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Phytochemical Screening and Estimation of Total Phenolic Content in *Aegle marmelos* Seeds

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ABSTRACT
The aim of present study was to investigate the phyto-constituents present within the aqueous and methanol extract of *Aegle marmelos* seeds and to estimate the total phenolic contents. The amount of total phenols, were analyzed using a spectrophotometric technique, based on Folin-ciocalteau reagent. Gallic acid was used as standard compound and the total phenols were expressed as mg / g gallic acid equivalents (Standard curve equation: $y= 0.0106x + 0.041$, $R^2 = 0.996$). The total phenols varied from 27.12 ± 0.6 to 65.20 ± 0.2 in extracts. The maximum phenolic content was found in the methanolic extract (65.20 ± 0.2 mg/g).

Keywords: *Aegle marmelos* seeds, Total phenolic contents, Folin-Ciocaiteau reagent.

INTRODUCTION

Bael (*Aegle marmelos* (Linn.), family Rutacae, is also known as Bale fruit tree, is a moderate sized, slender, aromatic tree, 6.0 -7.5 m in height, and 90 to 120 cm in girth, with a somewhat fluted bole of 3.0-4.5 meter growing wild throughout the deciduous forests of India. [1] It is indigenous to Indian sub continents and mainly found in tropical and subtropical regions. [2] The various phytoconstituents from various parts of *Aegle marmelos* tree have already been investigated and isolated. The marmesin–1"- α -L - rhamnopyranoside and 1, 5 -dihydroxy - 6 - methoxy -2 - methyl anthraquinone have been isolated from the stem bark of *Aegle marmelos* , together with lupeol and β-sitosterol. [3] The Aegeline [4], Lupeol [5], Cineol [6], Citral [7] and Eugenol [8] have also been isolated from the leaves of *Aegle marmelos*. Moreover, Marmelosin [9], Luvangetin [10] and Marmelide [11] have been reported and isolated from fruits of *Aegle marmelos*. The total phenol contents in aqueous, acetone and methanol extract of *Aegle marmelos* fruit have been estimated. [12]

MATERIAL AND METHODS

Chemical Used: The chemicals and reagents used for the purpose of preliminary phytochemical test were freshly prepared. Methanol, Gallic acid and Folin-ciocaltue reagent were of Merck Co. (Germany). All the chemicals and reagents used were of analytical grade.

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Collection and authentication of plant material

The fresh, unriped fruits of *Aegle marmelos* were collected from healthy trees were growing at very hygiene and polluted free area in the month of May-June, located at various regions of Jaipur, Rajasthan. The seeds and plants were identified and authenticated from the department of Botany, University of Rajasthan, Jaipur, Rajasthan, and voucher specimen was deposited, viz no. RUBL: 20866.

Preparation of extract

Freshly collected seeds of *Aegle marmelos* were dried at 30 °C and at 18.9 % relative humidity condition and milled with sieve to remove excess of mucilaginous hair. The plant extract was prepared using two different laboratory grade solvents (double distilled water & methanol).

Preparation of Aqueous Extract

The dried powdered plant part (1.0 kg of *Aegle marmelos* seeds) was extracted with 4.0 liters of double distilled water for 72 hours in a round bottom flask, by placing on water bath, attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (reddish brown) was obtained.

Preparation of Methanolic Extract

The powdered plant material (1.0 kg of *Aegle marmelos* seeds) was extracted with 4.0 liters of analytical grade methanol for 72 hours in a round bottom flask, on water bath attaching reflux water condenser. After filtering and concentrating under vacuum the crude extract (yellow reddish) was obtained.

The % yields of both the extracts (i.e. aqueous and methanol) were 19.71% and 10.84 %, respectively.

Phytochemical Screening

Qualitative phytochemical screening was done for preliminary chemical identification with freshly prepared reagents. The preliminary tests, for both the extracts (i.e. aqueous and methanol) were performed to confirm the presence of Alkaloids, Carbohydrates, Proteins and amino acids, Glycosides, Flavonoids, Tannins and Phenolics, Steroids, Volatile oils and fats.

Estimation of total phenolic contents

The total phenolic content in aqueous and methanolic extract of *Aegle marmelos* seeds were estimated by Folin-Ciocalteau reagent as method described by Singleton & Rossi (1965). [13] Gallic acid stock solution (1000µg/ml) was prepared by dissolving 100mg of gallic acid in 100ml of ethanol. Various dilutions of standard gallic acid were prepared from this stock solution. Folin-Ciocalteau reagent was prepared by mixing Folin's reagent with Phenol reagent (1:1), and diluted 1:1 in distilled water, before use.

Calibration curve (Fig. 1) was plotted by mixing 1ml aliquots of 1.0, 2.5, 5.0, 10, 25, 50 & 100µg/ml of gallic acid solutions with 5.0 ml of Folin-Ciocalteu reagent (diluted tenfold) and 4.0 ml of sodium carbonate solution (75g/l). The absorbance was measured after 30 min at 20°C at 765 nm. One ml of aqueous and methanol extract (1.0 g/100 ml) was mixed separately, with the same reagents as did in construction of calibration curve, and after 1 hour, the absorbance was measured for the determination of total phenolic compound in both the extract separately by using formula;

$$C=C1 \times V/m.$$

Where; C=Total content of phenolic compounds in mg/g, in GAE (gallic acid equivalent); C_1=The concentration of Gallic acid established from the calibration curve in mg/ml; V=The volume of extract in ml; M=The weight of plant extract in g,

RESULT AND DISCUSSION

Phytochemical Screening

Our observations revealed that both the aqueous and methanolic extracts of seeds of *Aegle marmelos* contain alkaloids, carbohydrates, proteins, glycosides and phenolics qualitatively, whereas amino acids are not found present in both the extracts after several trials.

Moreover; both the extracts were rich in carbohydrates, but were devoid of reducing sugar, monosaccharide, pentose sugar, hexose sugar. Since, the mucilaginous hairs were removed even though extracts (both, Aqueous and Methanol) were rich in mucilage. In addition to above, several tests for glycosides were found positive but both the extracts were failed to show presence of Cyanogenetic glycosides.

Nevertheless, aqueous extract of *Aegle marmelos* seeds was rich in Cardenoloids, but in methanolic extract Cardenoloids was not found present.

Also in addition to above, both the extracts were qualitatively studied for the presence of steroids by Liebermann's reaction and found positive in only aqueous extract.

Further Flavonoids, oils of volatile constituents and oils with long chain fatty acids were also examined qualitatively; except flavonoids in both the extracts other two were failed to show positive results. The preliminary phytochemical test results are shown in Table 1.

Estimation of Total Phenolic Content

The amount of total phenols was determined with Folin-Ciocalteu reagent. Gallic acid was used as standard compound. The absorbance for various dilutions of gallic acid with Folin-Ciocalteu reagent and sodium carbonate were found as described in Table 2. Found standard curve equation was; $y= 0.0106x + 0.041$, $R^2 =0.996$. The absorbance for aqueous and methanol extract of *Aegle marmelos* seeds were found 0.328 and 0.732. The total phenol contents (Gallic acid equivalents, mg/g) in methanol extract and aqueous extract were calculated as 65.20±0.2 and 27.12±0.6 mg/g, respectively.

Table 1: Results of Phytochemical Screening of *Aegle marmelos* Seeds

Phytochemicals		Aqueous extract	Methanolic extract
Alkaloids	General Test	+	+
Carbohydrates (Monosaccharides, Oligosaccharides & Polysaccharides)	General Test	+	+
	Reducing Sugars	-	-
	Monosaccharides	-	-
	Pentose Sugars	-	-
	Hexose Sugars	-	-
	Non Reducing Sugars	+	+
	Non Reducing Polysaccharides	+	+
	Gums	+	+
	Mucilage	+	+
Proteins & Amino acids	Proteins	+	+
	Amino Acids	-	-
Glycosides	General Test	+	+
	Cardiac Glycosides	+	+
	❖ Cardenoloids	+	-
	❖ Deoxysugars	+	+
	❖ Bufadenoloids	+	+
	Anthraquinone Glycosides	+	+
	Saponin Glycosides	+	+
	Cyanogenetic Glycosides	-	-
	Coumarin Glycosides	+	+
Flavonoids		+	+
Tannis & Phenolic Compounds	General Test	+	+
Steroids		+	-
Volatile Oils		-	-
Fats & Oils		-	-

(+) Confirms Presence and (-) confirms absence of above shown category of Phytoconstituents

Table 2: Absorbance recorded for Standard Gallic acid Curve at 765 nm

S. No.	Concentration of Standard Gallic Acid (µg/ml)	Absorbance (O.D)
1.	100	1.124±0.023
2.	50	0.542±0.112
3.	25	0.289±0.013
4.	10	0.149±0.030
5.	5	0.113±0.021
6.	2.5	0.101±0.011
7.	1	0.029±0.002

CONCLUSION

The qualitative phytochemical screening for aqueous and methanolic extract of *Aegle marmelos* seeds was performed then inferred to calculate total phenols which were estimated by Folin-Ciocalteu reagent. The results of phytochemical screening reveals that the seeds used for the extraction procedure were vital and enough matured to propagate and contain metabolites needed to show various protective mechanisms *in-vivo.* [14] The maximum phenolic content was found in methanolic extract between two (aqueous and methanol), Further, some pharmacological activities has to be performed to establish the importance of certain phyto constituents having protective action.

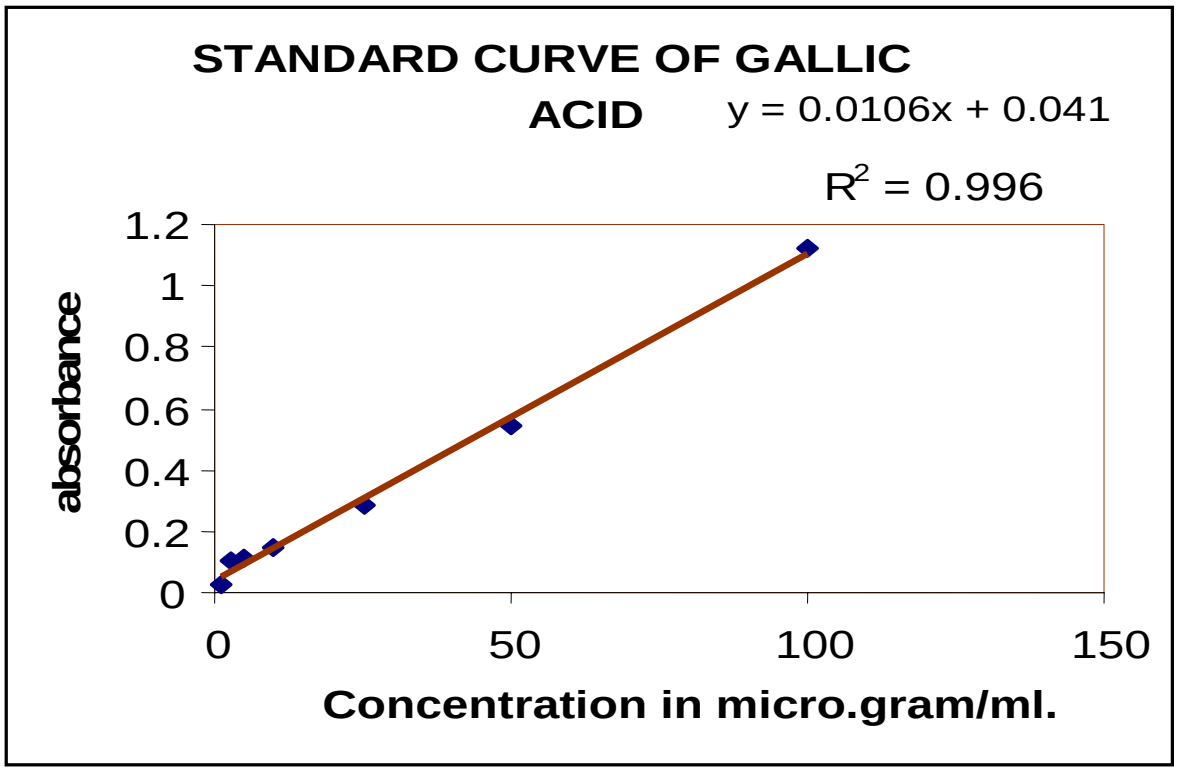

Fig. 1: Calibration curve of gallic acid

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