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Pharmacognostical Evaluation of Methanolic and Aqueous Extract of *Aegle marmelos* L.

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ABSTRACT

Traditional and folk medicine in India make use of the *Aegle marmelos* L. plant for the treatment of dysentery, diarrhoea, and infertility/abortion in women. Sacred to Hindus, the tree's fruits are also consumed and utilised in traditional medicine all across its area. People all over the world deal with gastric ulcers, and the only way to cure them is with synthetic medications, which might have negative side effects. The creation of herbal treatments for stomach ulcers is a recent development in the field of plant medicine. The antibacterial and antiulcer characteristics of *Aegle marmelos* make it a superior choice. Among its medicinal and therapeutic uses are its potent antioxidant and antiulcer properties, which it employs to treat stomach ulcers caused by Helicobacter pylori. This study seeks to provide scientific proof, based on supporting data and prior research, that *Aegle marmelos* fruits are effective against stomach ulcers. *Aegle marmelos* was determined to have standard-quality physicochemical characteristics; the methanolic extract (MEAM) was greenish-blue with a sticky consistency and yielded 41.69% w/w, while the aqueous extract (AEAM) was dark brown in colour and dry amorphous in consistency, giving 29.61% w/w. The total quercetin equivalents per gramme of extract for AEAM and MEAM were determined to be 19.71 ± 0.841 mg, whereas the total phenolic content for AEAM and MEAM was 22.48 ± 0.107 mg, and 42.08 ± 0.458 mg, respectively. Twenty chemicals were identified in the GC-MS study of the methanolic extract of *Aegle marmelos* Linn Fruits. Among them were n-Hexadecanoic acid, tetradecanoic acid, and stigmasterol.

Keywords: *Aegle marmelos* L.; Methanolic extract; Aqueous extract; GC-MS; Antioxidant activity.

1. INTRODUCTION

Aegle marmelos (L) Correa, also known as bael in India, is a sacred deciduous tree in the Rutaceae family. It has been used in Hindu rituals since ancient times, and its trifoliate leaves symbolize the Threefold Trinity. Bael fruit, which has medicinal properties, has been used in ethnomedicine for its anti-inflammatory, anti-dysentric, anti-diarroheal, and antipyretic effects. It has been shown to treat various illnesses, including hyperlipidemia, diabetes, and stomach ulcers. Some isolated compounds also exhibit antiviral, antioxidant, antibacterial, and radioprotective properties. The tree's life cycle, including stem, bark, root, leaves, fruit, and seeds, has been used in ethnomedicine to treat various illnesses. The fruit ripens in 10 to 11 months from March to June, with oval-shaped leaves and a pleasant scent. The therapeutic properties of bael have been documented in the Ayurvedic literature. The tree's medicinal properties are also found in the venerable Ayurvedic literature. The bael tree is well-liked in Shiva and Vishnu temples and can be cultivated in any home (1-3).

2. MATERIALS AND METHODS

2.1 Collection and Standardization of the Plant Material

The *Aegle marmelos* 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 (MEAM) and Aqueous (AEAM) extract of *Aegle marmelos* 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 *Aegle marmelos* fruit (MEAM) by GC-MS analysis

The study involved analyzing the lipid content of *Aegle marmelos* 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 and aqueous extracts of *Aegle marmelos* fruit (MEAM and AEAM) 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 and aqueous extracts of *Aegle marmelos* fruit (MEAM and AEAM) 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 *Aegle marmelos*

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

Table 1: Physicochemical evaluation of *Aegle marmelos*

Sr. No.	Standardization parameters	Value %w/w
01	**Ash analysis**	
	❖ Ash Content (Total Ash)	17.23 ± 0.3821
	❖ Acid In-Soluble Ash	1.843 ± 0.7005
02	**Extractive value (Maceration Process)**	
	❖ Alcohol soluble	9.655 ± 0.4328
	❖ Water soluble	27.11 ± 0.4010
03	**Moisture content(Loss On Drying)**	8.596 ± 0.1732
04	**pH (**1% aqueous solution)	5.970 ± 0.1528

3.2 Percentage (%) Yield

The aqueous extract (AEAM) of dark brown color and dry amorphous consistency was obtained with percent yield of 29.61% w/w. The methanolic extract (MEAM) of greenish blue with sticky consistency was obtained with percent yield of 41.69% w/w.

3.3 Phytochemical Screening and Quantitative Estimation

AEAM showed presence of carbohydrates, proteins, amino acids, alkaloids, saponins, sterols, tannins and phenolic compounds flavonoids. The total flavonoid content of AEAM and MEAM were found to be 19.71 ± 0.841 and 23.45 ± 0.881 mg quercetin equivalents/g of extract and total phenolic content of AEAM and MEAM were found to be 22.48 ± 0.107 and 42.08 ± 0.458 mg tannic acid equivalents/g of extract.

3.4 GC-MS Analysis of *Aegle marmelos* (L.) Methanol Extract

Twenty compounds were detected in the Methanolic extract of the Fruits of *Aegle marmelos* Linn. Among the twenty compounds, the GC-MS analysis of Methanol extract of the *Aegle marmelos* Linn revealed the presence of Stigmasterol, Tetradecanoic acid and n- Hexadecanoic acid (Table 2; Figure 1).

Table 2: GC-MS Analysis of *Aegle marmelos* (L.) Methanol Extract

S.No	Peak name	Formula	MW	Retention time
1	1-Hexanol,2-ethyl-	$C_8H_{18}O$	130	8.31
2	Nonanoic acid	$C_9H_{18}O_2$	158	13.20
3	1-Hexadecene	$C_{16}H_{32}$	224	14.93
4	1-Nonadecene	$C_{19}H_{38}$	266	19.10
5	1-Eicosanol	$C_{20}H_{42}O$	298	22.43
6	Tetradecanoicacid	$C_{14}H_{28}O_2$	228	24.15
7	á-Sitosterol	$C_{29}H_{50}O$	414	24.41
8	n-Hexadecanoicacid	$C_{16}H_{32}O_2$	256	25.74
9	Undecanoicacid	$C_{11}H_{22}O_2$	186	27.56
10	1-Docosene	$C_{22}H_{44}$	308	28.70
11	Squalene	$C_{30}H_{50}$	410	28.92
12	Campesterol	$C_{28}H_{48}O$	400	31.70
13	à-Tocopherolquinone	$C_{29}H_{50}O_3$	446	33.16
14	1-Undecanol	$C_{11}H_{24}O$	172	34.41
15	1,2-Benzenedicarboxylic acid,diisooctylester	$C_{24}H_{38}O_4$	390	34.81
16	Stigmasterol	$C_{29}H_{48}O$	412	35.31
17	Phytol	$C_{20}H_{40}O$	296	35.84

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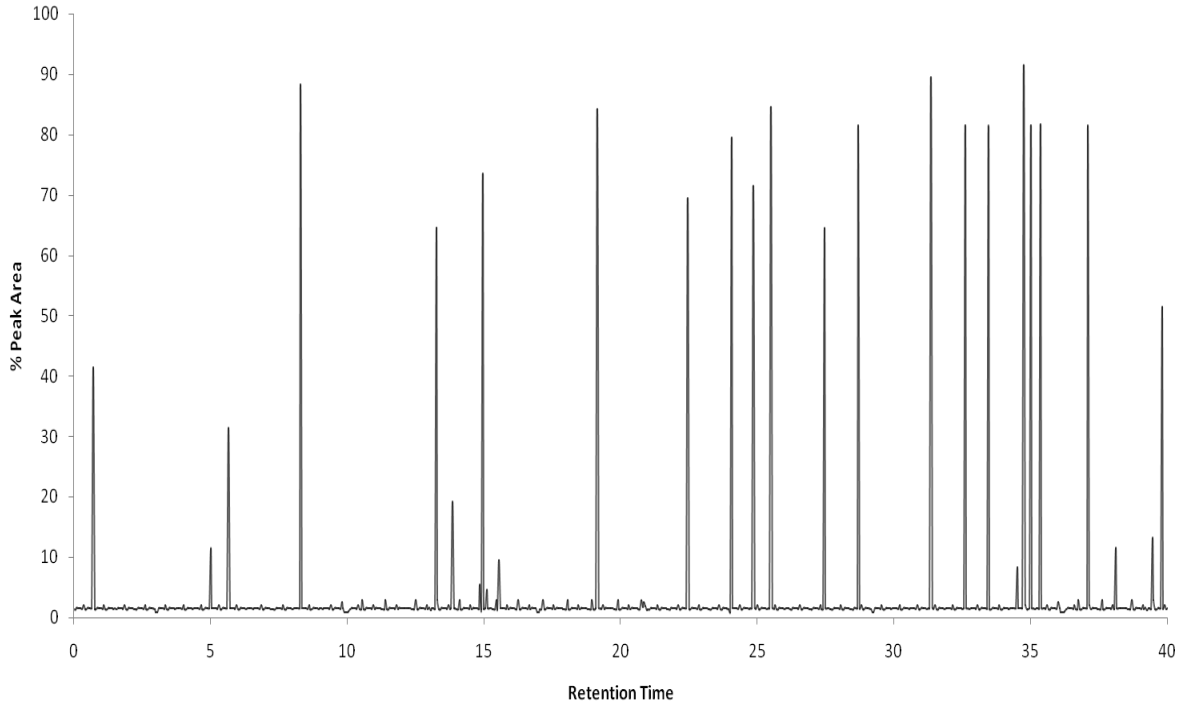

Figure 1: GC-MS Chromatogram of *Aegle marmelos* (L.) Methanol Extract

3.5 Assessment of Antioxidant Activity

3.5.1. DPPH scavenging assay

The AEAM and MEAM in concentration range of 100-1000 µg/ml inhibited DPPH radical formation as indicated by concentration dependent decrease in the purple colour of the solution. Similar effect was obtained with standard antioxidant- Butylated Hydroxy Toluene (BHT) in the concentration range of 10-100 µg/ml. In linear regression analysis of concentration versus percent DPPH inhibition was carried out. The linear regression coefficient of AEAM, MEAM and BHT were r^2=0.995, r^2=0.999 and r^2=0.998, respectively, suggesting that the DPPH scavenging was concentration dependent. The IC_{50} value of AEAM and BHT, obtained from regression analysis, were 602.733, 399.408 and 50.173 µg/ml, respectively (Table 3).

Table 3: Effect of AEAM and MEAM on DPPH radical scavenging

Concentration (µg/ml)		% DPPH Inhibition	IC_{50} Value
AEAM	**100**	16.357±0.315	**602.733 µg/ml**
	200	27.293±0.173	
	400	37.545±0.990	
	600	48.515±0.285	
	800	62.749±0.824	
	1000	75.064±0.223	
MEAM	**100**	21.25±0.145	**399.408 µg/ml**
	200	39.14±0.845	
	400	51.09±1.154	
	600	69.45±0.425	
	800	80.09±0.666	
	1000	95.14±0.856	
BHT	**10**	13.311±0.397	**50.173 µg/ml**
	20	25.706±0.529	
	40	47.305±0.496	
	60	65.163±0.636	

	80	75.064±0.223	
	100	80.271±0.257	

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

3.5.2. Reducing power assay

The AEAM 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. A concentration verses absorbance graph comparing ascorbic acid and AEAM and MEAM were plotted and depicted in (Figure 2).

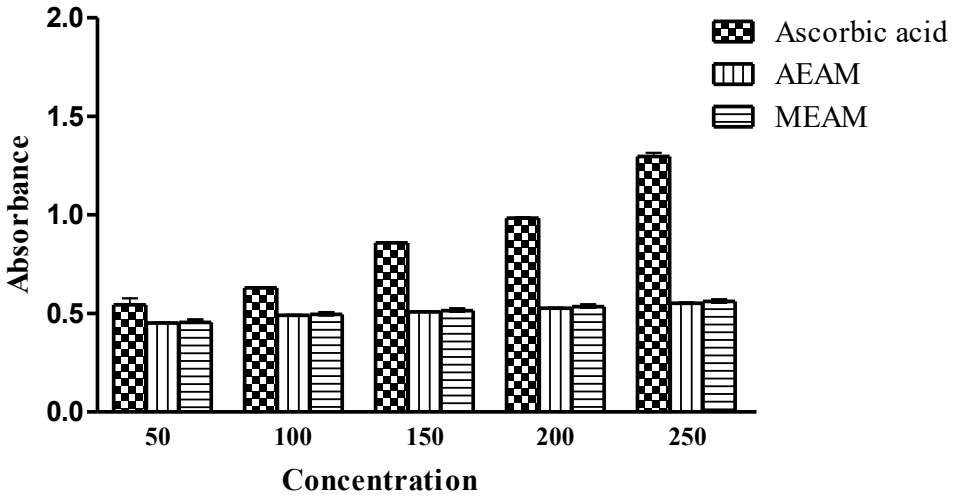

Figure 2: Effect of AEAM on reducing potential

(Results are expressed as Mean±SEM; n=3)

4. CONCLUSIONS

Aegle marmelos L., a native plant of India, is traditionally used in traditional and folk medicinal systems for treating diarrhea, dysentery, and infertility/abortion in women. The tree is considered sacred by Hindus and its fruits are used in traditional medicine and as food throughout its range. Gastric ulcers, a common issue among the global population, are treated with synthetic drugs that have harmful effects. Recent interest in the medicinal potential of plants has led to the development of herbal remedies for gastric ulcers. *Aegle marmelos* presents a better option due to its scientifically proven antibacterial and antiulcer properties. Its components have therapeutic and medical use, and it has strong antioxidant and antiulcer effects against Helicobacter pylori-caused ulcers on the stomach mucosa. This study aims to find scientific validation for the efficacy of *Aegle marmelos* fruits against gastric ulcers, based on supportive evidence and previous studies. The physicochemical constants of *Aegle marmelos* were found to be of standard quality, with the aqueous extract (AEAM) of a dark brown color and dry amorphous consistency yielding 29.61% w/w and the methanolic extract (MEAM) of a greenish blue with sticky consistency yielding 41.69% w/w. The total flavonoid content of AEAM and MEAM were found to be 19.71 ± 0.841 and 23.45 ± 0.881 mg quercetin equivalents/g of extract and total phenolic content of AEAM and MEAM were found to be 22.48 ± 0.107 and 42.08 ± 0.458 mg tannic acid equivalents/g of extract. The GC-MS analysis of the Methanolic extract of *Aegle marmelos* Linn Fruits revealed the presence of twenty compounds, including Stigmasterol, Tetradecanoic acid, and n-Hexadecanoic acid.

5. CONFLICT OF INTEREST

None

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