In vitro antioxidative acitivity of *Azadirachta indica* and *Melia azedarach* Leaves by DPPH scavenging assay

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Abstract: Medicinal plants are a major source of raw material for the traditional system like Ayurveda, Siddha & Unani. Even the modern system of medicine has more than 25 percent of drugs in use, which are either plant based or plant derived. Although several tree posses various medicinal properties, it has been ignored by indigenous & modern system of medicine. Among them ***Azadirachta indica* & *Melia azedarach*** belonging to family Meliaceae play a vital role in day to day usage of different indigenous communities due to its sacred and medicinal value. Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants. In the course of finding potential antioxidant from plant source, two medicinal tree species belonging to family Meliaceae has been selected. Leaves were dried and extracted with different solvent systems namely water, ethanol & methanol. Antioxidant activity using DPPH radical scavenging assay of six extracts from two genus of the family Meliaceae is reported & a comparison of the free radical scavenging ability of the extracts is emphasized. The result of the present study showed that the extract of Melia azedarach, which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparison to *Azadirachta indica* Neem. The high scavenging property of may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger. [Journal of American Science 2010;6(6):123-128]. (ISSN: 1545-1003).

Key words: Antioxidant activity, Azadirachta indica, Melia azedarach.

1. 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 (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. (McClements & Decker, 2000). Many antioxidant compounds, naturally occurring in plant sources, have been identified as a free radical or active oxygen scavengers (Zheng & Wang, 2001). A number of plants have been investigated for their biological activities and antioxidant principles (Baris et al., 2006; Saleem, et al, 2001). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants (Ito, et al, 1983). 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 (Hertog et al, 1993) such as flavonols (Salah et al, 1995), anthaquines (Yen, et al 2000), 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 (Aruoma, 1998). The intake of antioxidants such as polyphenols has been effective in the prevention of diseases (Cao et al 1997; Vinson et al 1995). 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 (Singh et al, 2002).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 no of pure antioxidants.

It is an established fact that polyphenolic compounds possess remarkable antioxidant activities which are present quite commonly in the plant family Meliaceae. ***A.indica*** is well known in India and its neighboring countries for more than 2000 years as one of the most versatile medicinal plants having a wide spectrum of biological activity. ***A. indica* and *M. azedarach*** are two closely related species of Meliaceae family. The former is popularly known as Indian Neem (Margosa tree) or India lilac, and the latter as Mahaneem or Persian lilac. All parts of the plant have been used for medicinal purposes including fruits, seeds, leaves, roots and barks (Anon 1985). Neem has been extensively used in Ayurveda,Unani and homoepathic medicine and has become a Synonym of modern medicine. The Neem tree contains more than 100 bioactive ingredients. The most important bioactive compound is azadirichtin.***Melia azedarach***, the Persian Lilac is popularly known as Maha neem tree and cultivated in all stations. It is a large evergreen tree found throughout India and very similar to Neem. It is native to upper Burmah region. It's Flowering time is May-June and Fruiting time is Nov-Dec. The inner bark contains a resinous alkaloid substance and is used as an anthelmintic. Various scientific studies reported the analgesic, anticancer, antiviral, antimalarial, antibacterial, and antifungal, antifeedent and antifertility activity of this plant. (Vishnukanta, 2008).

Leaf & bark extract of *A. indica* has been studied for its anti-oxidant activity (Ghimeray et al 2009; Sultane et al 2007). However anti-oxidant activity of *M.azedarach* another very important medicine plant has not been investigated. In present work leaves, extracted in water, ethanol & methanol of two trees, *A. indica* & *M. azedarach* belonging to family Meliaceae were investigated for the presence of phenol content & antioxidant activity in a comparative way.

2. Material and Methods

2.1. 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 Ascorbic acid 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.

2.1.2. Plant Materials

The leaves of the two species i.e. *A. indica* and *Melia azedarach* of Meliaceae family were collected from the Medicinal Garden of B.J.B (A) College, Bhubaneswar, Orissa. Fresh plant leaves were rinsed severally with clean tap water to make it dust and debris free. Then the leaves were spread evenly and dried in the shady condition for 3to4 days until they become crispy while still retaining the greenish coloration. Dried leaves were ground in electric chopper to get fine powder form for further use.

2.1.3. Instrumentations

Collection of multi-solvent extract was done by Soxhlet apparatus (J.S.G.W) with varying temperatures according to the B.P. of the solvents. The samples were evaporated through the Rotary vacuum evaporator at 60-100^0C according to the B.P. of supplied solvents. Absorbance spectrophotometery was carried out using a UV-vis spectrophotometer (EI, model-1371).Wavelength scans and absorbance measurements were in 1ml quartz cells of 1cm path length.

2.2 Preparation of plant extracts

The dried and powdered Neem and Maha-neem leaves (each 50g) were extracted successively with double distilled water, ethanol and methanol (each 400ml.) for 10-12 hrs.,using a Soxhlet apparatus. Then collected solutions were filtered through Whatman No-1 filer paper. The extracts were evaporated to dryness under reduced pressure at 90^0C by Rotary vacuum evaporator to obtain the respective extracts and stored in a freeze condition at -18^0C until used for further analysis. These extracts were designated as AW:]Aqueous Extract of Azadirachta, AE: Ethanolic Extract of Azadirachta, AM: Methanolic Extract of Azadirachta, MW: AqueousExtract of Melia, ME: Ethanolic Extract of Melia, MM: Methanolic Extract of Melia respectively.

2.3. 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). Reading samples on a UV-vis spectrophotometer at 650 nm. Results were expressed as catechol equivalents (µg/mg).

2.4. Antioxidative activity

The antioxidant activity of the Neem and Mahaneem (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) with slight modification. The following concentrations of extracts were prepared 0.02mg/mL, 0.04mg/mL,0.06mg/mL, 0.08mg/mL and 0.1mg/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. 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.

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

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

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

3. Results and Discussion

3.1. The effect of different solvents on the yields of Azadirachta and Melia leaf extracts.

The significant variation in the yields of **Azadirachta** and **Melia** extracts were shown using various fraction solvents. The yield of extracts using Water, Methanol and Ethanol in case of **Azadirachta** were 4.93gm, 4.34gm and 6.36gm respectively. Likewise the **Melia** leaf extract also followed the same order as the **Azadirachta** extracts, and they were 5.92gm, 5.62gm and 5.95gm. The variation in yield may be due to the polarity of the solvents used in the extraction process. (Table-1)

3.1.2. Free radical and antioxidative activity

Table-2 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result revealed that the ethanol fraction of **Melia** exhibited the highest radical scavenging activity with 68.23±0.03 followed by its aqueous extract with 64.34±0.04 and methanol extract with 61.17±0.05. In comparison to **Melia** the **Azadirachta** extract shows less scavenging activity. The **Azadirachta** extract of obtained from ethanol shows 50.48±0.03. i.e. highest scavenging activity followed by its aqueous extract with 49.48±0.03 and methnolic extract with 41.17±0.04. In overall comparison the ethanolic extract of both **Azadirachta** and **Melia** show the highest scavenging activity followed by the aqueous and then methanol. Methanol and ethanol has been proven as effective solvent to extract phenolic compounds (Siddhuraju & Becher, 2003). In the present study, the values of ethanolic and aqueous extracts were higher than those of methanolic ones. 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 (Karadeniz, et al 2005). Fig. 1. Shows the comparative study of radical scavenging activity between **Melia** and **Azadirachta** with respect to Ascorbic acid as standard.

3.1.3. Phenol content & antioxidant activity:

It is reported that the phenolics are responsible for the variation in the antioxidant activity of the plant (Cai et al., 2004). They exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals (Pokorney, 2001; Pitchaon et al., 2007). The total phenolic content varied significantly between the two species of Maliaceae family i.e. ***Azadirachta indica*** **and** ***Melia azedarach***. The contents of total phenolic compounds in crude ethanolic extracts obtained from these two **Azadirachta** plants are presented in Table-1. The results were reported as catechol equivalents (μg/mg). The highest concentration of total phenolics was 360μg/mg present in the ethanolic extract of **Melia** plant where as lowest in aqueous extract of **Azadirachta** plant i.e. 120μg/mg. The aqueous and methanolic fractions of **Melia** showed 140μg/mg and 268μg/mg of phenlic contents respectively. Similarly the **Melia** ethanolic extract and **Azadirachta** methanolic extract exhiited highest phenol contents of i.e.300μg/mg and 258μg/mg.

3.1.4 *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-1 reports no IC_{50} value in water and methanol extraction of Azadirachta indica. Only ethanolic extract of **Azadirachta** showed an IC_{50} value of 0.008μg/mg. In comparison of **Azadirachta**, all extracts of **Melia** showed lower IC_{50} value, however MnE being the lowest (Fig-2).The ethanolic extract of Mahaneem exhibited significant activity with low IC_{50} value in comparison to **Azadirachta**. The antioxidant activity of **Azadirachta** and **Melia** 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 of **Azadirachta** and Melia 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.

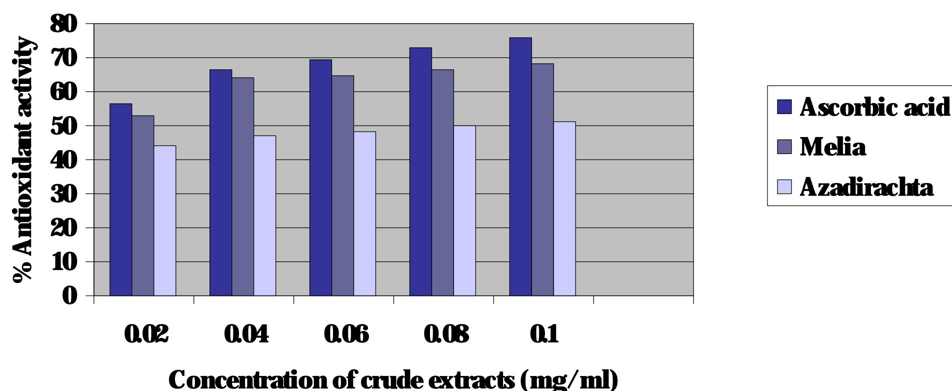

Fig. 1. Antioxidant activity of Melia and Azadirachta in comparison to Ascorbic acid.

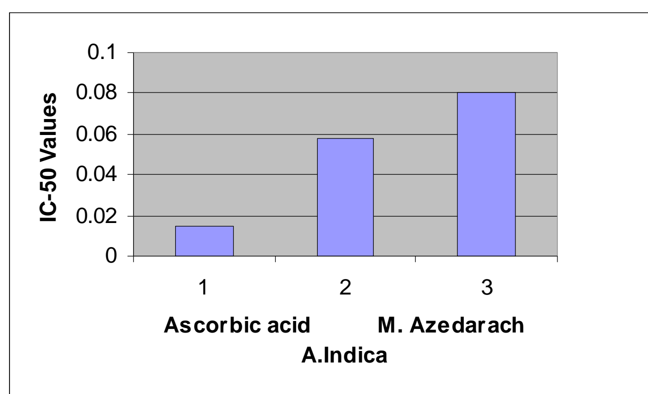

Fig. 2. IC_{50} value of Melia and Azadirachta leaf extracts obtained from ethanol.

Table-1-Crude extracts phenolic content & IC_{50} Value in Melia and Azadirachta leaves.

	Azadirachta indica			*Melia azedarach*		
Solvent used	Crude Extracts (gm)	Phenol content (µg/mg)	IC_{50} Value (µg/ml)	Crude Extracts (gm)	Phenol content (µg/mg)	IC_{50} Value (µg/ml)
Water	4.93	120	<50%	5.92	140	0.062
Methanol	4.34	258	<50%	5.62	268	0.066
Ethanol	6.36	300	0.080	5.95	360	0.058

Table-2-Antioxidant activities of Melia and Azadirachta in different solvents

Concentration of extracts (mg/ml)	Antioxidant activity (%)		Antioxidant activity (%)		Antioxidant activity (%)	
	AW	MW	AM	MM	ME	MW
0.02	45.24±0.04	53.69±0.03	34.11±0.04	55.29±0.03	44.10±0.01	52.94±0.05
0.04	44.18±0.03	55.75±0.05	35.84±0.06	56.47±0.06	47.05±0.03	64.11±0.03
0.06	47.48±0.02	60.43±0.03	39.43±0.06	59.98±0.09	48.20±0.06	64.70±0.04
0.08	48.47±0.05	63.14±0.04	40.00±0.10	60.59±0.04	50.02±0.13	66.47±0.03
0.1	49.48±0.03	64.34±0.04	41.17±0.04	61.17±0.05	50.48±0.03	68.23±0.03

AW: Aqueous Extract of Azadirachta, AE: Ethanolic Extract of Azadirachta, AM: Methanolic Extract of Azadirachta, MW: AqueousExtract of Melia, ME: Ethanolic Extract of Melia, MM: Methanolic Extract of Melia

3.1.5 Conclusion

The result of the present study showed that the extract of ***Melia azedarach***. which contains highest amount of phenolic compounds exhibited the greatest anti-oxidant activity in comparision to Azadirachta indica. The high scavenging property of **Melia** may be due to hydroxyl groups existing in the phenolic compounds chemical structure that can provide the necessary components as a radical scavenger.

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