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# Comparative analysis of phytochemical constituents present in various parts of *Aegle marmelos*

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## ABSTRACT

**Objective:** To perform phytochemical screening on all parts (Root, Stem bark, Stem, Leaf and Fruit) of *Aegle marmelos* so as to find out the identical phytochemicals and enhance its usage in drug preparation. **Methods:** Screening is performed for aqueous and methanol extract of various parts of the plant by High Performance Thin Layer Chromatography. **Results:** Stem bark and root of aqueous extracts have similar phytochemical compounds. Marmelosin is found in all parts of the plant and its concentration is high in fruit. **Conclusions:** Stem bark could be substituted or added along with the root in any of the drug preparation where root is of important. Since marmelosin is a coumarin and is found in all parts we can conclude that coumarins are present in all parts of the plant.

## 1. Introduction

*Aegle marmelos* is a mid-sized, slender, aromatic, armed, gum-bearing tree growing up to 18 meters tall. Older branches are neither straight sharp single nor paired, 2.5 cm long. Young branches are green slightly zigzag and compressed. Leaves are alternate, attenuate trifoliate, occasionally digitately 5-foliate; petiole is 2.5 to 6.3 cm long. All the parts of this tree including stem, bark, root, leaves and fruit at all stages of maturity have medicinal virtues and have been used as traditional medicine for a long time [1–3]. The plant is proven to have a number of pharmacological activities such as antifungal, antibacterial, antiprotozoal, antispermatogenic, anti-inflammatory, anthelmintic, antidiabetic, laxative, febrifuge and expectorant [4]. The roots of the plant are widely used in many ayurvedic formulations and hence trade data collected over years has indicated that the demand has exceeded the supply [5]. Also the plant has been stated to be in the red list in vulnerable status [6]. Therefore it is necessary to screen the phytochemicals

in all parts of the plant so as to find out if there are any similar compounds in one or the other parts to be used instead or along with the root. High Performance Thin Layer Chromatography is the best suited conventional method for phytochemical analysis with or without marker compound [7–11]. The marker used is marmelosin a furano coumarin, one of the active ingredients of the plant. This bioactive compound is responsible for the pharmacological activities such as hypoglycemic, anti-inflammatory, antioxidant, antidiabetic, anthelmintic and antibacterial [12]. With the help of marker compound the screening is made more efficient by determining the identical phytochemicals in all the parts of the plant. Therefore the present study is to develop a fingerprint for various parts of *Aegle marmelos* by HPTLC.

## 2. Materials and Method

**Collection and Authentication of plant samples:** The various parts (Root, Stem bark, Stem, Leaf and Fruit) of *A. marmelos* were purchased from AVN Ayurveda Formulations Pvt Ltd., Madurai. They were identified and authenticated by Dr.K.M.Rajasekaran, Head & Professor, Department of Botany, Madura College, Madurai.

**Pretreatment of samples:** The various parts were

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individually washed with de-ionized water and shade dried for 3 days. Some amount of samples were simply cut, crushed and used as such. Others were cut into small pieces, crushed, grinded and sieved into fine powders.

### 2.1. Aqueous and Methanol extract

**Powder extract:** 5g of powder sample was taken in two RB flasks each containing 100 ml of de-ionized water and 100 ml of methanol. They were then boiled at 100°C for 1 hour. The solution was then filtered using Whatman No.1 filter paper and both extracts were stored separately at 4°C.

**Material extract:** 5g of material sample was taken in two RB flasks each containing 100 ml of de-ionized water and 100 ml of methanol. They were then boiled at 100°C for 1 hour. The solution was then filtered using Whatman No.1 filter paper and both extracts were stored separately at 4°C.

**Preparation of marmelosin standard:** Stock solution was prepared by dissolving 1mg of marmelosin in 1ml of methanol. From this working standard has been prepared with the concentration of 5 µg/ml.

**HPTLC analysis:** 2 µl of aqueous and methanol extracts (Powder extract and Material extract) of various parts of *Aegle marmelos* and 2 µl of marmelosin (marker compound) were applied to plates as 8.0mm bands on 20 cm x 10cm precoated silica gel 60 F254 TLC plates with layer thickness 0.2 mm using micro syringe by means of Linomat 5 applicator. Plates were developed in twin trough chamber using the mobile

phase (Toluene: Ethyl acetate (7.5: 2.5)) for different extracts up to 80 mm distance. The plates were dried and scanned using Camag Reprostar 3 TLC Scanner at 254 and 366 nm.

### 3. Results

The results of the preliminary phytochemical studies confirmed the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannins, flavanoids and steroids in the aqueous and methanolic extracts of *A. marmelos* root, stem bark, stem, fruit and leaves. The aqueous powder extract of root, stem bark, stem, fruit and leaf showed 11 different phytochemicals with 11 different Rf values from 0.04 to 0.47 (Table 1). Among these, three compounds with Rf value 0.18, 0.34, 0.38 were similar to root, stem bark, stem and leaf. The aqueous material extract of root, stem bark, stem, fruit and leaf showed 16 different compounds with 16 different Rf values from 0.06 to 0.51 (Table 2). Among these, seven compounds with Rf 0.08, 0.19, 0.22, 0.29, 0.34, 0.39, 0.48 were similar to root, stem bark, stem and leaf. The methanol powder extract of root, stem bark, stem, fruit and leaf showed one Rf value more or less equivalent to Rf value 0.70 of marmelosin. The methanol material extract of root, stem bark, stem, fruit and leaf showed one Rf value more or less equivalent to Rf value 0.69 and 0.75 of marmelosin.

**Table 1.**

Rf value and area under the curve for Aqueous powder extract samples at 366nm

FPE		LPE		SPE		SBPE		RPE	
Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area
0.07	222.0	0.04	248.5	0.07	422.3	0.18	13265.2	0.18	2375.5
0.18	590.0	0.09	125.3	0.21	900.1	0.34	5089.4	0.34	938.8
0.33	293.8	0.21	493.4	0.34	259.9	0.38	1872.5	0.38	383.2
0.39	1850.8	0.32	1223.9	0.38	306.0				
		0.39	1169.1						
		0.47	712.2						

FPE– Fruit Powder Extract, LPE– Leaf Powder Extract, SPE– Stem Powder Extract, SBPE– Stem Bark Powder Extract & RPE– Root Powder Extract

**Table 2.**

Rf value and area under the curve for Aqueous material extract samples at 366nm

FME		LME		SME		SBME		RME	
Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area
0.07	82.8	0.06	285.5	0.08	355.8	0.08	50.7	0.20	120.2
0.18	1512.0	0.08	392.7	0.19	799.7	0.19	262.5	0.23	77.9
0.34	429.7	0.12	273.0	0.22	1250.8	0.22	146.7	0.41	264.5
0.39	320.3	0.19	669.1	0.29	379.2	0.29	66.8	0.51	117.1
		0.22	526.0	0.34	359.2	0.34	73.1		
		0.26	453.0	0.39	2134.9	0.39	518.5		
		0.34	586.5	0.49	99.8	0.48	146.0		
		0.39	454.2						
		0.49	336.9						

FME– Fruit Material Extract, LME– Leaf Material Extract, SME– Stem Material Extract, SBME– Stem Bark Material Extract & RME– Root Material Extract

**Table 3.**

Rf value and area under the curve for Marker and Methanol powder samples at 366 nm.

FPE		LPE		SPE		SBPE		RPE		STD	
Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area
0.06	122.0	0.09	77.6	0.07	448.3	0.18	77.0	0.09	79.4	0.70	621.0
0.10	148.6	0.18	127.9	0.18	4455.2	0.39	407.1	0.72	1108.5	0.76	740.0
0.19	1094.8	0.28	218.3	0.29	377.5	0.72	333.9				
0.28	1415.1	0.38	175.9	0.39	15206.1						
0.39	4778.0	0.44	149.1	0.50	229.0						
0.62	512.9	0.57	209.6	0.54	1463.6						
0.73	4388.0	0.72	125.8	0.62	1168.3						
0.82	684.4	0.82	115.2	0.67	339.0						
				0.82	324.2						

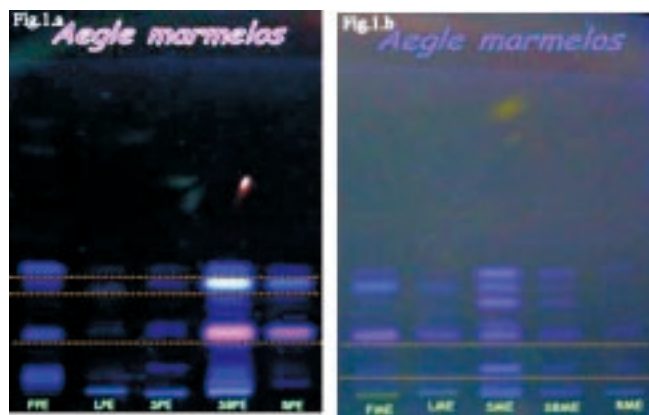
FPE– Fruit Powder Extract, LPE– Leaf Powder Extract, SPE– Stem Powder Extract, SBPE– Stem Bark Powder Extract, RPE– Root Powder Extract & STD– Marmelosin.

**Table 4.**

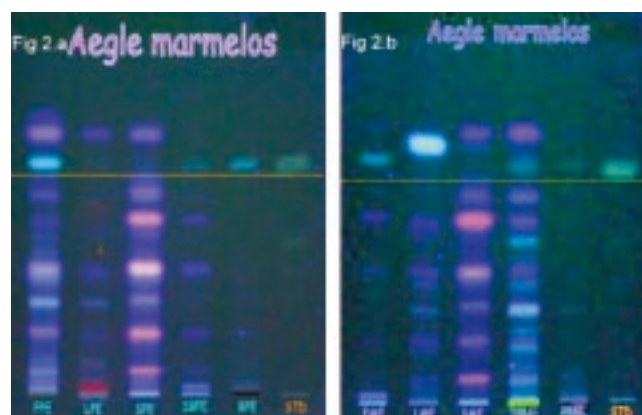
Rf value and area under the curve for Marker and Methanol material extract samples at 366 nm.

FME		LME		SME		SBME		RME		STD	
Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area	Rf	Area
0.41	368.0	0.18	317.0	0.18	1200.8	0.11	459.4	0.28	117.0	0.69	713.0
0.58	280.0	0.28	349.6	0.26	50.2	0.16	1121.1	0.39	198.5	0.75	687.3
0.76	1792.0	0.36	325.6	0.29	290.3	0.21	222.4	0.73	599.4		
		0.40	709.4	0.40	10120.6	0.28	3212.2				
		0.55	510.8	0.56	2981.8	0.35	239.9				
		0.80	7976.4	0.63	1448.8	0.43	345.9				
				0.69	443.8	0.49	719.9				
				0.85	557.8	0.56	504.3				
						0.63	1997.7				
						0.73	1418.1				
						0.83	841.4				

FME– Fruit Material Extract, LME– Leaf Material Extract, SME– Stem Material Extract, SBME– Stem Bark Material Extract, RME– Root Material Extract & STD– Marmelosin.



**Figure 1.** HPTLC fingerprint profile of aqueous extract of *Aegle marmelos* at 366nm. a. Powder extract; b. Material extract; Horizontal lines indicate the identical compounds with same Rf value. FPE– Fruit Powder Extract, LPE– Leaf Powder Extract, SPE– Stem Powder Extract, SBPE– Stem Bark Powder Extract, RPE– Root Powder Extract; FME– Fruit Material Extract, LME– Leaf Material Extract, SME– Stem Material Extract, SBME– Stem Bark Material Extract, RME– Root Material Extract.



**Figure 2.** HPTLC fingerprint profile of methanol extract of *Aegle marmelos* at 366nm. a. Powder extract; b. Material extract; Horizontal lines indicate the identical compounds with same Rf value. FPE– Fruit Powder Extract, LPE– Leaf Powder Extract, SPE– Stem Powder Extract, SBPE– Stem Bark Powder Extract, RPE– Root Powder Extract; FME– Fruit Material Extract, LME– Leaf Material Extract, SME– Stem Material Extract, SBME– Stem Bark Material Extract, RME– Root Material Extract, STD– Marmelosin.

#### 4. Discussion

The phytochemicals with same Rf value found in different plant parts of both powder and material aqueous extract indicates that they are same compounds. Since root is of main importance due to its demand, HPTLC profile of root

is compared with other parts of the plant. The root and stem bark have more identical compounds and so they have same pharmacological activities and therefore stem bark could be substituted or used along with root in any of drug preparation where root is of important. The marker compound marmelosin is present in all parts of the plant

and its concentration is high in fruit. Also, the Rf of other phytochemicals of methanol extracts which are near to marmelosin range may be other compounds under the class coumarins. Therefore, we can infer that coumarins are largely present in all parts of the plant and there is more number of other coumarins.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgement

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