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PHYTOCHEMICAL STUDIES AND ANTIOXIDANT ACTIVITY OF *MELIA AZEDARACH* LINN LEAVES BY DPPH SCAVENGING ASSAY

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ABSTRACT

The aim of this study was to investigate the antioxidant activity and phytochemical analysis of the leaves extracts of *Melia azedarach* L**.** The phytochemical screening was carried on the leaves extracts of *Melia azedarach,* revealed the presence of some active ingredients such as Alkaloids, Tannins, Saponins, Phenols, glycosides, steroids, terpenoids and flavonoids. The ethanolic leaves extract were also evaluated for their total phenolic contents and antioxidant activity using DPPH radical scavenging assay**.** The result of the present study showed that the ethanolic leaves extract of *Melia azedarach*. which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity than petroleum ether and aqueous extracts. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds.

Key words: *Melia azedarach,* DPPH, Phytochemical screening, Phenolic compounds and Antioxidant activity.

INTRODUCTION

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. 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. Within the human body, millions of chemical reactions are occurring constantly .These processes require oxygen. Reactive oxygen spices (ROS), sometimes called active oxygen species, are various from of activated oxygen, which include free radicals such as superoxide ions (O2 -) and hydroxyl radicals (OH-), as well as non-free-radicals species such as hydrogen peroxide (H2O2)[1,2]**.** Free radicals are the compounds

generated from normal body processes and also from environmental pollutions .They tend to attack the healthy cells DNA as well as proteins and fats, causing them to deteriorate. Anti-oxidants are compounds that protect cells against the damaging effects of reactive oxygen specious, such as singlet oxygen, super oxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage. Oxidative stress has linked to cancer, ageing, atherosclerosis, and ischemia injury, inflammation and neurodegenerative diseases (Parkinson's and Alzheimer's) [3].

Certain antioxidant enzymes are produced within the body .The most commonly recognized of naturally occurring antioxidants are superoxide Dismutase, Catalase, and Glutathione. Superoxide Dismutase changes the structure of oxidants and breaks them down into hydrogen peroxide. Catalase in turn breaks down hydrogen peroxide into water and tiny oxygen particles or gasses. Glutathione is a detoxifying agent, which binds with different toxins to change their form so that they are able to leave the body as waste. Other antioxidant agents are found in foods, such as green leafy vegetables. Items high in vitamin A, vitamin E, and beta-carotene are believed to be the most beneficial. *Melia azedarach Linn* (Meliaceae) is an indigenous plant possessing several medicinal properties.

Melia azedarach Linn (synonym: media dubia Cav, Indian lilac, Persian lilac) belongs to the family meliaceae and is a tree found in India. It is popular as Indian lilac. Different phytochemicals have been isolated from fruit include melianoninol (I), melianol (II), melianone (III), meliandiol (IV), vanillin (V) and vanillic acid (VI)[4]. Leaves have been shown to contain nimbinene, meliacin, quercetrin, quercetin-3-0-b-rutinoside, kaempferol- 3-0-b rutinoside, rutin and kaempferol-3-L-rhamno-Dglucoside [5, 6]. Hot methanolic extract of Melia azedarach leaves contain dipentadecyl ketone, glycerol 1,3-bis-undec-9- enoate 2-dodec-9-enoate and glycerol tris-tridec-9-enoate.[7].

The plant is traditionally used for the treatment of leprosy, inflammations, and cardiac disorders. Its fruits extracts possess ovicidal [8] and larvicidal activity [9]. The leaf extracts also possess antiviral [10] and antifertility activity [11]. As the role of free radicals has been documented in many of these conditions, the present study was directed to investigate the antioxidant activity and phytochemical analysis of leaves extract of *Melia azedarach* L .

MATERIALS and METHOD

Chemicals and Reagents

Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Sodium chloride (S.D. Fine Chem, India), Sodium carbonet (Merck Pvt. Ltd, India), Catechol (Himedia Lab., India), 2, 2-Diphenyle-2-picryl hydrazyl (DPPH) and Vitamin C are obtained from (Himedia Lab., India).All solutions, including freshly prepared doubled distilled water. Stock solutions of the test extracts were prepared in ethanol. Appropriate blanks were used for individual assays.

Plant collection and identification

The basic plant material of *Melia azedarach Linn* used for the investigation was obtained from Mount Opera Garden, Near Ramoji Film City, Nalgonda Dist. The plant can be identified authenticated by Department of Botany, research office (Botanist), Anwar-ul-loom College of Pharmacy, Hyderabad.

Extraction

The leaves were dried under shade and powdered in a mechanical grinder. The powdered material (200gms) was extracted successively in Ethanol, Petroleum Ether and

distilled water by cold percolation method using Soxhlet apparatus at 55°C for 18 h. The extracts was concentrated in vacuo and kept in a vacuum desiccators for complete removal of solvent and weighed.

Phytochemical investigation

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups such as alkaloids, tannins, glycosides and saponins etc present in ethanol, petroleum ether and aqueous extracts [12- 14].

Phenolic Estimation

The total phenolic content of plant extracts were determined by using Folin-Ciocalteu Spectrophotometric method according to the method described by(Kim et al (2007)[15]. Reading samples on a UV-vis spectrophotometer at 650 nm. Results were expressed as catechol equivalents (µg/mg).

Antioxidative activity

The antioxidant activity of the *Melia azedarach* (Leaves) 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 by Brand-Williams et al.(1995)[16] with slight modification. The following concentrations of ethanol extract were prepared 20µg/mL, 40µg/mL, 60µmg/mL, 80µmg/mL and 10µg/mL. 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 517nm. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. Vitamin C (0.1 mg/ml) 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 methanol) was calculated and expressed as % scavenging of DPPH radical. The capability to scavenge the DPPH radical was calculated by using the following equation.

Scavenging effect (%) = (1-As/Ac) ×100

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

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

Statistical analysis

All data were expressed as mean value ± standard deviation (SD) of the number of experiments (n=3).

RESULTS AND DISCUSSION

The effect of different solvents on the yields of *Melia azadirachta* leaf extracts.

The significant variation in the yields of *Melia azadirachta leaves* extracts was shown using various fraction solvents. The yields of extracts using aqueous, petroleum ether and ethanol in Melia azadirachta were 21.20gm, 20.35gm and 22.13gm respectively. The variation in yield may be due to the polarity of the solvents used in the extraction process (Table-2). The results on the quantitative analysis of phytochemical constituents of different extract of *Melia azadirachta* leaves are shown in Table-1

Table 1: Results on the quantitative analysis of phytochemical constituents of different extracts of *Melia azadirachta leaves*.

Sl. No	Constituents	Aqueous Extract	Petroleum Ether Extract	Ethanol Extract
1	Alkaloids	+	+	+
2	Steroids	+	+	+
3	Tannins	+	+	+
4	Phenols	+	+	+
5	Flavonoids	+	+	+
6	Glycosides	+	+	+
7	Saponins	+	+	+
8	Terpenes	+	+	+
9	Reducing Sugar	+	-	-
10	Anthraquinone	+	-	+

+ = The presence of phytochemical constituents.

- = The absence of phytochemical constituents:

Table-2: Crude extracts, phenol contents & IC_{50} Value in *Melia azedarach* leaves.

Solvent used	*Melia azedarach*		
	Crude Extracts (gm)	Phenol content (µg/mg)	IC 50Value (µg/ml)
water	21.20	305	0.0064
Petroleum Ether	20.35	412	0.0086
Ethanol	22.13	492	0.0054

Table-3: Antioxidant activities of *Melia azedarach* in different solvents.

Concentration of extracts (µg/ml)	Antioxidant activity (%)		
	Aqueous extract	**Petroleum Ether extract**	**Ethanol extract**
20	56.43±0.01	54.31±0.05	54.98±0.05
40	58.45±0.07	55.99±0.04	66.65±0.07
60	62.65±0.06	58.12±0.01	68.38±0.06
80	65.32±0.041	59.37±0.08	69.11±0.03
100	68.87±0.09	61.76±0.06	71.42±0.04

Each value is expressed as the mean ± SD (n = 3).

Fig 1: Antioxidant activities of *Melia azedarach* in different solvents.

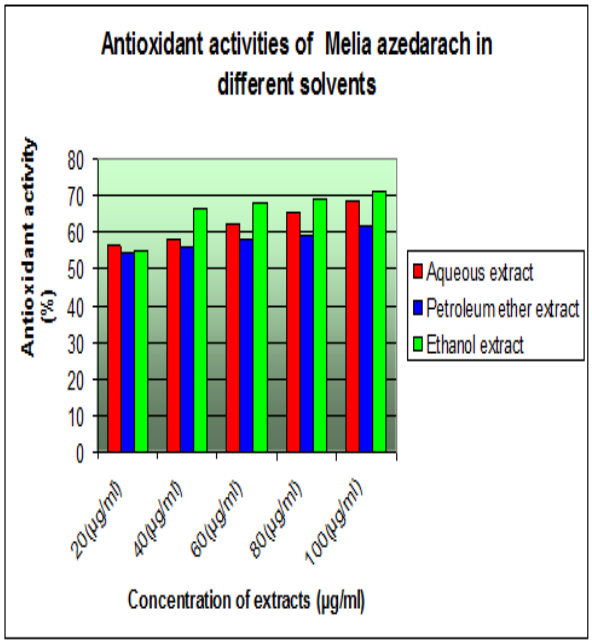
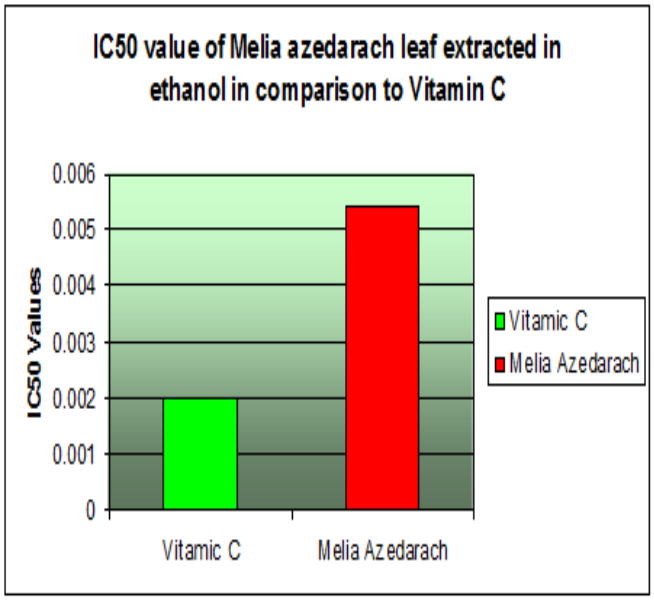


Fig 2:IC_{50} values of *Melia azedarach* leaf extracted in ethanol in comparison to Vitamin C



Free radical and antioxidative activity:

Table-3 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result revealed that the ethanol fraction of *Melia azedarach* exhibited the highest radical scavenging activity with 71.42±0.04 followed by its aqueous extract with 68.87±0.09 and Petroleum Ether extract with 64.76±0.06. In overall comparison of different extracts the ethanolic extract of *Melia azedarach* show the highest scavenging activity followed by the aqueous and then Petroleum Ether. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds [17]. In the present study, the values of ethanolic and aqueous extracts were higher than Petroleum Ether. Among solvents used in this study ethanol has showed the best effectiveness extracting phenolic components. Ethanol is preferred for the extraction of antioxidant compounds mainly because its lowers toxicity [18]. **Fig. 1**. Shows the antioxidant activities of *Melia azedarach* in different solvents.

Phenol content & antioxidant activity

Therefore, we assume that DPPH free radical scavenging activity is related to the presence of bioactive compounds such as phenolic

compounds in extract. The total phenolic content varied significantly between the different extracts of *Melia azedarach.* The contents of total phenolic compounds in crude extract are shown in Table-1. The results were reported as catechol equivalents (µg/mg). The highest concentration of total phenol was 492µg/mg present in the ethanolic extract, followed by Petroleum Ether (412µg/mg) and aqueous extract (305µg/mg) of *Melia azedarach*.

IC_{50} value

IC_{50} value is defined as the concentration of substrate that causes 50% loss of the DPPH activity and was calculated by linear regression mentioned of plots of the percentage of antiradical activity against the concentration of the tested compounds. Results showed in table-2 reports IC_{50} all extracts of *Melia azedarach* showed lower IC_{50} value, however ethanolic extract of *Melia azedarach* being the lowest. **Fig. 2** Shows IC_{50} values of *Melia azedarach* leaf extracted in ethanol in comparison to Vitamin C**.** The ethanolic extract of *Melia azedarach* exhibited significant activity with low IC_{50} value. The antioxidant activity of *Melia azedarach* extracts rise with the rising of polyphenol content of the extract. A linear relationship between the reciprocal of IC_{50} value and the total polyphenol content was observed in this study, indicating that increasing the polyphenol content strengths the antioxidant activity. This finding is similar to that reported by Katsube et al. (2004) [19].

CONCLUSION

The result of the present study showed that the ethanolic leaf extract of *Melia azedarach* contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity. The high scavenging property of *Melia azedarach* may be due to hydroxyl groups existing in the phenolic compounds. It is reported that phenols are responsible for the variation in the antioxidant activity of the plant [20]. They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals [21, 22]. Polyphenols are one of the major plant compounds with antioxidant activity. The –OH groups in phenolic compounds are thought have a significant role in antioxidant activity [23]. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties [24].

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