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Pharmacognostical Characterization and Evaluation of Invitro Antioxidant activity of Melia azedarach fruits

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ABSTRACT

Melia azedarach L, also known as Chinaberry tree, is a Southeast Asian plant with anti-inflammatory, antipyretic, analgesic, and insecticidal properties. It contains bioactive compounds like flavonoids, alkaloids, terpenoids, and saponins. Recent studies have shown that extracts from *Melia azedarach* L have anticancer properties and antimicrobial activity against various pathogens. Ayurveda, a traditional Indian medicinal system, has been practiced for thousands of years. *Melia azedarach* Linn, also known as mahanimba, is used for its anthelmintic, antilithic diuretic, emmenagogue, astringent, and stomachic properties. The tree is highly nutritious and has a calorific value of 5100 kcal/kg. It is also used in manufacturing agricultural implements, furniture, plywood, and fuel wood. In present study, pharmacognostical examination is done prior to its preclinical activity in order to check for its purity and having a record of possible phytochemicals present in the plants which may be responsible for its pharmacological action. Recent research on MEMA reveals its potent antioxidant capacity due to its high flavonoid content, which reduces damaging radicals. The presence of polyphenols in MEMA results in reduced oxidative stress indicators and increased protective capacity of antioxidants. This suggests that the methanol extract (MEMA) of *Melia azedarach* fruit has stronger antioxidant activity than the aqueous extract (AEMA), indicating its potential as a potential health supplement.

Keywords: *Melia azedarach; methanol extract; aqueous extract; pharmacognostical examination; antioxidants.*

1. INTRODUCTION

Melia azedarach L, also known as Chinaberry tree, is a well-studied plant native to Southeast Asia. Its leaves, bark, and fruits have been traditionally used for their antiinflammatory, antipyretic, analgesic, and insecticidal properties (1). The plant contains bioactive compounds like flavonoids, alkaloids, terpenoids, and saponins. Recent studies have shown that extracts from *Melia azedarach* L have anticancer properties and antimicrobial activity against various pathogens. Ayurveda, a traditional Indian medicinal system, has been practiced for thousands of years. Research on pharmacognosy, chemistry, and clinical therapeutics has been conducted on ayurvedic medicinal plants (2). Modern medicine, or allopathy, has evolved over time, but its foundation remains rooted in traditional medicine and therapies. *Melia azedarach* Linn, also known as mahanimba, is a large evergreen tree found throughout India, used for its anthelmintic, antilithic diuretic, emmenagogue, astringent, and stomachic properties (3). The pharmacognostic evaluation of *Melia azedarach* includes the identification and characterization of its chemical constituents, which are essential for formulating an effective antidiabetic product. Assessing the antidiabetic potential of *Melia azedarach* fruit extract involves conducting preclinical and clinical studies to determine its efficacy and safety profile. These studies play a crucial role in establishing the plant's credibility as a natural remedy for managing diabetes.

2. MATERIALS AND METHODS

2.1 Collection and Standardization of the Plant Material

The *Melia azedarach* fruit were collected in February-March 2024 from the nearby areas of from Almora, Uttrakhand. The voucher specimen was submitted to the Herbarium and Museum Section of the Institute. The crude plant material was evaluated for physicochemical parameters using standardized techniques (4).

2.2 Extraction and Phytochemical Evaluation of the Methanolic (MEMA) and Aqueous (AEMA) extract of *Melia azedarach* fruit

Fruits were shade dried, ground, and ground into a coarse powder. The powder was extracted using methanol and water using a Soxhlet apparatus. The powder was defatted to remove wax and lipids, then refluxed with petroleum ether to remove fat. The defatted marc was soaked in purified water and kept in a cool, dark place for 48 hours. The filtrate was filtered through Whatman filter paper and dried in a rotary evaporator. The dried residue was used as a crude extract for further research. The percentage yield was calculated and quantitative (4) and qualitative (5) phytochemical evaluation was done for the extract.

2.3 Metabolic profiling of Methanolic extracts of *Melia azedarach* fruit (MEMA) by GC-MS analysis

The study involved analyzing the lipid content of *Melia azedarach* fruit using GC-MS. The Methanolic extract was suspended in a methoxylamine hydrochloride solution and GC grade pyridine. The lipid content was analyzed using Thermo Trace GC Ultra coupled with Thermo fisher DSQ II mass spectrometers. Chromatographic separations of metabolites were performed on a 30 m x 0.25 mm Thermo TR50 column. Xcalibur software was used to process the data. The GC oven temperature was maintained at 70°C for 5 minutes, then raised to 290°C. The sample was injected in split mode with helium as a carrier gas. The resulting GC-MS profile was analyzed using Replib, WILLY, and NIST mass spectral libraries (6). The concentration of metabolites was calculated on the percent peak area basis.

2.4 Evaluation of Invitro Anti Oxidant Activity

2.4.1 DPPH scavenging assay

This investigation involves a process of dissolved DPPH in methanol to create a stock solution with a concentration of 0.1 mM. The Methanolic extracts of *Melia azedarach* fruit (MEMA) is then diluted with methanol to create different concentrations. Each test tube receives 3 mL of the stock solution, and the mixture is incubated at room temperature for half an hour. A spectrophotometer is used to test the absorbance at 517 nm, indicating higher antioxidant activity (7).

2.4.2 Reducing power assay

This investigation involves creating a stock solution of Methanolic extracts of *Melia azedarach* fruit (MEMA) at varying concentrations, preparing dilutions, and combining phosphate buffer, potassium ferricyanide, and the test chemical solution. The reaction mixture is incubated for 20 minutes at 50°C, then centrifuged to remove precipitated proteins. Distilled water and ferric chloride solution are then added to the supernatant. The complex's absorbance at 700 nm is determined using spectrophotometric measurement (8).

3. RESULTS AND DISCUSSION

3.1 Standardization of *Melia azedarach* fruit

The physicochemical constants of *Melia azedarach* fruit were found to be as mentioned in the Table 1. The physicochemical studies viz. ash content, extractive value, moisture content, pH indicated that the *Melia azedarach* fruits are of standard quality.

Table 1: Physicochemical evaluation of *Melia azedarach* fruit

Sr. No.	Standardization parameters	Value %w/w
01	**Ash analysis**	
	❖ Ash Content (Total Ash)	10.27 ± 0. 821
	❖ Acid In-Soluble Ash	0.943 ± 0. 005
02	**Extractive value (Maceration Process)**	
	❖ Alcohol soluble	15.655 ± 0.328
	❖ Water soluble	33.11 ± 0.010
03	**Moisture content(Loss On Drying)**	10.496 ± 0.732
04	**pH** (1% aqueous solution)	8.010 ± 0.128

3.2 Percentage (%) Yield and Phytochemical Screening and Quantitative Estimation

The aqueous extract (AEMA) of dark brown color and dry amorphous consistency was obtained with percent yield of 32.69%

w/w. The methanolic extract (MEMA) of greenish blue with sticky consistency was obtained with percent yield of 59.71% w/w. AEMA showed presence of carbohydrates, proteins, amino acids, alkaloids, saponins, sterols, tannins and phenolic compounds flavonoids. The total flavonoid content of AEMA and MEMA were found to be 20.71 ± 0.841 and 25.45 ± 0.881 mg quercetin equivalents/g of extract and total phenolic content of AEMA and MEMA were found to be 26.48 ± 0.107 and 45.08 ± 0.458 mg tannic acid equivalents/g of extract.

3.3 GC-MS Analysis of Methanol Extract of *Melia azedarach* (L.) fruits

Twenty compounds were detected in the Methanolic extract of the *Melia azedarach* (L.) fruits. A total of 25 compounds were identified and are listed in Table 2 and Figure 1, along with their respective retention times and peaks.

Table 2: GC-MS Analysis of Methanol Extract of *Melia azedarach* (L.) fruits

S.No	Peak name	Formula	MW	Retention time
1	Ethylbenzene	106.16	0.86	4.361
2	p-Xylene	106.16	3.67	4.534
3	o-Xylene	106.16	1.53	4.986
4	1-Ethyl-2-methylbenzene	120.19	2.20	6.341
5	1-Eethyl-3-methylbenzene	120.19	1.08	6.408
6	1,3,5-Trimethylbenzene	120.19	1.13	6.508
7	1,2,4-Trimethylbenzene	120.19	16.28	6.961
8	1,2,3-Trimethylbenzene	120.19	8.98	7.457
9	Indane	118.18	0.88	7.718
10	1-Methyl-3-propylbenzene	134.22	1.01	8.003
11	4-Ethyl-1,2-dimethylbenzene	134.22	4.35	8.099
12	1-Ethyl-3,5-dimethylbenzene	134.22	2.19	8.419
13	2-Ethyl-1,3-dimethylbenzene	134.22	2.49	8.467
14	2-Ethyl-1,4-dimethylbenzene	134.22	5.33	8.568
15	1-Ethyl-2,3-dimethylbenzene	134.22	0.89	8.900
16	1,2,3,4-Tetramethylbenzene	134.22	7.67	9.100
17	1,2,4,5-Tetramethylbenzene	134.22	11.37	9.156
18	1H-Indene,2,3-dihydro-5-methyl	132.20	1.02	9.508
19	1,2,3,4-Tetramethyl-5-methylene-1,3-cyclopentadiene	134.22	4.34	9.662
20	Azulene	128.17	7.75	10.251
21	Pentamethylbenzene	148.24	2.01	10.353
22	Di-n-octylphthalate	390.6	1.16	24.218
23	Heneicosane	296.6	2.26	25.403
24	Nonacosane	408.8	6.14	26.767
25	Tetracontane	563.1	3.42	28.068

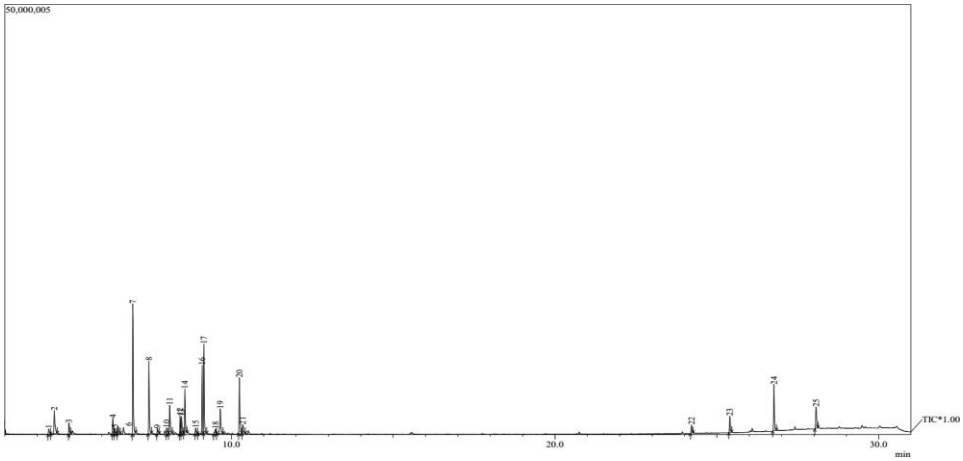

Figure 1: GC-MS Chromatogram of Methanol Extract of *Melia azedarach* (L.) fruits

3.4 Evaluation of Antioxidant Activity

3.4.1 DPPH scavenging assay

The study found that AEMA and MEMA, when combined with standard antioxidant-Butylated Hydroxy Toluene (BHT), effectively inhibited DPPH radical formation in a concentration-dependent manner. The linear regression analysis showed that DPPH scavenging was concentration-dependent, with IC_{50} values of 602.733, 399.408, and 50.173 µg/ml, respectively. This suggests that the antioxidants' effectiveness in DPPH scavenging is dependent on their concentration (Table 3).

Table 3: Effect of AEMA and MEMA on DPPH radical scavenging

Concentration (µg/ml)		% DPPH Inhibition	IC_{50} Value
AEMA	**100**	20.57±0.315	**382.803 µg/ml**
	200	37.23±0.173	
	400	57.45±0.990	
	600	68.15±0.285	
	800	70.49±0.824	
	1000	85.04±0.223	
MEMA	**100**	38.25±0.145	**392.475 µg/ml**
	200	49.14±0.845	
	400	59.09±1.154	
	600	71.45±0.425	
	800	89.09±0.666	
	1000	95.14±0.856	
BHT	**10**	13.31±0.397	**50.173 µg/ml**
	20	25.06±0.529	
	40	47.05±0.496	
	60	65.63±0.636	
	80	75.04±0.223	

	100	80.21±0.257	

(Values are mean± SEM; n=3; IC_{50}= 50% Inhibitory concentration)

3.4.2. Reducing power assay

The AEMA and MEMA in the concentration range of 50-250 µg/ml showed concentration related reduction of ferricyanide to ferrocyanide as indicated by increase in the green colour absorbance measured at 700 nm. Similar effect was obtained with standard antioxidant- ascorbic acid in the concentration range of 50-250 µg/ml (Figure 2).

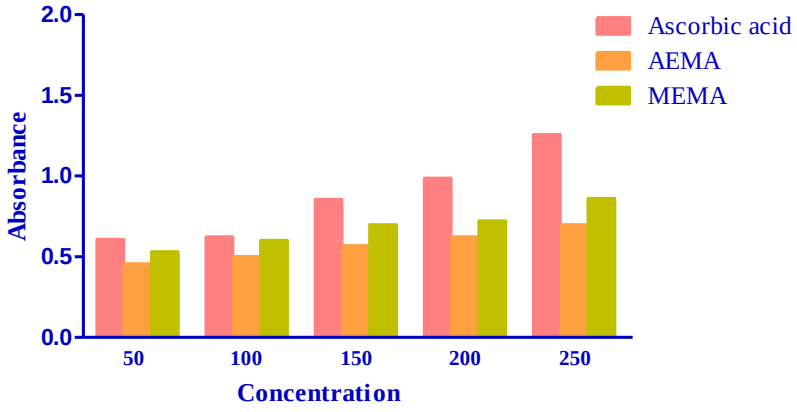

Figure 2: Effect of AEMA and MEMA on reducing potential

4. CONCLUSIONS

Recent discoveries on MEMA have demonstrated that it possesses a potent antioxidant capacity. This is attributed to the fact that it contains a high flavonoid content, which is characterised by the scavenging action of damaging radicals by reduction. The presence of polyphenols is responsible for the effects that were seen by MEMA in the current investigation. These effects include a reduction in oxidative stress indicators and an increase in the protective capacity of antioxidants. When all of these data are taken into consideration, it is possible to draw the conclusion that the methanol extract (MEMA) of *Melia azedarach* fruit possesses strong antioxidant activity than that of aqueous extract (AEMA).

5. CONFLICT OF INTEREST

None

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