



Evaluation of Antioxidant Activity of Hydro Distilled Extracts of Leaf, Heart Wood and Flower of *Azadirachta Indica*

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ABSTRACT

The present study on evaluation of antioxidant activity of hydro distilled extracts of Leaf, heart wood and flower of *Azadirachta indica* Juss. revealed that the hydro distilled extract of heart wood at 500 µg/ml producing the highest free radical scavenging activity i.e. 70.66±0.78 %. The Neem heart wood has the highest amount of total phenol content (160 µg/ml) which is responsible for highest percentage of inhibition of DPPH radical. In conclusion Neem leaf, flower, and heart wood have potential for use in human health which is used as food by common people and in diabetes and different extracts of Neem is widely used for variety of diseases and also antioxidant potential for use in different pharmaceutical industries.

Keywords: Neem, Flower, Antioxidant activity, DPPH scavenging assay.

INTRODUCTION

Plants have a great potential for producing new drugs for human benefit. Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and even infectious diseases. According to a report of World Health Organization, more than 80% of world's populations depend on traditional medicine for their primary health care needs. The demand for more and more drugs from plant sources is continuously increasing. It is therefore essential for systematic evaluation of plants used in traditional medicine for various ailments. The increased interest in plant derived drugs is mainly because of the wide spread belief that 'herbal medicine' is safer than costly synthetic drugs which possesses side effects. Hence, there is need to screen medicinal plants for promising biological activity. Further, there is a continuous development of resistant strains which pose the need for search and development of new drug to cure diseases

The different parts of Neem (*Azadirachta indica*) seeds, leaves, flowers and bark have a vast pharmacological activity and are used as raw materials for pesticide, medicine and other commodities¹⁻³. Essential oils may have antioxidant properties and their consumption can influence immune cell functions^{4,6}. The biological activity of the oils can be compared with the activity of synthetically produced pharmacological preparations and should be investigated in the same way.^{7,8} The high antioxidant activities of plant phenolic compounds are attractive to the food industry, prompting their use as replacements for synthetic antioxidants and also as nutraceuticals have a significant role in preventing many diseases. The Neem flowers are also useful in medicine, food and pharmaceutical fields⁵. The present study has been undertaken to evaluate the antioxidant activity as well as the compounds related to antioxidant such as

phenol in hydro distilled extracts of Neem flower, leaf and heart wood which are not documented .

MATERIALS AND METHODS

Plant material

Leaves, heartwood and flowers of the plant have separately been collected from plant and crushed separately using mechanical grinder and then subjected to hydro distillation.

Hydro distillation of volatiles from neem leaf, heart wood and flowers

Hydro distillation of freshly collected mature leaves, heartwood and flowers was carried out separately immediately after the material was crushed to prevent the loss of volatiles. Clevenger apparatus B.P. used for hydrodistillation. Volatiles was abbreviated as AZL for leaves, AZHW for heartwood and AZF for flowers.

Chemicals and Reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonate (Merck Pvt. Ltd, India), Catechol (Himedia Lab., India), 2, 2-Diphenyl-2-picryl hydrazyl (DPPH) and Ascorbic acid are obtained from (Himedia Lab., India). Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

Antioxidant activity

Phenolic Content estimation

The total phenolic content of hydro distilled extract of leaf, heart wood and flowers were determined by using Folin-Phenol Spectrophotometric method. In this method the blue color formed due to the phenol present in extract measured using UV-vis Spectrophotometer at 760 nm. The results were expressed as gm/100gm of Tocopherol.



Free radical scavenging activity by DPPH Method

The antioxidant activity of hydro distilled extract of leaf, heart wood and flowers the on the basis of the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described with slight modification⁹. The different concentrations of extracts were prepared. All the solutions were prepared with methanol 5 ml of each prepared concentration was mixed with 0.5mL of 1mM DPPH solution in methanol. Experiment was done in triplicate. The test tubes were incubated for 30 min. at room temperature and the absorbance measured at 517 nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid was used as a standard and the same concentrations were prepared as the test solutions. The different in absorbance between the test and the control (DPPH in ethanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

$$\text{Scavenging effect (\%)} = (1 - A_s/A_c) \times 100$$

As is the absorbance of the sample at t = 0 min.

Ac is the absorbance of the control at t = 30 min.

RESULTS AND DISCUSSION

Free Radical and Antioxidant Activity

Table-2 shows the results of the free radical (DPPH) scavenging activity in % inhibition in AZL AZHW and AZF. The result revealed that hydro distilled fraction of heartwood exhibited the highest radical scavenging activity with 70.66 ± 0.78 followed by leaf with 48.04 ± 0.98 and flowers with 26.29 ± 0.81

Fig.1. Shows the comparative study of radical scavenging activity between. AZL, AZHW and AZF with respect to Ascorbic acid as standard.

Table 1: Total Polyphenolic content of AZL, AZHW and AZF

Extract	Total Phenolic content ($\mu\text{g/ml}$)
AZL	120
AZHW	160
AZF	50

Table 2: Antioxidant activity of AZL, AZHW and AZF

Concentration ($\mu\text{g/ml}$)	% Antioxidant activity		
	AZL	AZHW	AZF
100	13.45 ± 0.83	28.95 ± 0.25	7.33 ± 0.44
200	18.58 ± 0.88	43.00 ± 0.47	11.87 ± 0.55
300	28.08 ± 0.89	54.50 ± 0.57	17.29 ± 0.61
400	37.95 ± 0.91	63.08 ± 0.67	22.62 ± 0.78
500	48.04 ± 0.98	70.66 ± 0.78	26.29 ± 0.81

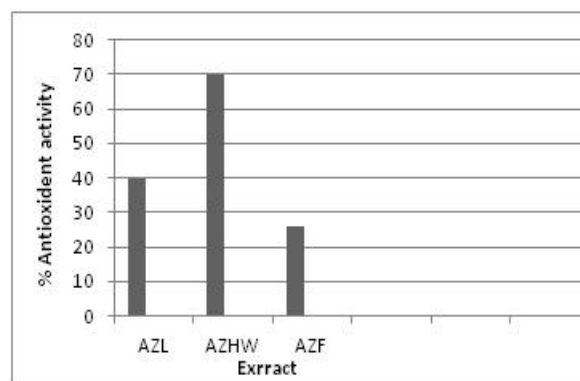


Figure 1: % Antioxidant activity of AZL, AZHW and AZF

Phenol Content and Antioxidant Activity

It is reported that phenols are responsible for the variation in the antioxidant activity of the plant¹⁰ They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydro peroxides into free radicals.¹¹ Phenolic compounds are considered to be the most important ant oxidative components of herbs and other plant materials, and a good correlation between the concentrations of plant phenolic and the total antioxidant capacities has been reported¹². The total phenolic content varied significantly in different parts of plant. Total phenolic compounds in hydro distilled extracts obtained from the Neem plants are presented in Table-1. The order of total phenol content as follow: heart wood > leaf > flower. In present study it was found that there is a positive correlation between total phenolic content and antioxidant activity in different parts of neem tree. Some studies have demonstrated a correlation between phenolic content and antioxidant activity.¹³ The correlation between total phenolic content and antioxidant capacity is possible due to the presence of phenolic compounds or polyphenols or flavonoids or tannins

CONCLUSION

In conclusion, the hydrodistilled extract of *Azadirachta indica* heartwood have potential antioxidant Activity in comparison to leaf and flower which is a very important medicinal plant belonging to family Meliaceae, which can be exploited for combating diseases related to oxidative stress.

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