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Research Article

ANTI OXIDANT ACTIVITY OF STEM BARK OF *AZADIRACHTA INDICA* IN DIFFERENT SCAVENGING ACTIVITY

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ABSTRACT

The aim of this study was to investigate the antioxidant activity of the multi-solvent extracts (aqueous, ethanolic and ascorbic acid Bark of Medicinal plant i.e. *Azadirachta indica* using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) - scavenging assay and Nitric oxide radical scavenging activity, Superoxide radical scavenging activity, ferric ion scavenging activity, were compared with standard antioxidant like ascorbic acid. All the extracts showed good antioxidant activity. The plant Azadirachta indica and its plant parts may be exploited for clinical medicine as potent factor because of its high antioxidant activity.

Kevwords: *Azadirachta indica*. Antioxidant activitv. Radical. Stem Bark.

INTRODUCTION

India has a wealth of medicinal plants most of which have been traditionally used in Ayurveda, Unani systems of medicine and by tribal healers for generation. In ancient Indian literature, it is mentioned that every plant on this earth is useful for human beings, animals and other plants. Medicinal plants constitute the major constituents of most indigenous medicines and a large number of Western medical preparations contain one or more ingredients of plant origin. Medicines that are used today are not definitely the same as those that were used in ancient times or even in the recent past. Several modifications, improvement, sophistication and newer discoveries contribute continuously to the type, quality, presentation and concept of medicinal preparation. The therapeutic use of development of human knowledge, scientists endeavored to isolate different chemical constituents from plant, put them to biological and pharmacological tests and thus have been used to prepare modern medicines. There is an increasing interest in the measurement and use of plant antioxidant for scientific research as well as industrial (e.g., dietary, pharmaceutical and cosmetics) purposes. This is mainly due to their strong biological activity, excluding those of many synthetic antioxidants which have possible activity as promoters of carcinogenesis. Therefore, the need exists for safe, economic, powerful and natural antioxidants to replace these synthetic ones. Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts[1]. Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers[2]. A number of plants have been investigated for their biological activities and antioxidant principles[3]. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants[4]. In addition, naturally antioxidants have the capacity to improve food quality and stability and also act as nutraceuticals to terminate free radical chain reaction in biological systems, and thus may provide additional health benefits to consumers. Recent works have highlighted the role of polyphenolic compounds of the higher plants[5]. such as flavonols[6]. anthaquines[7]. Xanthaninsms that contribute to their anticarcinogen or cardioprotective effects. Increasing experimental evidence has suggested that these compounds can affect a wide range of cell biological function by virtue of their radical scavenging properties[8]. The intake of

antioxidants such as polyphenols has been effective in the prevention of diseases[9-10]. In the search of plants as a source of natural antioxidants, some medicinal plants and fruits have been extensively studied for their antioxidant activity and radical scavenging in the last few decades[11]. Some antioxidant compounds are extracted from easy sources, such as agricultural and horticultural crops, or medicinal plants. Among them the medicinal plants are taking the main role for providing a large number of pure antioxidants.

MATERIAL AND METHODS

Collection of plant material

The fresh leaves of *Azadirachta indica* (Neem) were purchased from local nursery garden during the month of July 2016. The plant material was identified and authenticated at, Sri Krishna devaraya University. Botany Department by Dr. S. Thimma Naik. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Material used for the antioxidant studies

All chemicals and solvents were of analytical grade and were obtained from Ranbaxy fine Chemicals, Mumbai, India. 1, 1-diphenyl,2-picrylhydrazyl (DPPH), Sulphanilamide, nitroblue tetrazolium (NBT), *o*-phenanthroline were obtained from sigma chemicals, USA. The other chemicals used were sodium nitroprusside, potassium Superoxide (DMSO), ethylene diamine tetra acetic acid (EDTA) and sodium hydroxide (NaOH), potassium dihydrogen phosphate, ferric chloride Ranbaxy Fine chemicals, Mumbai, India.

DPPH radical scavenging activity

DPPH scavenging activity was measured by the spectrophotometric method[12-13]. To an ethanolic solution of DPPH (1mM), equal volume **o**f test compounds dissolved in ethanol was added at different concentrations (2-1000µg/mL). An equal amount of ethanol was added to the control. After 20 min the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated.

$$\% \text{ scavenging activity} = \frac{\text{A Control - test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,

A control= absorbance of control

A test or A Std= absorbance of test or std

Scavenging of nitric oxide radical

Nitric oxide was generated from sodium nitroprusside and measured by griess reaction[14]. Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration (2-1000µg/mL) of the ethanol extract dissolved in phosphate buffer (0.025M, pH:7.4) and the tubes were incubated at 25^0C for 5 h. Control experiments without the test compound but with equivalent amount of buffer were conducted in an identical manner. After 5 h, 0.5mL of incubation solution was removed and diluted with 0.5mL of Griess reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotion of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546nm. The experiment was repeated in triplicate.

The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control - test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,

A control=absorbance of control;

A test A Std=absorbance of test or std.

Scavenging of superoxide radical

The scavenging activity towards the superoxide radical (O_2) was measured in terms of inhibition of generation of O_2[15-16]. The method was performed by using alkaline DMSO method. Potassium superoxide and dry DMSO were allowed to stand in contact for 24 h and the solution was filtered immediately before use. 200 µL of the filter was added to 2,8mL of an aqueous solution containing NBT (56µ M), EDTA (10µM) and potassium phosphate buffer (10mM). Test compounds (1mL) at various concentrations (2-1000µg/mL) were added and the absorbance was recorded at 560nm against a control in which pure DMSO was added instead of alkaline DMSO.

The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control - test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control= absorbance of control:
A test or A Std = absorbance of test or std.

Scavenging of ferric ions
Ortho substituted Phenolic compounds were found to be more active than un sub stiuted phenol. Hence these compounds may exert pro-oxidant effect by interacting with iron similar to ascorbate and gallic acid. It is concluded that antioxidant property of the compound is due to Phenolic group, which can react with a free radical to from the phenoxyl radical. The reaction mixture containing *O*-phenanthroline, ferric chloride, and drug at different concentration ranging from 2g to 1000g/mL in a final volume of 5mL was incubated for 10 min at ambient temperature. The absorbance at 510nm was recorded. Blank was carried out without drug[17]. Experiment was performed in triplicate.
The percentage inhibition was calculated using the formula:

$$\% \text{ scavenging activity} = \frac{\text{A Control} - \text{test or Std}}{\text{A control}} \times 100 \text{ Control}$$

Where,
A control=absorbance of control; A test or A Std=absorbance of test or std.

RESULTS AND DISCUSSION
DPPH model (colorimetric method)
The **Figure-1** shows concentration dependent DPPH scavenging of Ethanol fraction of ***Azadirachta indica Stem bark*** with maximum scavenging activity (67.07%) at 200µg/mL extract, and the scavenging activity water extract the maximum scavenging activity (53.23%) was observed at 200µg/mL and it where as the standard reference substance ascorbic acid showed maximum scavenging activity of 92.02% at 200 µg/mL **(Table**
Scavenging of nitric oxide radical: Figure -2 shows concentration dependent scavenging of nitric oxide radical by ethanol fraction of ***Azadirachta indica Stem bark*** with maximum scavenging activity (47.07%) at 200µg/mL extract, and the scavenging activity water extract the maximum scavenging activity (32.23%) was observed at 200µg/mL and it where as the standard reference substance ascorbic acid showed maximum scavenging activity of 68.02% at 200 µg/mL **(Table 2).**

Scavenging of superoxide radical
Figure-3 shows concentration dependent scavenging of super oxide radical by the ethanol fraction of ***Azadirachta indica Stem bark.*** The maximum scavenging activity (27.05%) was observed at 200µg/mL and the scavenging activity water extract the maximum scavenging activity (19.13%) was observed at 200µg/mL and it and it was comparable to that of the standard reference substance ascorbic acid which was showed 48.42 % of scavenging activity at 200µg/mL**(Table3).**

Scavenging of ferric ions
Figure-4 shows concentration dependent ferric ion scavenging activity of ethanol fraction of ***Azadirachta indica Stem bark***. The maximum scavenging activity (25.35%) was observed at 200µg/mL and minimum of 8.42% at 10µg/mL and the scavenging activity water extract the maximum scavenging activity (17.23%) was observed at 200µg/mL and it was comparable to that of the standard reference substance ascorbic acid which was showed scavenging activity of 41.42%at 200µg/mL (**Table-4).**

Table 1: Antioxidant activity of Alcohol and aqueous extract of *Azadirachta indica Stem bark* and Ascorbic acid (DPPH)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
10	23.34 ± 0.6	10.78 ±0.5	32.87 ±0.8
20	25.14 ± 0.5	14.32 ±0.7	35.83 ±0.4
40	28.12 ±1.2	16.35 ±0.2	43.91 ±1.0
60	30.34 ±1.0	17.12 ±23	51.23 ±2.3
80	32.02 ±0.54	19.14 ±0.5	58.23 ±0.8
100	35.45 ±1.2	19.54 ±3.4	68.87 ±1.1
120	52.23 ±1.3	32.45 ±1.2	78.94 ±1.4
140	59.23 ±1.1	42.16 ±0.4	81.32 ±0.6
180	62.12 ±23	50.23 ±1.2	87.45 ±0.8
200	67.07 ±0.7	53.23 ±2.3	92.02 ±0.8

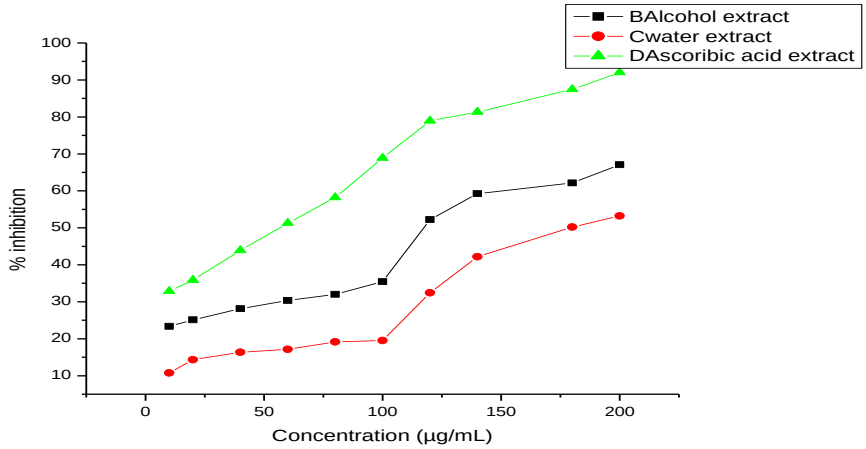

Fig. 1: DPPH radical scavenging model of *Azadirachta indica Stem bark* extracts

Table 2: Antioxidant activity of Alcohol and aqueous extract of *Azadirachta indica Stem bark* and Ascorbic acid (Nitric acid)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
10	13.34 ± 0.6	6.78 ±0.5	18.87 ±0.8
20	15.14 ± 0.5	9.32 ±0.7	22.83 ±0.4
40	18.12 ±1.2	11.35 ±0.2	26.91 ±1.0
60	20.34 ±1.0	13.12 ±23	31.23 ±2.3
80	22.02 ±0.5	14.14 ±0.5	36.23 ±0.8
100	25.45 ±1.2	15.54 ±3.4	42.87 ±1.1
120	32.23 ±1.3	21.45 ±1.2	47.94 ±1.4
140	39.23 ±1.1	24.16 ±0.4	54.32 ±0.6
180	42.12 ±23	27.23 ±1.2	62.45 ±0.8
200	47.07 ±0.7	32.23 ±2.3	68.02 ±0.8

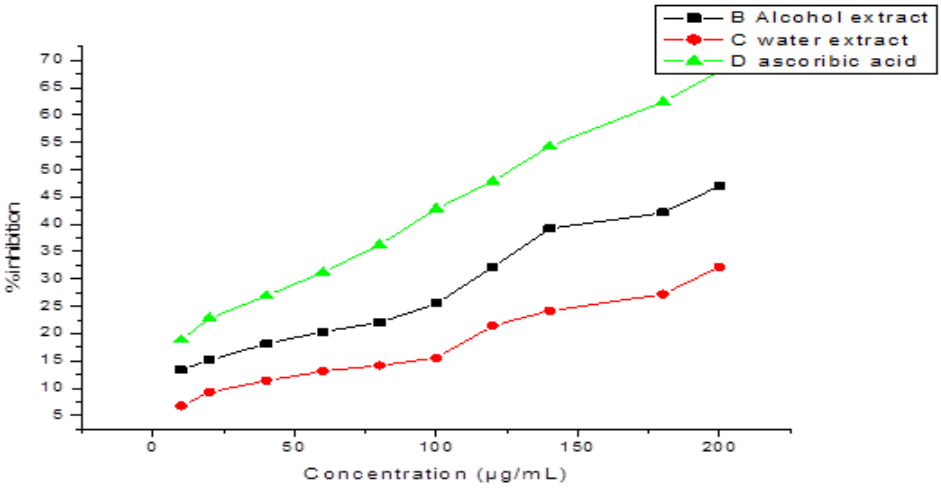

Fig. 2: Nitric acid radical scavenging model of *Azadirachta indica Stem bark* extracts

Table 3: Antioxidant activity of Alcohol and aqueous extract of *Azadirachta indica Stem bark* and Ascorbic acid (Superoxide radical)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
10	9.12± 0.6	3.78 ±0.4	12.17 ±0.8
20	11.12± 0.5	5.32 ±0.6	18.43 ±0.4
40	12.13±1.2	8.35 ±0.4	24.21 ±1.0
60	14.15±1.0	9.12 ±1.3	27.43 ±2.3
80	16.05±0.5	10.14 ±1.5	30.23 ±0.8
100	18.15±1.2	12.34 ±3.4	32.17 ±1.1
120	19.16±1.3	13.25 ±1.2	35.34 ±1.4
140	23.13±1.1	14.16 ±0.4	39.12 ±0.6
180	25.14 ±23	16.23 ±1.2	42.25 ±0.8
200	27.05 ±0.7	19.13 ±2.3	48.42 ±0.8

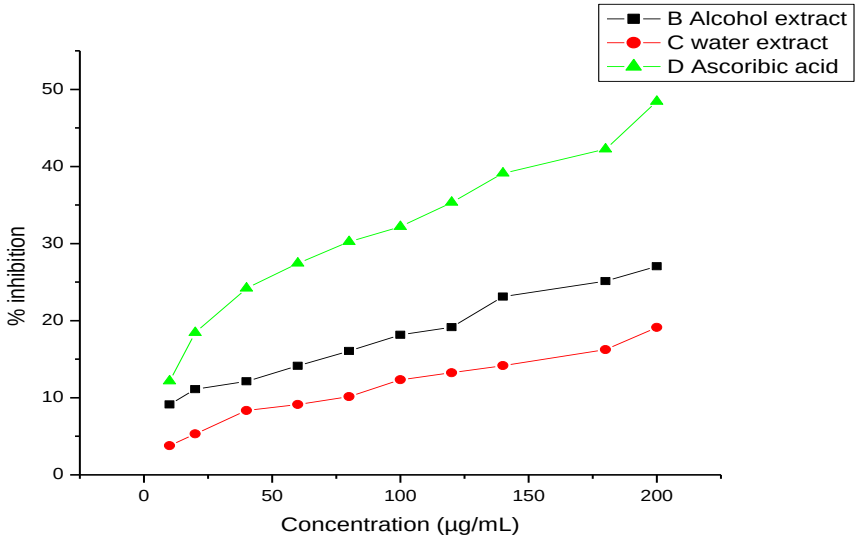

Fig. 3: (Superoxide radical) radical scavenging model of *Azadirachta indica Stem bark* extracts

Table 4: Antioxidant activity of Alcohol and aqueous extract of *Azadirachta indica Stem bark* and Ascorbic acid (ferric ion)

Conc. of extract (µg/ml)	% Inhibition *		
	Alcohol Extract	Water Extract	Ascorbic acid
10	8.42± 0.6	4.58 ±0.4	13.45 ±0.8
20	10.32± 0.5	6.42 ±0.6	16.13 ±0.4
40	11.13±1.2	8.85 ±0.4	20.41 ±1.5
60	12.35±1.0	9.02 ±1.3	22.43 ±1.3
80	14.25±0.5	10.78 ±1.5	26.53 ±0.8
100	16.15±1.2	12.02 ±3.4	29.24 ±1.5
120	17.26±1.3	12.54 ±1.2	30.12 ±1.7
140	20.23±1.1	13.16 ±0.4	32.16 ±0.6
180	22.14 ±23	15.12 ±1.2	37.26 ±0.3
200	25.35 ±0.7	17.23 ±2.3	41.42 ±0.8

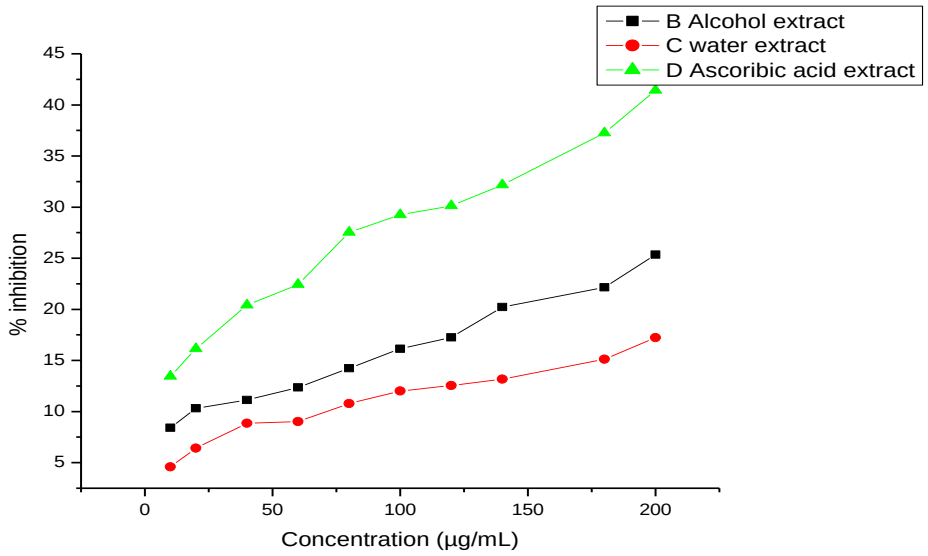

Fig. 4: ferric ion scavenging model of *Azadirachta indica Stem bark* extracts

CONCLUSION

The present study demonstrated that methanol stem extract of *Azadirachta indica* Showed promising antioxidant and radical scavenging activity, from the observation it can concluded that the stem of *Azadirachata indica* are the good sources of natural antioxidants and might be useful in treating the diseases associated with oxidative stress.

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