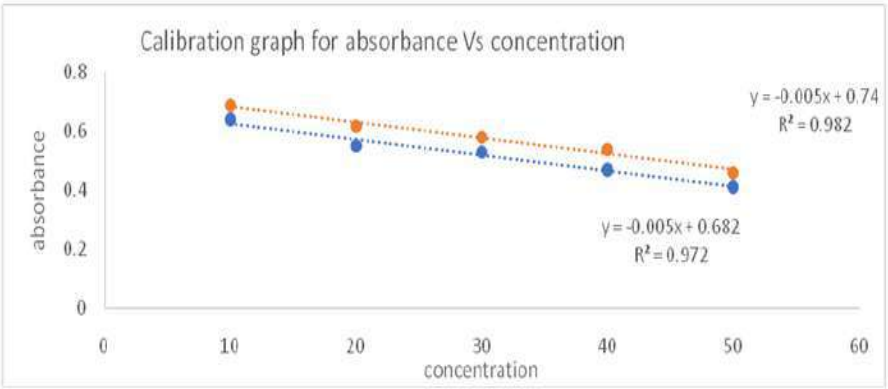

Fig. 1: Calibration graph for absorbance Vs concentration of methanolic extract of Azadirachta indica flowers (Orange) and Ascorbic acid (Blue).

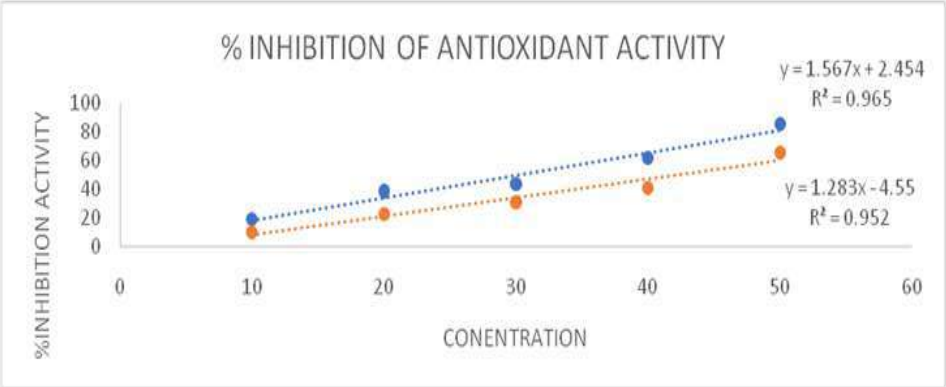

Fig. 2: Calibration graph for %inhibition Vs concentration of methanolic extract of *Azadirachta indica* flowers (Orange) and Ascorbic acid (Blue).

From the Calibration graph, it was observed that as the concentration of the extract increases there is increase in the percent inhibition on Hydrogen peroxide, shown the linearity. We calculated the IC50 value, which is 19.7 µg/ml of extract, is required to show the minimal activity.

CONCLUSION:

In the present study we evaluated the anti-oxidant activity of methanolic extract of Azadirachta indica flowers and identified the presence of phytochemicals like Carbohydrates, Flavonoids, Saponins and Phenols. It has shown potent Antioxidant activity against Hydrogen peroxide when compared standard Ascorbic acid. In future we want continue work by isolating active ingredients of this extract.

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