PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF EXTRACTS OF *AZADIRACHTA INDICA* PLANT.

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Abstract: *Azadirachta indica* is the medicinally potential plant which is used to cure various skin diseases such as acne, psoriasis and used for the treatment of diabetes and athlete's. It is commonly used in shampoos for treating dandruff and in a soaps or creams for skin conditions. The present investigation was undertaken to screen the phytochemical analysis and antioxidant activity of *Azadirachta indica* plant extracts. The phytochemical investigation showed positive result for carbohydrates, reducing sugars, protein, amino acids, alkaloids, flavonides in case of aqueous extract and ethanol extracts.

Keywords: Azadirachta indica, Phytochemical analysis, antioxidant activity, IC_{50}

I. INTRODUCTION

Most of us may have heard the name of an indigenous plant found in our country Neem this is the local name for a Plant called Margosa - the Indian Neem. Its scientific name Azadirachta indica. Neem is a native of India sub- continent the name of the genus Azadirachta is derived from the Persian name of the tree i.e. Azad-darakhat-e- hind the earlier mention was made by Abu Mansour in 970 A. D which means " the free tree of India" suggesting that it is intrinsically free from insect and disease problem. It is one of the important tree species belonging to Maliaceae family. Neem has also been called "Heal all", "Divine Tree", "Village Pharmacy" and even " Nature's drug store"

It is an evergreen tree, cultivated in various parts of subcontinent. Every part of the tree has been used as traditional medicine for household remedy against various oilments from antiquity. Neem has been extensively used as Ayuveda, Homeopathic medicine. The Sanskrit name of Neem tree is Arishtha, meaning reliever of sickness, Chemical investigation on the products of Neem tree was extensively undertaken by the many researchers. Extracts from the Neem tree (*A.indica*) also called 'Dogonyaro' in Nigeria are most consistently recommended in ancient medical texts for gastrointestinal upsets, diarrhoea and intestinal infections, skin ulcers and malaria. Its leave can be used as drug for diabetes, eczema and reduce fever. Barks of Neem can be used to make toothbrush and the roots has an ability to heal diseases and against insects. The seed of Neem tree has a high concentration of oil. Neem oil is widely used as insecticides, lubricant, drugs for variety of diseases such as diabetes and tuberculosis. In Africa, extracts from Neem leaves have provided various medicinal preparations. [1,9,10]

Neem plant also used for the smeared skin disorders with neem leaf juice, taken neem tea as a tonic and placed neem leaves in their beds, books, grain bins, cupboard and closets to keep away troublesome bugs. The number of benefits of neem is listed in ancient documents like 'Charak Samhita and Susruta Samhita.[2]

MATERIALS AND METHOD

The plant materials of Neem were collected from nearby area. The Leaves of Neem plants were shade dried at room temperature and ground in a manual mill to get coarse powder. The coarse powdered materials of leaves were kept in the airtight polythene bag and stored in dry place. These powders were extracted with ethanol and water by using soxhlet apparatus. The extracts were concentrated at 40^0C using rotary evaporator. Finally it was dried, crushed and stored in air tight bottles at 4^0C for further study.

PHYTOCHEMICAL SCREENING [1,4,6]

The chemical tests were performed for testing of different functional groups present in ethanolic and aqueous extract of leaves of test plants.

Table 1: Phytochemical analysis of Test plant Extracts.

Sr. No.	Chemical Constituents	Aqueous extract	Ethanol extract
1	Carbohydrates	+	+
2	Reducing sugar	+	+
3	Protein	+	+
4	Amino acid	+	+
5	Alkaloids	+	+
6	Cardic Glycosides	_	+
7	Flavonoids	+	+
8	Tannins	_	+
9	Steroids or Terpenoids	_	_
10	Saponins	_	+
11	Anthroquinones	_	_

PREPARATION FOR IDENTIFICATION OF QUERCETIN AND RUTIN [2]

(1) Preparation of leaves extract:

The Azadirachta indica leaves were washed under running tap water; it was then dried under shade and ground into course powder in the electronic grinder

(1**) Reagents and Materials** - Methanol and acetic acid were of HPLC grade. Deionized Water was prepared by water purification system. Rutin and Quercetin Were Obtained from SD fine chemicals

(2) plant material-

Preparation of Sample Solution-

An amount of 0.1-0.5 of ground plant material was extracted with 10 ml of solution Methanol and acetic acid –water [50:1:50] for 1 hour on a shaker at a laboratory temperature, 2ml of the extract were centrifuged for 10 min at a 2000 rot /min

Then solution was filtered through a micro filter with a regenerated cellulose membranes of the pore size 0.22 the filtrate was applied for HPLC

Preparation of standard solution

Standard stock solution of rutin and quercetin prepared in ethanol at concentration of 1, 5 , 10 and 15 ppm . All sample solution were filtered membrane filter. Rutin (Ru) and quercetin (Qu) where quntified by HPLC separation at 355.5 and 368 nm and the retention time for Ru and Qu was 6.7 and 9.8 min respectively.

STUDY OF ANTIOXIDANT ACTIVITY BY DPPH [5,6,7,8]

The antioxidant activity of the ethanol extracts of Leaves of *Azadirachtaindica,* plants were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). The diluted working solutions of the test plant extracts were prepared in ethanol. 0.004% of DPPH was prepared in ethyl alcohol and 3 ml of this solution was mixed with 3 ml of sample solutions. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using UV Visible spectrophotometer. Alcohol (3 ml) with DPPH solution (0.004%, 3 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below

Percentage (%) Inhibition of DPPH (% AA) = **A – B /A × 100**

Where A=Optical density of the blank and B=Optical density of the sample.

RESULTS AND DISCUSSION

The stock solution 1 mg/ml of ethanol was prepared. The required dilutions 0.1 mg/ml to 0.9 mg/ml were prepared by appropriate dilutions. The optical density and percent antioxidant activity were calculated (Table 2, 3; Figures 1, 2).

Table No. 2: O.D AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *A. indica*:

(O.D. of Black DPPH = 0.595)

Conc.mg/ml	*0.01*	*0.02*	*0.03*	*0.04*	*0.05*	*0.06*	*0.07*	*0.08*	*0.09*	*0.1*
O.D. of A.indica	*0.380*	*0.375*	*0.369*	*0.352*	*0.334*	*0.322*	*0.314*	*0.295*	*0.289*	*0.259*
%A A of *A.indica*	36.13	36.97	37.98	40.84	43.86	45.88	47.22	50.42	51.42	56.47

IC50 = 0.065 mg/ml

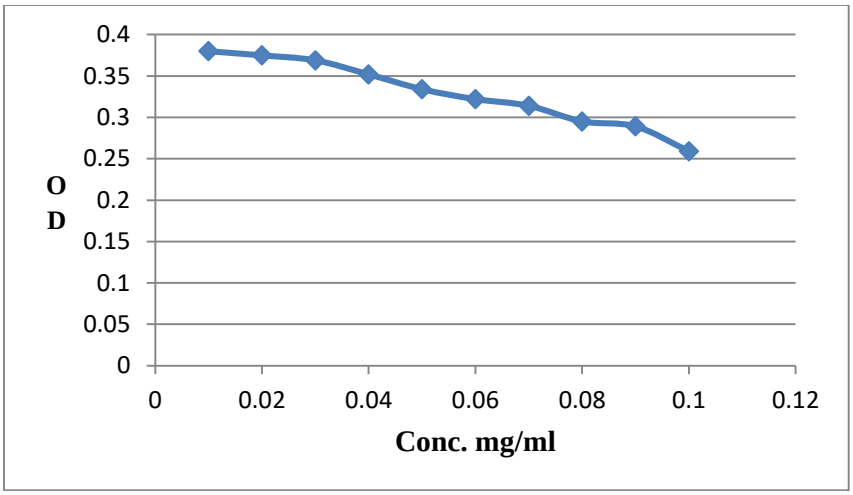

Fig. O. D. Extract of *A. Indica of Leaves*

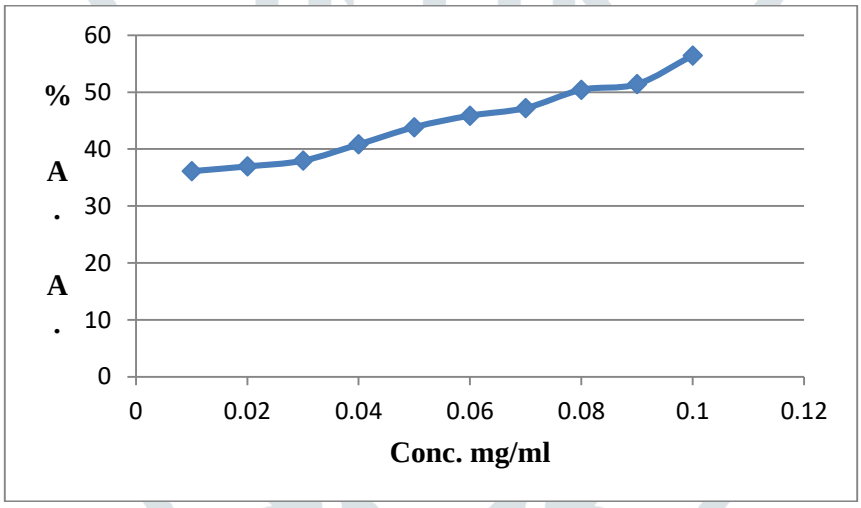

Fig. %A .A of Extract of *A. Indica of Leaves*

Calculation of IC_{50} Value for *Azadirachta indica* = max - ½ (max-min)
= 56.47 - ½ (56.47 – 36.13)
= 56.47– ½ (20.34)
= 56.47-10.17
=46.30
IC_{50} = 0.065 mg/ml

Table No. 3: O.D. and Antioxidant activity of Aqueous extract of A. indica leaves:-

Conc.mg/ml	*0.01*	*0.02*	*0.03*	*0.04*	*0.05*	*0.06*	*0.07*	*0.08*	*0.09*	*0.1*
O.D. of A.indica	0.520	0.515	0.502	0.485	0.450	0.395	0.352	0.298	0.271	0.250
%A A of *A.indica*	12.60	13.44	15.63	18.48	24.36	33.61	40.84	49.91	54.45	57.98

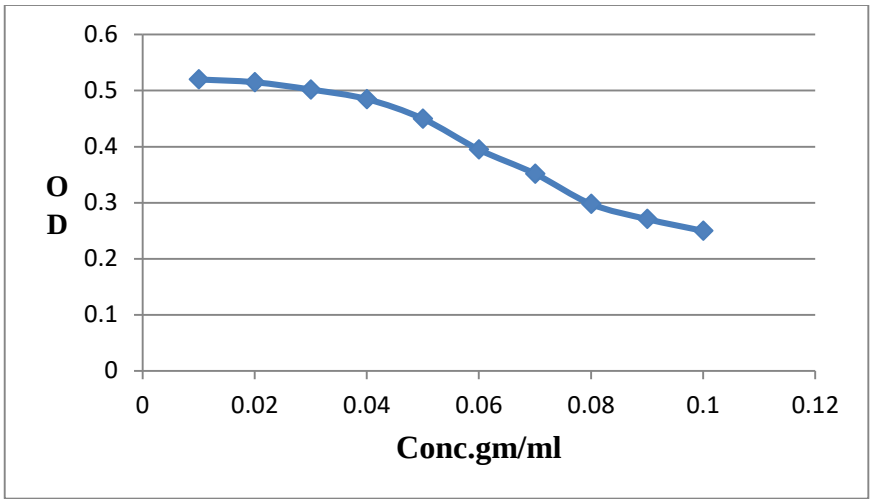

Fig. O. D. Extract of *A. Indica of Leaves*

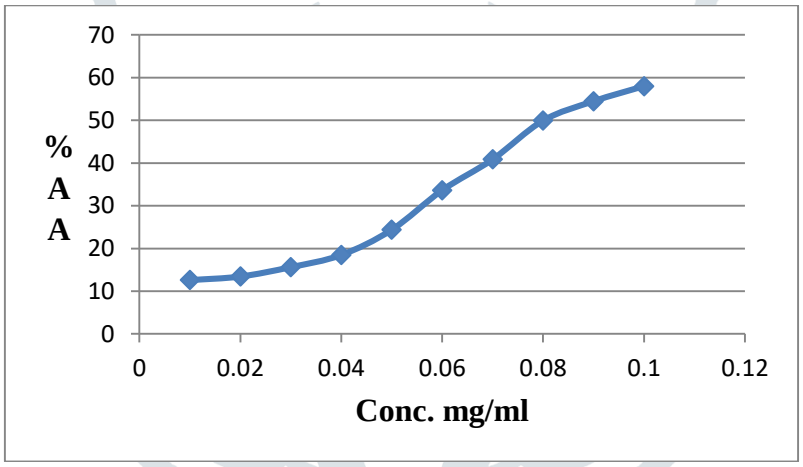

Fig. % A A of Extract of *A. Indica of Leaves*

Calculation of IC_{50} Value for *Azadirachtaindica* extract = max - ½ (max-min)
= 57.98 - ½ (57.98 – 12.60)
= 57.98– ½ (45.38)
= 57.98-22.69
=35.29
IC_{50} = 0.063 mg/ml

CONCLUSION

The phytochemical investigation of the plant extract showed the various phytochemicals found in the both extracts of the leaves. The results obtained for the antioxidant assay by DPPH for ethanol extracts and water extract of Leaves of *A.indica* plants are reported. Remarkable decrease in O. D. value of test plant samples were observed from the graph, showed antioxidant activity. The IC50 value for ethanol and aqueous extracts of Leaves of *A.indica* plants were found to be 0.075 mg/ml, 0.063 mg/ml respectively.

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