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Physico Phytochemical Analysis, Authentication And *In Vitro* Antioxidant Study on the *Aegle marmelos*

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Abstract

Traditional system of medicine consists large number of medicinal plants, which conveyed their potential therapeutic utilities. *Aegle marmelos* is commonly known as Bael belongs to Rutaceae family, widely grown in India, Tropical and Subtropical countries. It possesses great mythological significance and medicinal uses in ancient system of medicine. The *Aegle marmelos* plant leaves powder is analyzed for organoleptic characters, fluorescence studies, phytochemical screening, physicochemical parameters, qualitative analysis and ethanolic extract of the plant leaves powder is analyzed for quantitative elemental analysis, HPTLC studies for authentication of *Aegle marmelos* plant and *invitro* antioxidant study by DPPH method. It is proven that it contains antioxidant property.

Keywords

Aegle marmelos, physicochemical parameters, Elemental analysis, HPTLC and antioxidant study.

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INTRODUCTION:

Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts. It is also known as botanical medicine, medical herbalism ,herbal medicine, herbology and phytotherapy.(1).Plants are rich source of ecological developed secondary metabolites which are potential remedies for different ailments(2).*Aegle marmelos(L)* commonly known as Bael or Bilva belonging to the family Rutaceae has been widely used in indigenous system of Indian medicine due to its various medicinal properties(3).The Bael tree has its origin from Eastern Ghats and Central India. It is found growing along foothills of Himalayas, Uttaranchal, Jharkhand, Madhya Pradesh, the Deccan plateau and along the East coast (4). The present study on this plant provide information on chemical marker, inter specific variations, HPTLC study for authentification and to find out whether plant posses anti oxidant potential.

MATERIALS AND METHODS:

Aegle marmelos leaves are collected from Ammapettai of Thanjavur district, Tamilnadu, India. The voucher specimen are authenticated by the Rapinat Herbarium, St.Joseph's college, Tiruchirappalli, Tamil Nadu, India.

The leaves of plant are rinsed with water thrice to remove the fine dust materials. The leaves of plant are air dried for 15 days and then they are kept in air hot oven at 60^{o} for 36 hrs. The leaves are ground to a fine powder.50g of powdered material of sample is packed in soxlet thimble and extracted using ethanol, ethyl acetate, water, toluene and benzene

as a solvent. The extract is filtered with the help of filter paper. Then the extract is kept individually in refrigerator for future experiments. The analysis such as organoleptic character (5),fluorescence studies on day light and UV light (6), phytochemical screening (7-8),physicochemical properties (9-10) are carried out according to the standard methods, elemental analysis (11) and antioxidant potential are carried out using DPPH method(12-15).The HPTLC finger print analysis are done by taking ethanolic extract. The sample extract 10, 15 and 20µl of extract are applied for high performance thin layer chromatographic study with solvent system toluene, ethyl acetate and formic acid (7:3:0.5) using TLC Chamber. After development, the plates are photo documented using camag's TLC visualizer under uv 254nm,366nm and visible light after derivatized using vanillin sulphuric acid. The Rf values of the spots are recorded (16).

Elemental Analysis of *Aegle marmelos* leaf powder (11)

1g of *Aegle marmelos* leaf powder are digested individually in 10 ml of ultrapure metal free nitric acid in a microwave digester. After digestion, the content is diluted to 25 ml with distilled water. Estimation of elements are performed using inductively coupled plasma with Optical Emission Spectroscopy (ICP-OES). Perkin Elmer Optima (5300 DO). The microwave digested sample is aspirated into ICP-OES to estimate elements (12). like Cr, Cu, Mn, Zn, Se, V, Si.

The Calibration standards are prepared by diluting the stock multi-elemental standard solution (1000mg/l) in HNO_3.

Determination of antioxidant study by DPPH method (12 -15)

Free radical scavenging activity of ethanolic extract are tested against a methanolic solution of 1,1-diphenyl-2-picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1,1-diphenyl-2-picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the absorbance produced at 517nm has been used as a measure of antioxidant activity. The sample of extract are prepared in various concentrations viz 20,40,60,80µ g/ml. 1 ml of sample are mixed with equal volume of 0.1mM methanolic solution of DPPH at various concentrations. Ascorbic acid is used as a standard. After incubation for 30minutes in dark, absorbance is recorded at 517nm.Experiments are performed in triplicates. % scavenging is calculated by using the formula.

Calculation:

Percentage of anti-radical activity= [A-B/A] ×100

Where A= absorbance of control and B= absorbance of sample

Table 1: Organoleptic characters

S.No	Character	Leaf powder
1	Colour	Green
2	Odour	No Smell
3	Taste	Astringent
4	Texture	Rough

Table 2: Fluorescence studies

Reagents used	Daylight	Uv at 365 nm
S+1N NaOH	Brown	Dark green
S+1N Hcl	Greenish brown	Dark green
S+H_2So_4	Greenish brown	Dark green
S+ConcHNO_3	Brown	Dark green
S+$Fecl_3$	Green	Dark green
S+NH3	Dark green	Dark green
S+Sodium nitro prusside	Green	Dark green
S+KOH in H2O	Brownish green	Dark green
S+picric acid	Dark green	Dark green
S+Acetic acid	Green	Dark green

S-Sample of *Aegle marmelos* leaf powder

Table 3: Physico-chemical properties of *Aegle marmelos* leaf powder

S.No	Characters	*Aegle Marmelos*(g)
1	Total ash	0.34
2	Water soluble ash	0.25
3	Acid insoluble ash	0.24
4	Sulphated ash	1.24
5	Moisture content	0.005

***Aegle marmelos* leaf powder taken 5g**

Table 4: Extractive values for *Aegle marmelos* leaf powder is in different solvent

S.No	Solvents	*Aegle marmelos* extract values(g)
1	Water	1.56
2	Ethanol	1.72
3	Ethyl acetate	1.26
4	Toluene	0.86
5	Benzene	0.73

***Aegle marmelos* leaf powder taken 5g**

Table5: Qualitative Analysis of Water,Ethanol, Ethyl Acetate,Toluene and Benzene Extract of *Aegle marmelos* leaf powder

S.No	Name of the Test	Phytochemical constituents	Water extract	Ethanol extract	Ethyl Acetate extract	Toluene extract	Benzene extract
	Mayer's test		+	+	+	-	-
1.	Dragondraff test	**Alkaloids**	+	+	+	-	-
	Wagner Test		-	+	+	-	-
2.	Molish Test	**Carbohydrates**	+	-	-	-	-
3.	Foam Test	**Saponins**	-	-	-	-	-
4.	Lead Acetate	**Tannins**	+	+	-	+	+
5.	Liebermann's	**Steroidal Glycosides**	+	-	++	-	-
6.	Ammonia	**Flavonoids**	-	+	+	-	-
7.	Ferric chloride	**Phenols**	+	+	+	+	+
8.	Salkowski's Test	**Terpenoid**	+	+	+	+	+

+ shows the presence of the constituents,
- shows the absence of the constituents

Table6: Elemental Analysis of *Aegle marmelos* leaf powder

Elements	Cr	Cu	Mn	Zn	Se	V	Si
Aegle marmelos(mg/l)	0.237	0.071	0.526	1.802	2.391	0.091	0.214

Fig 1-TLC Photo documentation of *Aegle marmelos* (Ethanol extract)

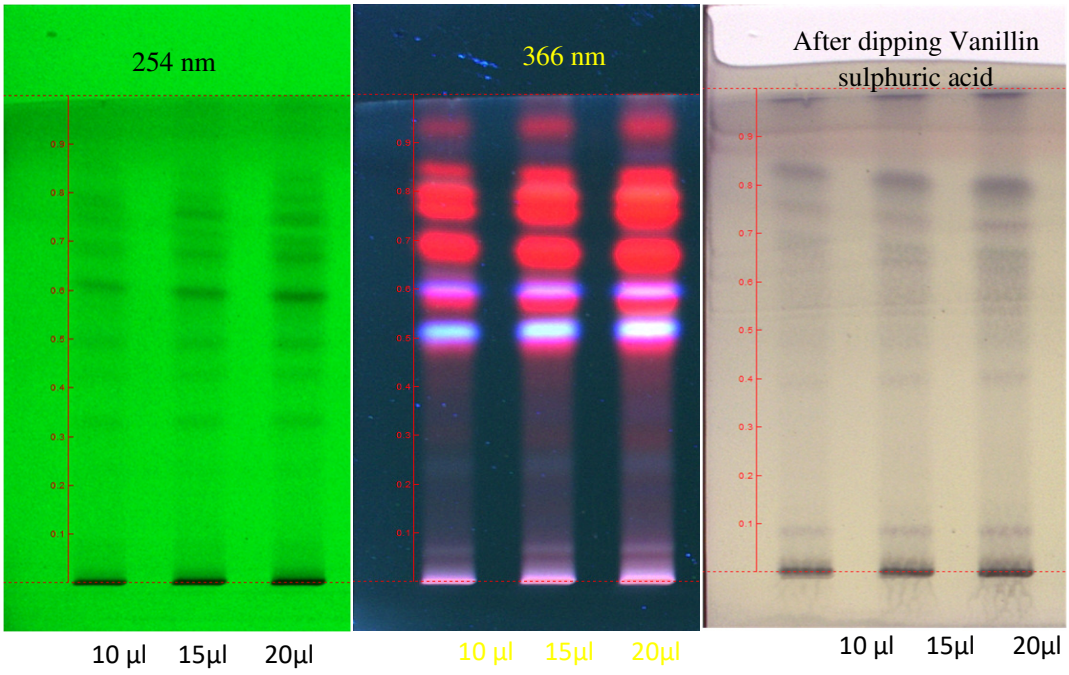
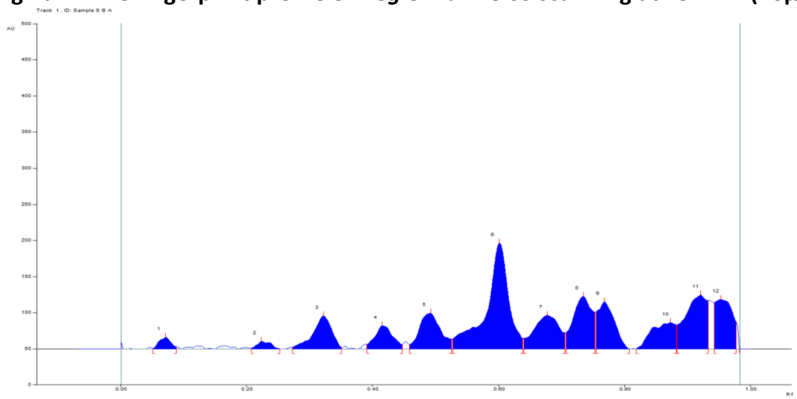


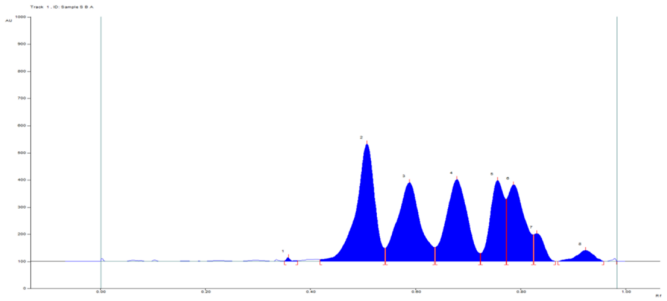
Fig 2a-HPTLC fingerprint profile of *Aegle marmelos* scanning at 254 nm (10µl)



Track 1, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.7 AU	0.07 Rf	16.2 AU	2.44 %	0.09 Rf	2.9 AU	261.2 AU	1.41 %
2	0.21 Rf	1.4 AU	0.22 Rf	10.6 AU	1.60 %	0.25 Rf	0.2 AU	198.5 AU	1.07 %
3	0.27 Rf	2.2 AU	0.32 Rf	45.8 AU	6.90 %	0.35 Rf	1.7 AU	1193.8 AU	6.46 %
4	0.39 Rf	6.3 AU	0.42 Rf	32.3 AU	4.86 %	0.45 Rf	5.9 AU	801.1 AU	4.34 %
5	0.46 Rf	6.1 AU	0.49 Rf	49.7 AU	7.47 %	0.53 Rf	13.8 AU	1446.4 AU	7.83 %
6	0.53 Rf	14.0 AU	0.60 Rf	146.5 AU	22.04 %	0.64 Rf	14.8 AU	4481.4 AU	24.26 %
7	0.64 Rf	14.8 AU	0.68 Rf	46.3 AU	6.96 %	0.71 Rf	22.3 AU	1615.8 AU	8.75 %
8	0.71 Rf	22.7 AU	0.74 Rf	72.8 AU	10.95 %	0.75 Rf	51.0 AU	1890.4 AU	10.23 %
9	0.76 Rf	51.5 AU	0.77 Rf	65.1 AU	9.80 %	0.81 Rf	0.1 AU	1521.6 AU	8.24 %
10	0.82 Rf	0.3 AU	0.87 Rf	36.3 AU	5.46 %	0.88 Rf	33.5 AU	1201.5 AU	6.51 %
11	0.89 Rf	33.5 AU	0.92 Rf	74.5 AU	11.21 %	0.93 Rf	67.0 AU	2211.8 AU	11.97 %
12	0.94 Rf	64.2 AU	0.95 Rf	68.5 AU	10.31 %	0.98 Rf	37.6 AU	1646.9 AU	8.92 %

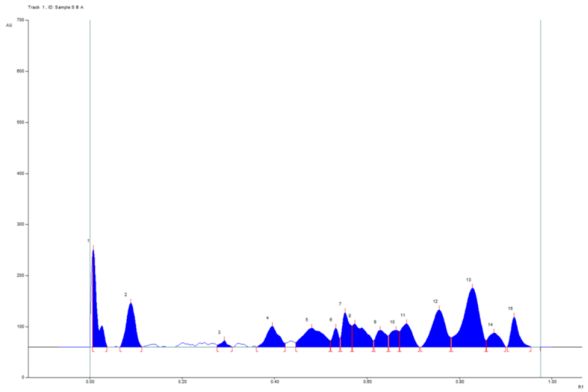
Fig 2b-HPTLC fingerprint profile of *Aegle marmelos* scanning at 366 nm (10µl)



Track 1, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.35 Rf	1.5 AU	0.36 Rf	14.0 AU	0.79 %	0.38 Rf	2.2 AU	98.6 AU	0.19 %
2	0.42 Rf	6.7 AU	0.51 Rf	432.7 AU	24.60 %	0.54 Rf	48.2 AU	13227.5 AU	25.43 %
3	0.54 Rf	49.2 AU	0.59 Rf	289.7 AU	16.47 %	0.64 Rf	51.4 AU	10817.6 AU	20.80 %
4	0.64 Rf	51.5 AU	0.68 Rf	301.3 AU	17.13 %	0.72 Rf	29.2 AU	10321.4 AU	19.85 %
5	0.73 Rf	29.6 AU	0.76 Rf	297.8 AU	16.93 %	0.77 Rf	27.6 AU	6896.8 AU	13.26 %
6	0.77 Rf	229.3 AU	0.79 Rf	281.8 AU	16.02 %	0.83 Rf	97.7 AU	7814.7 AU	15.03 %
7	0.83 Rf	98.2 AU	0.83 Rf	101.8 AU	5.79 %	0.87 Rf	0.1 AU	1685.5 AU	3.24 %
8	0.87 Rf	0.1 AU	0.93 Rf	40.0 AU	2.27 %	0.96 Rf	0.1 AU	1144.6 AU	2.20 %

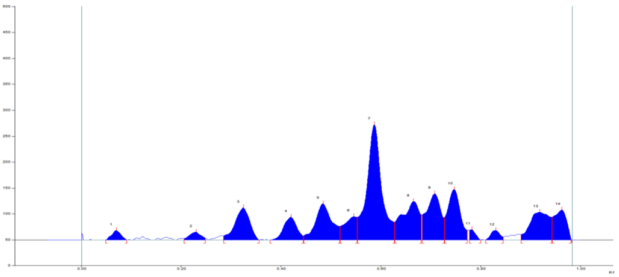
Fig 2c-HPTLC fingerprint profile of *Aegle marmelos* scanning at 520 nm (10µl)



Track 1, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	185.9 AU	0.01 Rf	192.1 AU	21.28 %	0.04 Rf	2.0 AU	1644.4 AU	9.80 %
2	0.07 Rf	0.1 AU	0.09 Rf	86.2 AU	9.55 %	0.11 Rf	0.5 AU	1414.7 AU	8.43 %
3	0.28 Rf	3.7 AU	0.29 Rf	12.5 AU	1.39 %	0.31 Rf	0.3 AU	152.1 AU	0.91 %
4	0.36 Rf	0.0 AU	0.40 Rf	40.5 AU	4.48 %	0.42 Rf	7.4 AU	925.7 AU	5.52 %
5	0.45 Rf	8.7 AU	0.48 Rf	36.7 AU	4.07 %	0.52 Rf	12.2 AU	1419.4 AU	8.46 %
6	0.52 Rf	12.6 AU	0.53 Rf	37.2 AU	4.12 %	0.54 Rf	16.0 AU	409.0 AU	2.44 %
7	0.54 Rf	18.3 AU	0.55 Rf	67.5 AU	7.48 %	0.57 Rf	41.5 AU	927.6 AU	5.53 %
8	0.57 Rf	41.8 AU	0.57 Rf	45.0 AU	4.98 %	0.61 Rf	12.5 AU	1127.3 AU	6.72 %
9	0.62 Rf	12.7 AU	0.63 Rf	32.7 AU	3.62 %	0.65 Rf	21.8 AU	622.8 AU	3.71 %
10	0.65 Rf	22.2 AU	0.66 Rf	32.9 AU	3.64 %	0.67 Rf	30.6 AU	538.9 AU	3.21 %
11	0.67 Rf	31.1 AU	0.69 Rf	45.4 AU	5.03 %	0.71 Rf	0.3 AU	926.3 AU	5.52 %
12	0.72 Rf	0.1 AU	0.76 Rf	72.6 AU	8.05 %	0.78 Rf	18.9 AU	1848.0 AU	11.01 %
13	0.78 Rf	18.9 AU	0.83 Rf	115.3 AU	12.77 %	0.86 Rf	12.4 AU	3387.1 AU	20.19 %
14	0.86 Rf	13.0 AU	0.88 Rf	27.4 AU	3.03 %	0.90 Rf	1.0 AU	594.3 AU	3.54 %
15	0.91 Rf	0.8 AU	0.92 Rf	58.6 AU	6.50 %	0.96 Rf	0.2 AU	840.4 AU	5.01 %

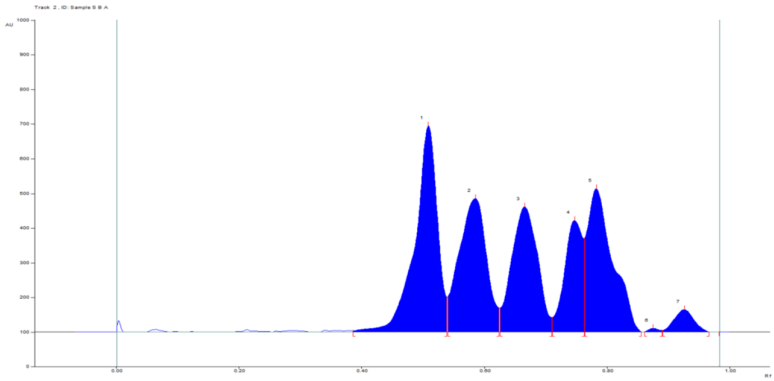
Fig 3a - HPTLC fingerprint profile of *Aegle marmelos* scanning at 254 nm (15µl)



Track 2, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.1 AU	0.07 Rf	18.4 AU	2.07 %	0.09 Rf	0.0 AU	289.7 AU	1.32 %
2	0.21 Rf	2.8 AU	0.23 Rf	15.4 AU	1.73 %	0.25 Rf	3.4 AU	303.0 AU	1.38 %
3	0.29 Rf	7.8 AU	0.33 Rf	62.0 AU	6.99 %	0.36 Rf	0.0 AU	1603.6 AU	7.29 %
4	0.38 Rf	0.1 AU	0.42 Rf	43.9 AU	4.95 %	0.45 Rf	8.0 AU	1016.6 AU	4.62 %
5	0.45 Rf	8.4 AU	0.49 Rf	69.8 AU	7.87 %	0.52 Rf	26.1 AU	2012.5 AU	9.14 %
6	0.52 Rf	26.3 AU	0.55 Rf	44.9 AU	5.07 %	0.55 Rf	43.8 AU	961.7 AU	4.37 %
7	0.55 Rf	44.0 AU	0.59 Rf	222.5 AU	25.09 %	0.63 Rf	34.2 AU	5649.6 AU	25.67 %
8	0.63 Rf	34.4 AU	0.67 Rf	74.4 AU	8.39 %	0.68 Rf	48.4 AU	2249.5 AU	10.22 %
9	0.68 Rf	48.6 AU	0.71 Rf	88.9 AU	10.03 %	0.73 Rf	42.7 AU	2223.1 AU	10.10 %
10	0.73 Rf	43.9 AU	0.75 Rf	97.1 AU	10.95 %	0.77 Rf	18.7 AU	2119.3 AU	9.63 %
11	0.78 Rf	18.6 AU	0.78 Rf	19.8 AU	2.23 %	0.80 Rf	0.0 AU	211.6 AU	0.96 %
12	0.81 Rf	0.1 AU	0.83 Rf	18.3 AU	2.06 %	0.85 Rf	5.9 AU	295.9 AU	1.34 %
13	0.88 Rf	10.6 AU	0.92 Rf	53.5 AU	6.03 %	0.94 Rf	43.8 AU	1835.4 AU	8.34 %
14	0.95 Rf	44.2 AU	0.96 Rf	57.9 AU	6.53 %	0.98 Rf	2.5 AU	1239.0 AU	5.63 %

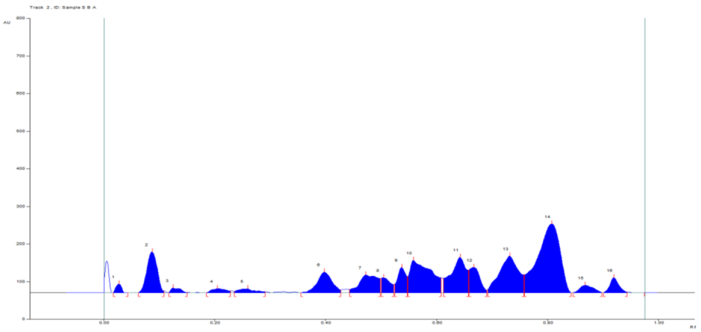
Fig3b-HPTLC fingerprint profile of *Aegle marmelos* scanning at 366 nm (15µl)



Track 2, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.39 Rf	3.6 AU	0.51 Rf	594.3 AU	27.67 %	0.54 Rf	00.1 AU	18909.5 AU	26.23 %
2	0.54 Rf	100.6 AU	0.59 Rf	384.1 AU	17.88 %	0.63 Rf	69.0 AU	15027.2 AU	20.84 %
3	0.63 Rf	69.2 AU	0.67 Rf	360.9 AU	16.80 %	0.71 Rf	41.6 AU	13274.3 AU	18.41 %
4	0.71 Rf	41.9 AU	0.75 Rf	321.3 AU	14.96 %	0.76 Rf	68.7 AU	8277.9 AU	11.48 %
5	0.77 Rf	271.3 AU	0.78 Rf	413.0 AU	19.22 %	0.86 Rf	0.6 AU	14678.9 AU	20.36 %
6	0.86 Rf	0.3 AU	0.88 Rf	10.2 AU	0.47 %	0.89 Rf	4.8 AU	143.4 AU	0.20 %
7	0.89 Rf	5.0 AU	0.93 Rf	64.4 AU	3.00 %	0.97 Rf	0.1 AU	1785.9 AU	2.48 %

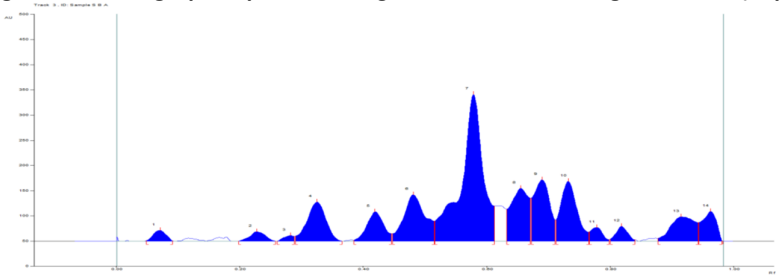
Fig3c-HPTLC fingerprint profile of *Aegle marmelos* scanning at 520 nm (15µl)



Track 2, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.02 Rf	0.1 AU	0.03 Rf	23.1 AU	2.39 %	0.04 Rf	0.0 AU	208.3 AU	0.89 %
2	0.06 Rf	0.1 AU	0.09 Rf	108.6 AU	11.27 %	0.11 Rf	4.9 AU	1764.7 AU	7.57 %
3	0.12 Rf	0.5 AU	0.13 Rf	12.8 AU	1.33 %	0.15 Rf	0.9 AU	198.3 AU	0.85 %
4	0.19 Rf	0.1 AU	0.21 Rf	10.7 AU	1.11 %	0.23 Rf	4.6 AU	245.9 AU	1.05 %
5	0.24 Rf	4.1 AU	0.26 Rf	10.6 AU	1.10 %	0.29 Rf	2.6 AU	277.6 AU	1.19 %
6	0.36 Rf	0.2 AU	0.40 Rf	54.8 AU	5.69 %	0.43 Rf	7.5 AU	1281.8 AU	5.50 %
7	0.44 Rf	8.6 AU	0.47 Rf	47.4 AU	4.92 %	0.50 Rf	38.3 AU	1392.2 AU	5.97 %
8	0.50 Rf	38.7 AU	0.51 Rf	39.8 AU	4.13 %	0.52 Rf	22.2 AU	583.1 AU	2.50 %
9	0.53 Rf	22.8 AU	0.54 Rf	67.1 AU	6.97 %	0.55 Rf	42.8 AU	881.4 AU	3.78 %
10	0.55 Rf	43.0 AU	0.56 Rf	86.4 AU	8.97 %	0.61 Rf	39.9 AU	2877.4 AU	12.34 %
11	0.61 Rf	39.1 AU	0.64 Rf	94.3 AU	9.78 %	0.66 Rf	60.4 AU	2220.0 AU	9.52 %
12	0.66 Rf	61.1 AU	0.67 Rf	67.4 AU	6.99 %	0.69 Rf	7.1 AU	1104.8 AU	4.74 %
13	0.69 Rf	8.1 AU	0.73 Rf	97.4 AU	10.11 %	0.76 Rf	47.9 AU	2926.9 AU	12.56 %
14	0.76 Rf	48.1 AU	0.81 Rf	183.2 AU	19.01 %	0.84 Rf	0.1 AU	6362.7 AU	27.29 %
15	0.85 Rf	0.4 AU	0.87 Rf	19.6 AU	2.04 %	0.90 Rf	0.3 AU	424.0 AU	1.82 %
16	0.90 Rf	0.2 AU	0.92 Rf	40.3 AU	4.18 %	0.95 Rf	1.7 AU	563.0 AU	2.42 %

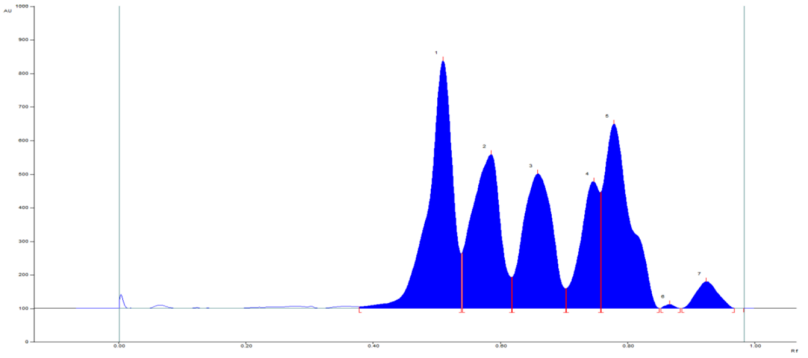
Fig 4a-HPTLC fingerprint profile of *Aegle marmelos* scanning at 254 nm (20µl)



Track 3, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	1.3 AU	0.07 Rf	21.7 AU	2.00 %	0.09 Rf	0.3 AU	389.0 AU	1.38 %
2	0.20 Rf	0.2 AU	0.23 Rf	19.1 AU	1.76 %	0.26 Rf	0.3 AU	411.5 AU	1.46 %
3	0.26 Rf	0.1 AU	0.28 Rf	11.3 AU	1.04 %	0.29 Rf	9.7 AU	160.8 AU	0.57 %
4	0.29 Rf	9.9 AU	0.33 Rf	77.8 AU	7.19 %	0.37 Rf	0.1 AU	2052.9 AU	7.29 %
5	0.39 Rf	2.1 AU	0.42 Rf	58.5 AU	5.41 %	0.45 Rf	14.8 AU	1382.2 AU	4.91 %
6	0.45 Rf	14.9 AU	0.48 Rf	92.2 AU	8.53 %	0.52 Rf	39.9 AU	2791.2 AU	9.91 %
7	0.52 Rf	40.3 AU	0.58 Rf	291.2 AU	26.91 %	0.61 Rf	69.6 AU	9011.9 AU	32.00 %
8	0.63 Rf	63.7 AU	0.66 Rf	104.9 AU	9.70 %	0.67 Rf	85.5 AU	2635.3 AU	9.36 %
9	0.67 Rf	85.6 AU	0.69 Rf	121.8 AU	11.26 %	0.71 Rf	42.1 AU	2787.8 AU	9.90 %
10	0.71 Rf	42.3 AU	0.73 Rf	118.9 AU	10.99 %	0.77 Rf	18.3 AU	2762.8 AU	9.81 %
11	0.77 Rf	18.9 AU	0.78 Rf	27.2 AU	2.51 %	0.80 Rf	3.2 AU	445.4 AU	1.58 %
12	0.80 Rf	3.7 AU	0.82 Rf	29.8 AU	2.76 %	0.84 Rf	3.1 AU	484.3 AU	1.72 %
13	0.88 Rf	5.8 AU	0.92 Rf	48.4 AU	4.48 %	0.94 Rf	37.1 AU	1670.0 AU	5.93 %
14	0.95 Rf	37.3 AU	0.96 Rf	59.2 AU	5.47 %	0.98 Rf	0.9 AU	1179.9 AU	4.19 %

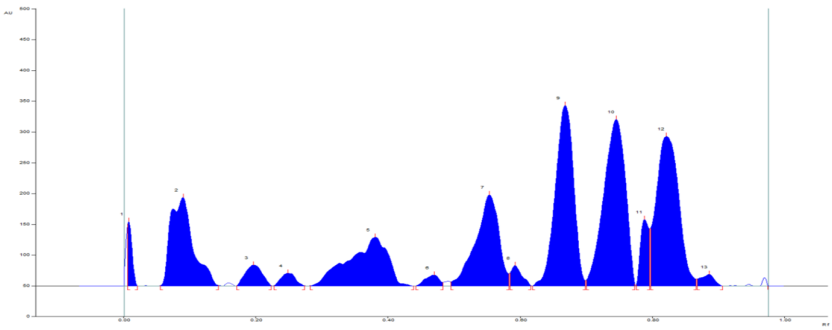
Fig4b-HPTLC fingerprint profile of *Aegle marmelos* scanning at 366 nm (20µl)



Track 3, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.38 Rf	4.4 AU	0.51 Rf	737.7 AU	28.21 %	0.54 Rf	62.3 AU	23895.2 AU	26.85 %
2	0.54 Rf	165.5 AU	0.59 Rf	458.8 AU	17.55 %	0.62 Rf	92.6 AU	17472.9 AU	19.63 %
3	0.62 Rf	93.1 AU	0.66 Rf	401.1 AU	15.34 %	0.70 Rf	59.0 AU	15963.1 AU	17.94 %
4	0.71 Rf	59.6 AU	0.75 Rf	377.3 AU	14.43 %	0.76 Rf	44.3 AU	9707.0 AU	10.91 %
5	0.76 Rf	346.8 AU	0.78 Rf	549.0 AU	21.00 %	0.85 Rf	0.1 AU	19451.6 AU	21.86 %
6	0.85 Rf	0.5 AU	0.87 Rf	11.2 AU	0.43 %	0.88 Rf	0.0 AU	140.1 AU	0.16 %
7	0.89 Rf	0.1 AU	0.93 Rf	79.7 AU	3.05 %	0.97 Rf	0.2 AU	2364.6 AU	2.66 %

Fig4c-HPTLC fingerprint profile of *Aegle marmelos* scanning at 520 nm (20µl)



Track 3, ID: Sample S B A

Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.01 Rf	0.4 AU	0.03 Rf	34.0 AU	2.75 %	0.04 Rf	0.1 AU	327.6 AU	1.05 %
2	0.06 Rf	0.3 AU	0.09 Rf	117.9 AU	9.52 %	0.11 Rf	0.2 AU	1892.7 AU	6.09 %
3	0.19 Rf	2.1 AU	0.21 Rf	13.0 AU	1.05 %	0.22 Rf	7.0 AU	227.7 AU	0.73 %
4	0.37 Rf	0.1 AU	0.40 Rf	73.2 AU	5.91 %	0.43 Rf	13.9 AU	1674.4 AU	5.39 %
5	0.44 Rf	12.0 AU	0.48 Rf	70.3 AU	5.68 %	0.50 Rf	58.0 AU	1986.2 AU	6.40 %
6	0.50 Rf	58.0 AU	0.51 Rf	60.6 AU	4.90 %	0.52 Rf	45.7 AU	1006.1 AU	3.24 %
7	0.53 Rf	46.0 AU	0.54 Rf	95.0 AU	7.67 %	0.55 Rf	73.9 AU	1767.6 AU	5.69 %
8	0.55 Rf	73.9 AU	0.57 Rf	125.2 AU	10.11 %	0.59 Rf	84.1 AU	3156.3 AU	10.16 %
9	0.60 Rf	84.2 AU	0.63 Rf	113.9 AU	9.20 %	0.64 Rf	04.8 AU	2963.1 AU	9.54 %
10	0.64 Rf	105.0 AU	0.65 Rf	115.9 AU	9.36 %	0.68 Rf	24.9 AU	2904.1 AU	9.35 %
11	0.68 Rf	25.1 AU	0.72 Rf	142.3 AU	11.49 %	0.74 Rf	49.6 AU	3396.4 AU	10.94 %
12	0.74 Rf	50.0 AU	0.80 Rf	230.2 AU	18.59 %	0.83 Rf	0.0 AU	8949.7 AU	28.82 %
13	0.84 Rf	2.1 AU	0.88 Rf	11.9 AU	0.96 %	0.89 Rf	0.5 AU	306.2 AU	0.99 %
14	0.90 Rf	0.6 AU	0.91 Rf	34.8 AU	2.81 %	0.95 Rf	0.0 AU	495.9 AU	1.60 %

Tab7 - DPPH radical scavenging activity of *Aegle marmelos*

Plant extract(µg/ml) Concentration	% scavenging activity	% scavenging of Ascorbic acid (Standard)
20	68.18±4.77	25.6±2.04
40	72.72±5.09	61.26±4.90
60	81.18±5.68	88.98±7.11
80	88.3±6.16	99.34±7.94

Experiments are performed in triplicates

RESULTS AND DISCUSSION

In the present study, the results of organoleptic character, fluorescence characteristics, physicochemical parameter of the leaves are shown in the table 1,2,3,4,5. The leaves are appeared in green colour and there is no odour. The leaves are astringent in taste and the texture is rough. The plant powder when treated with 1N NaOH if viewed under normal light appear brown and dark green under uv light at 365nm.The results with various biochemical reagents are shown in the table2.The results indicates the narrow ranging of colour variation. The physicochemical parameters of study plants are shown in table 3. The total cash value is 0.34mg, water soluble ash value is 0.25mg. On comparing the sulphated ash value is seen to be very high 1.24 mg. This preliminary finding helps to access the inner nature of the plant. The extractive values are shown in the table4 for different solvents like water, ethanol, ethyl acetate, toluene and benzene. Among these, the polar solvents water, ethanol values found to be more when compared to non-polar solvents. This information gives that polar solvents has more capacity to remove the phytoconstituents from the plant. The elements like copper and chromium are very much helpful in metabolism. People with ischemic heart disease have decreased cardiac, leucocyte copper and decreased activities of some copper dependent enzymes. Abnormalities of lipid metabolism, blood pressure control electrocardiogram impaired glucose tolerance. Chromium is an essential mineral that appears to have a beneficial role in regulation of insulin action, metabolic syndrome and to reduce the risk of cardiovascular disease. (17-20).

For authentication and standardization of study plant one of the important techniques is HPTLC finger print analysis. In HPTLC, plates are shown in fig1.The RF values obtained at 254nm for various concentration 10µl,15µl and 20µl are shown in fig 2a,3a,4a. Similarly, at 366nm for 10µl,15µl and 20µl are shown in fig 2b,3b,4b and at 520nm are shown in fig 2c,3c,4c.

The peaks and peak areas are shown in figures. The Rf values information are very much helpful in standardization of plant drugs. If there is any contamination or impurity during plant collection there will be a change in the peaks which clearly indicates the adulteration. So, the finger print chromatogram is used for the authentification of the study plant *Aegle marmelos*.

The *invitro* antioxidant of plant are studied using DPPH method with different concentration of ethanolic extract are shown in the table 6. The antioxidant activity of the study plant extracts are compared with standard Ascorbic acid at different concentration. At 80µl, Percentage of free radical scavenging activity 88.3 whereas for standard 99.34. The value is lower but nearer to the standard. So, the plant extract has good antiradical scavenging property.

CONCLUSION

The results of the study clearly indicate that the plant possess many important bioactive constituents like alkaloids, steroids, flavonoids, tannins and phenols. The study plant extractive values indicate that the ethanol has a more power in extracting bio-active principles than other solvents. The HPTLC results of study plant is very much helpful in authentication of the study plant by Rf values, number of peaks and peak intensities. The elemental analysis is helpful in knowing the elements are presents which are helpful in the metabolism. The elements present in the study plant are within permissible limits which are needed for the daily requirements of human metabolism. The plant has excellent antioxidant activity achieved by scavenging activity against DPPH radical. Further investigation is needed to explore the active components which are responsible for the antioxidant potential.

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