Investigation on the Antioxidant Activity of Leaves, Fruit and Stem Bark of Dhraik (Melia azedarach)

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Abstract: The present study was designed to check the antioxidant activity of Melia azedarach commonly known as bead-tree or cape lilac. The extraction of leaves, fruit and stem bark of Melia azedarach was carried out by using solvent aqueous methanol. The purpose of extraction was to check the antioxidant activity of testing plant Melia azedarach by using different in vitro antioxidant assays. The TPC (Total Phenolic Contents) and TFC (Total Flavonoid Contents) contents in different parts of sun dried extracts of the said plant Melia azedarach were found to be in the range of 74.43-112.10 mg GAE/g DW and 13.32-28.11 mg CE/g DW, while in ambient dried it was found to be in the range of 66.89-103.34 mg GAE/g DW and 10.67-23.45 mg CE/g DW, respectively. The DPPH scavenging activity and linoleic inhibition capacity of sun dried was found to be in the range of 55.43-63.86% and 35.57-52.11%, respectively while for ambient dried was found to be in the range of 48.54-61.00% and 33.87-50.33%, correspondingly. The reducing potential of sun dried and ambient dried at concentration of 10.0 mg/mL was found in the range of 0.727-1.211 and 0.601-0.890, respectively. In conclusion, the sun dried extracts of Melia azedarach had higher antioxidant activity whereas, among the plant parts, the stem bark exhibited better antioxidant activity.

Key words: Antioxidant activity • Melia azedarach • Flavonoids • Phenolics • Scavenging activity

INTRODUCTION

Natural antioxidants have attracted a great deal of public and scientific interest because of their anticarcinogenic potential and other health promoting effects. Plants and vegetables are good source of phenolic components, ascorbic acids, tocopherols, glutathione, vitamin C and E, carotenoids, flavonoids that may contribute to protection against oxidative damage. These phytochemicals from plants have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human beings such as anti-allergic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects [1].

Melia azedarach commonly known as bead-tree or cape lilac. This is a species of deciduous tree in the mahogany family, Meliaceae, that is native to Pakistan, India, Indochina, Southeast Asia and Australia. Meliaceae is native to India, southern China and Australia. It has become naturalized to tropical and warm temperate regions of the Americas and is planted in similar climates around the world. Besides the problem of toxicity, its usefulness as a shade tree in urban areas is diminished by its tendency to sprout where unwanted and to turn sidewalks into dangerously slippery surfaces when the fruits fall, though this is not a problem where songbird populations are in good shape [2]. Common names include Persian Lilac, White Cedar, Chinaberry, Texas Umbrella, Bead Tree, Lunumiddle, Ceylon Cedar, malai vemba, Bakain and Dharek/Dhraik. Melia azedarach in keeping with other members of the family Meliaceae has a timber of high quality, but as opposed to many almost-extinct species of Mahogany it is under-utilised. Seasoning is relatively simple in that plant dry without cracking or warping and are resistant to fungal infection [3].

The present research work will provide the antioxidant activity of different parts i.e. leaves, stem bark and fruit of Melia azedarach fruit indigenous to Pakistan.
MATERIALS AND METHODS

Collection of Samples: Fresh samples of leaves, fruit and stem bark of *Melia azedarach* were obtained from the botanical garden, University of Agriculture, Faisalabad, Pakistan. The samples were further identified and authenticated from Department of Botany, University of Agriculture, Faisalabad.

Pretreatment of Samples: Different parts of the *Melia azedarach* like leaves, fruit and stem bark were separated manually using sharp steel knife. The samples were washed with distilled water and were dried under sun and ambient then ground into fine powder.

Extraction: Ground Dhraik (*Melia azedarach*) sample (10g) was extracted separately with 100 mL of aqueous methanol (methanol:water, 80:20 v/v) and shaken for 24 h at room temperature in an orbital shaker (Gallenkamp, UK). All extracts were separated from the residues by filtering through Whatman No. 1 filter paper. The residues were extracted twice with the same manner and extracts combined. The combined extracts were concentrated and freed of solvent under reduced pressure at 45°C, using a rotary evaporator. The dried, crude concentrated extracts were weighed to calculate the yield and stored at -4°C until used for further analysis.

Determination of Total Phenolics (TP): Amount of TP was assessed using Folin–Ciocalteu reagent procedure as described by [4]. In detailed, 50 mg of dry mass of each extract was mixed with 0.5 mL of Folin–Ciocalteu reagent and 7.5 mL deionized water. The mixture was kept at room temperature for 10 min and then 1.5 mL of 20% NaCO₃ (w/v) was added. The mixture was then heated in a water bath at 40°C for 20 min and cooled in an ice bath; finally absorbance was measured at 755 nm (Hitachi U-2001 Spectrophotometer, Model 121-0032). The results were expressed as gallic acid equivalents (GAE) per dry matter. All samples were analyzed in triplicate.

Determination of Total Flavonoids (TF): Amount of TF was determined following the procedure described by [5]. 1 mL of aqueous extract containing 0.1 g/mL of extract was placed in a 10 mL volumetric flask, then 5 mL of distilled water added followed by 0.3 mL of 5% NaNO₂. After 5 min, 0.6 mL of 10% AlCl₃ was added then passing more 5 min 2 mL of 1 M NaOH was added and volume made up with distilled water. The solution was mixed and absorbance measured at 510 nm. TF amounts were expressed as catechin equivalents.

Determination of Antioxidant Activity in Linoleic Acid System: The antioxidant activity of *Melia azedarach* extracts was also determined in terms of measurement of inhibition of peroxidation in linoleic acid system following a reported method of [6]. Each extracts (5 mg) added to a solution mixture of linoleic acid (0.13 mL), 99.8% ethanol (10 mL) and 10 mL of 0.2 M sodium phosphate buffer (pH 7). Total mixture was diluted to 25 mL with distilled water. The solution was incubated at 40°C and the degree of oxidation was measured following thiocyanate method with 10 mL of ethanol (75%), 0.2 mL of an aqueous solution of ammonium thiocyanate (30%), 0.2 mL of sample solution and 0.2 mL of ferrous chloride (FeCl₂) solution (20 mM in 3.5% HCl) being added sequentially. After 3 min of stirring, the absorption values of mixtures measured at 500 nm were taken as peroxide contents. A control was performed with linoleic acid but without extracts. Synthetic antioxidants; butylated hydroxytoluene (BHT) and ascorbic acid (200 ppm) were used as positive control. The maximum peroxidation level observed as 360 h (15 days) in the sample that contained no antioxidant component was used as a test point. Percent inhibition of linoleic acid peroxidation was calculated using the following formula.

\[ 100 - \left(\frac{\text{Abs. increase of sample at 360 h}}{\text{Abs. increase of control at 360 h}}\right) \times 100 \]

Determination of Reducing Power: The reducing power of the extracts was determined according to the procedure described by [7], with slight modification. Equivalent volume of extracts containing 2.5-0.0 mg of extract was mixed with sodium phosphate buffer (5.0 mL, 0.2 M, pH 6.6) and potassium ferricyanide (5.0 mL, 1.0%); the mixture was incubated at 50 °C for 20 min. Then 5 mL of 10% trichloracetic acid added and centrifuged at 980g for 10 min at 5°C in a refrigerated centrifuge (CHM-17; Kokusan Denki, Tokyo, Japan). The upper layer of the solution (5.0 mL) was diluted with 5.0 mL of distilled water and ferric chloride (1.0 mL, 0.1%) and absorbance read at 700 nm (Hitachi U-2001). The measurement was run in triplicate and results averaged.

DPPH Radical Scavenging Assay: Free radical scavenging activities of *Melia azedarach* extracts was measured by using procedure described by [6]. Briefly, to 1.0 mL of each extract (25 mg/mL of methanol), 5.0 mL of freshly prepared solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) at concentration 0.025 g/L was added. Absorbance at 0, 0.5, 1, 2, 5 and 10 min was measured at 515 nm (Hitachi U-2001 Spectrophotometer, Model
121-0032). The remaining amount of DPPH radical was calculated from calibration curve. Absorbance measured at 5th minute was used in comparison of radical scavenging activity of each extracts.

Statistical Analysis: Three samples of each part of Melia azedarach were assayed. Each sample was analyzed individually in triplicate and data was reported as mean (n = 3×3). T-test was performed by using Minitab 2000 Version 13.2 statistical software (Minitab Inc. USA).

RESULTS AND DISCUSSION

Percentage Yield of Extract: Table 1 showed that percentage yield of stem bark, leaves and fruits of sun dried and ambient dried extract of Melia azedarach was found to be in the range of 10.11-17.30 and 8.20-15.60%, respectively. It was cleared from the results that the Melia azedarach samples dried under sun showed the highest %age yield. Whereas the comparison among the different parts depicted that the sun dried stem bark exhibited higher % age yield.

Total Phenolic and Total Flavonoid Contents: The TPC and TFC contents in different parts i.e. stem bark, leaves and fruits of sun dried extracts of Melia azedarach were found to be in the range of 74.43-112.10 mg GAE/g DW and 13.32-28.11 mg CE/g DW, respectively. Whereas, in ambient dried it was found to be in the range of 66.89-103.34 mg GAE/g DW and 10.67-23.45 mg CE/g DW, respectively. The total phenolic components of stem bark extract of sun dried sample was found to be the highest (112.10 mg GAE/g DW) while fruit extract of ambient dried sample possessed the lowest value (66.88 mg GAE/g DW). The comparison of sun dried and ambient dried showed that all parts of sun dried plants Melia azedarach exhibited the highest values of TPC and TFC. The total phenolic contents of the presently investigated was better compared to Cassia auriculata (L) [8] and also compared with the Azadirachta indica [8, 9]. Kayani et al. [10] also stated that shoot contained greater amount of TPC (63-524 µg g⁻¹) as compared to the root of the same studies 37 plant species. Whereas, Achakzai et al. [11] revealed that old leaves contained high level of TPC as compared to old stem.

The maximum value of total flavonoids (28.11mg/g DW) was obtained from stem bark dried under sun while the minimum value (10.67 mg/g DW) was obtained from ambient dried fruit samples. The total flavonoid contents of the presently investigated plants and their parts were better compared to Azadirachta indica reported by [9].

Antioxidant Activity in Linoleic Acid System: The extracts of samples of both ambient dried and sun dried exhibited appreciable inhibition of oxidation. Sun dried stem bark, leaves and fruit exhibited inhibition of oxidation...
values in the range of 35.57-52.11% and ambient dried sample exhibited inhibition of oxidation values in the range of 33.87-50.33%. The comparison between sun dried and ambient dried showed that all the parts of sun dried Melia azedarach exhibited highest percentage value. The comparison among different parts revealed that the sun dried stem bark exhibited the highest % age inhibition value (52.11%). The results described here summed to be comparable with those results reported by [12] who reported that the peroxidation inhibition activity of the neem extract using methanol was 55.46% and the leaf extracts exhibited 47% in methanol.

**Free Radical Scavenging Activit:** Melia azedarach extracts at ambient dried and sun dried conditions exhibited appreciable scavenging activity. Different parts i.e. stem bark, leaves and fruit of sun dried Melia azedarach exhibited the DPPH scavenging values in the range of 55.43-63.86% while ambient dried in the range of 48.54-61.00%. Sun dried stem bark under had highest percentage value 63.87% and ambient dried fruit the lowest value which is 48.54%. The extracts of the tested medicinal plant materials possessed free radical scavenging properties, but to varying degrees. Our results are in the range of results as reported by [13,14].

**Reducing Power:** The data for the reducing potential of different parts extracts of Melia azedarach in Table 3 is presented. The reducing potential of the bark, leaves and fruit extracts measured for the concentration up to 10.0 mg/mL, showed general increase in activity when concentration increased. Reducing potential of different part extracts of sun dried at concentration of 10 mg/ml ranged from 0.727 to 1.211%. While the reducing power of ambient dried sample was found to be in the range of 0.601-0.890%. The reducing power of the presently investigated work was better compared to Azadirachta indica bark [9].

**CONCLUSIONS**

The highest antioxidant activity was showed by stem bark in all assays within the extracts sun dried sample showed high antioxidant activity as compared to air dried extract. Results showed that with the increase in concentration antioxidant activity of all plant extracts increases in linoleic acid system % inhibition increased with the increase in incubation. The results of present study revealed that Melia azedarach extracts are rich in poly phenolic contents and they are potential sources of antioxidant.

**REFERENCES**