JOURNAL OF NATURAL REMEDIES
DOI: 10.18311/jnr/2015/498

Effect of Extraction Solvent on the Phytoconstituents of *Aegle marmelos* (L.) Correa

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Abstract

The objective of the present study was to evaluate the effect of extraction solvents such as absolute ethanol, absolute methanol, aqueous ethanol (ethanol: water, 80:20 v/v), aqueous methanol (methanol: water, 80:20 v/v), hydro alcohol (ethanol: water 50:50 v/v) and water on phytochemicals of different parts of *Aegle marmelos*. The chemical comparison was done using thin layer chromatography. Major class of compounds such as phenolics and flavonoids were quantitatively estimated spectrophotometrically. Among the six solvents used for the extraction, hydro alcohol showed the maximum yield of extract. In the case of root, the yield of hydro alcoholic extract was 42.05 % higher than that of aqueous extract. The increase of yield for stem and fruit were found as 27.5 % and 46.35 % respectively. Due to the higher yield of extract, use of hydro alcohol instead of water for the preparation of herbal formulations can be considered and it may lead to the judicious use of raw materials.

Keywords: *Aegle marmelos*, extraction solvent, phenolics, TLC

1. Introduction

The demand for ayurvedic formulations is increasing both in the domestic market as well as internationally. The medicinal plant industry is posing great threat due to the unavailability of genuine raw drugs thereby resulting in the use of several substitutes / adulterants as the source plant. In the near future, many species may be totally unavailable for the use of industry due to over exploitation [1].

The tree *Aegle marmelos* (Rutaceae) commonly known as *Bael* is indigenous to India and found wild all over the Sub-Himalayan forests, in Central, and South India [2]. It is a rich source of coumarins, vitamin C, and riboflavin. The bark as well as fruit is reputed to be a valuable Ayurvedic medicine for dysentery and various intestinal complaints [3, 4]. It possesses potentmicrofilarial [5], radio protective [6], analgesic [7], antihyperglycemic, antidyslipidemic [8], anticancer [9, 10] and antidiabetic activity [11, 12].

Solvent extraction is most frequently used technique for isolation of plant metabolites. However, the extract yields of the plant materials are strongly depend on the nature of extracting solvent, due to the different solubility of the chemical compounds present in it. Polar solvents are frequently employed for the recovery of polyphenols from a plant matrix.

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Identification of most effective extraction solvents to increase the yield of raw materials is of great importance as it helps reducing the quantity required for medicine manufacture. The possibilities of using organic solvents such as hydro alcohol instead of water for the preparation of Herbal drugs have been evaluated in the present investigation. For the present study, *A. marmelos* an important medicinal plant belonging to *Dasamoola* group, has been selected.

2. Materials and Methods

2.1 Collection of Plant Materials

The different parts such as root stem and fruit of *A. marmelos* were collected from Herb garden of Arya Vaidya Sala, Kottakkal, Kerala, India during April, 2013, and authenticated by Plant Systematics and genetic resources division of Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal, Kerala. The voucher specimens were deposited in CMPR Herbarium.

2.2 Chemicals

Folin-Ciocalteu reagent was procured from Sisco Research Laboratory (SRL), Mumbai, India, Gallic acid and quercetin were procured from Sigma Chemicals Co. (Bangalore, India). All other chemicals employed were of standard analytical grade from Merck India.

2.3 Extraction

The shade dried ground plant materials such as root, stem and fruit (5 g for each sample) were extracted with 250 ml each of the solvents – absolute ethanol, absolute methanol, aqueous ethanol (ethanol: water, 80:20 v/v), aqueous methanol (methanol: water, 80:20 v/v) hydro alcohol (ethanol: water 50:50) and water for 6 hours by reflux method. The extracts were concentrated into dryness in a rotary evaporator at 40^0C under reduced pressure. The yields of extracts were calculated. 10 mg of each extract was dissolved in 10 ml of respective solvents and these extracts were used for total phenolic and flavonoid estimations.

2.4 Estimation of Polyphenolic Compounds

The total phenolic content (TPC) was determined spectrophotometrically using Folin-Ciocalteu reagent [13]. Different Gallic acid standards were used for obtaining a standard curve. TPC was expressed as gallic acid equivalents (GAE) in mg / g of sample. Total flavonoid content (TFC) was measured by aluminium chloride colorimetric assay [14]. The flavonoid content was calculated from the calibration curve of standard quercetin. TFC was expressed as mg quercetin equivalents (mg EQ). The absorbance against the reagent blank was determined at 550 nm with UV-Visible spectrophotometer (Pharmaspec-1700, Shimadzu, Japan).

2.5 Thin Layer Chromatographic Analysis

Thin Layer Chromatography (TLC) Profiling of different extracts of *Aegle marmelos* was carried out on a precoated silica plate (F_{254} Merck) using toluene, ethyl acetate and methanol as mobile phase in the ratio of 8:2:0.5. Two marker compounds, umbelliferone and scopoletin (Sigma Aldrich, Bangalore, India) were also spotted for their identification in the extracts. The developed plate was visualized and documented at UV 254 nm and 366 nm.

3. Results and Discussion

3.1 Percentage of Yield of Extracts

The efficiency of extraction is influenced by several parameters such as nature of phytochemicals, the method used for extraction, particle size, the solvent used, as well as the effect of nosy substances [15]. The percentage yield of extracts is given in Table 1. The yield of extract varied with extraction solvents. The variation was also observed with respect to parts used. Hydro alcohol showed maximum yield for all the extracts. The use of aqueous alcoholic solvent may facilitate the extraction of chemicals that are soluble in water and/or organic solvent. This may be the reason why yields of hydro alcohol extracts are higher [16]. Hydro alcoholic extract of fruit showed highest extractive value (32.06%) whereas absolute methanolic stem extract showed minimum yield (2.82%). The variation of yield of extracts compared with water extract is given in Fig. 1. When compared to aqueous extract, root showed an increase of 42.05% in hydro alcohol. This supports the possibilities of using hydro alcohol as extraction solvents in Ayurvedic medicine manufacturing industry and which may lead

Table 1: Percentage of yield of Extracts

Sl. No	Extraction Solvents	Root (% W/V)	Stem (% W/V)	Fruit (% W/V)
1	Aqueous ethanol (80:20)	16.4	4.034	19.52
2	Aqueous methanol (80:20)	17	2.95	26.44
3	Absolute ethanol	18.624	3.96	9.04
4	Absolute methanol	17.4	2.82	5.54
5	Hydro alcohol (50:50)	18.76	4.97	32.06
6	Water	10.87	3.6	17.2

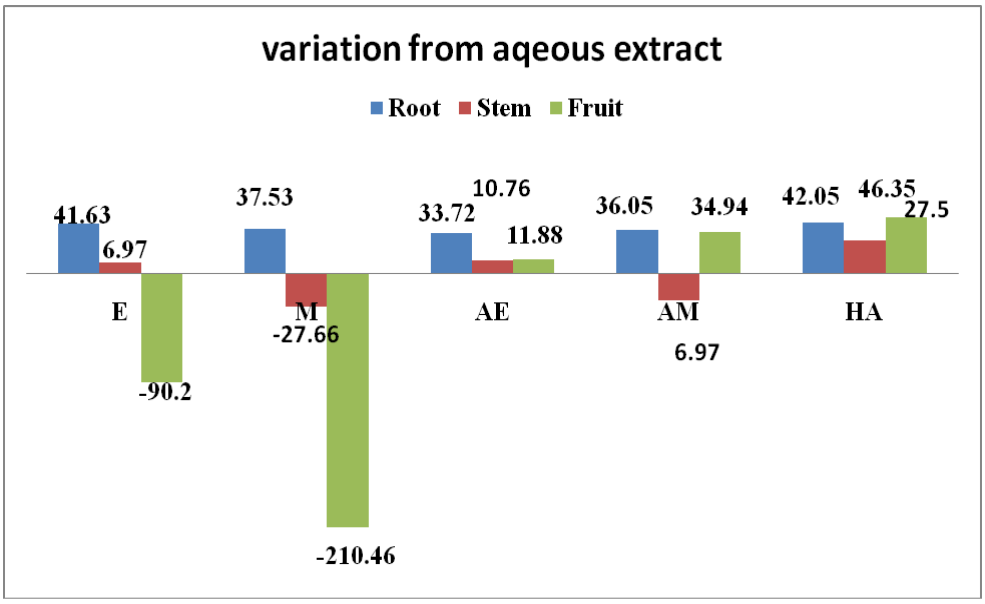

Fig. 1. Difference in yield of extract from aqueous extract.

to the judicious use of raw materials especially where the roots are useful parts.

3.2 Total Phenolics (TPC) and Total Flavonoids (TFC)

Total polyphenolic content was estimated spectrophotometrically using gallic acid and Quercetin as standards for plotting calibration curve (Fig. 2 & 3). Efficiency of solvents and methods is strongly dependent on plant matrix used for extraction. Solvents, such as methanol, ethanol, acetone, propanol and ethyl acetate have been commonly used for the extraction of phenolics [17]. The properties of extracting solvents significantly affected the measured total phenolics content. The highest extract yields (up to 22.8%) were obtained with polar alcohol based solvents [18]. The present reports also showed that alcohol based extracts contain more phenolics.

The total phenolic and total flavonoid contents of various extracts of root of *A. marmelos* is given in Table 2. The highest phenolic content was observed in absolute ethanolic extract (34.9 mg EGa). Aqueous extract showed maximum flavonoid content (19.88 mg EQ). Aqueous extract contains 30.37 mg EGa of TPC and 19.88 mg EQ of TFC. Hydro alcoholic extract contains 21.35 mg EGa of TPC and 15.78 mg EQ of TFC.

The TPC & TFC of stem is presented in Table 3. Aqueous extract showed highest phenolic contents (58.34 mg EGa) followed by aqueous ethanol (54.54), aqueous methanol (50.81), hydro alcohol (46.28), absolute methanol (46.1) and absolute ethanol (42.25). The TFC follows the order water (44.4) > absolute ethanol (34.34) > aqueous ethanol (33.71) >hydro alcohol (31.34) aqueous methanol (31.17) >absolute methanol (24.1).

The phenolics and flavonoids of various fruit extracts are presented in Table 4. The highest TPC was observed

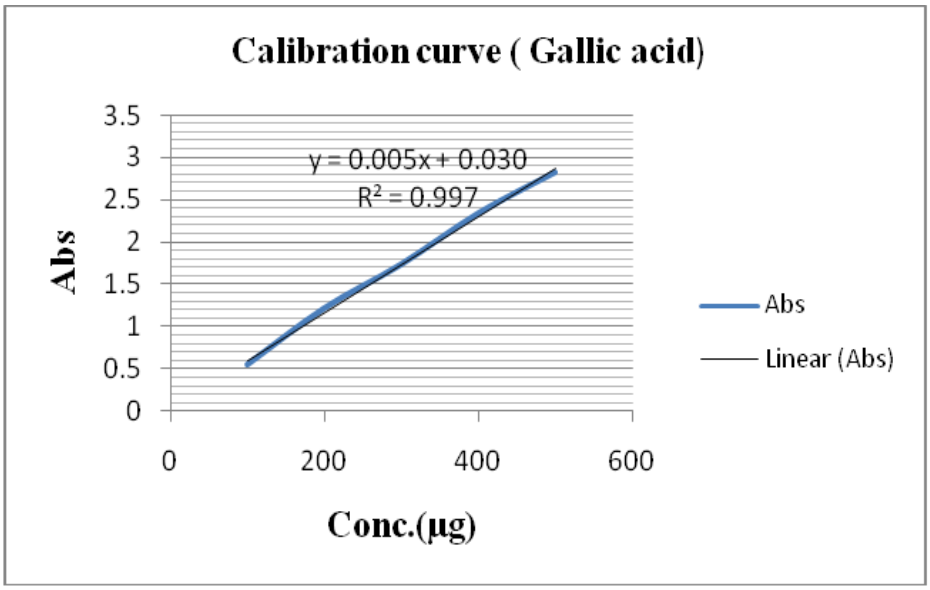

Fig. 2. Calibration (TPC).

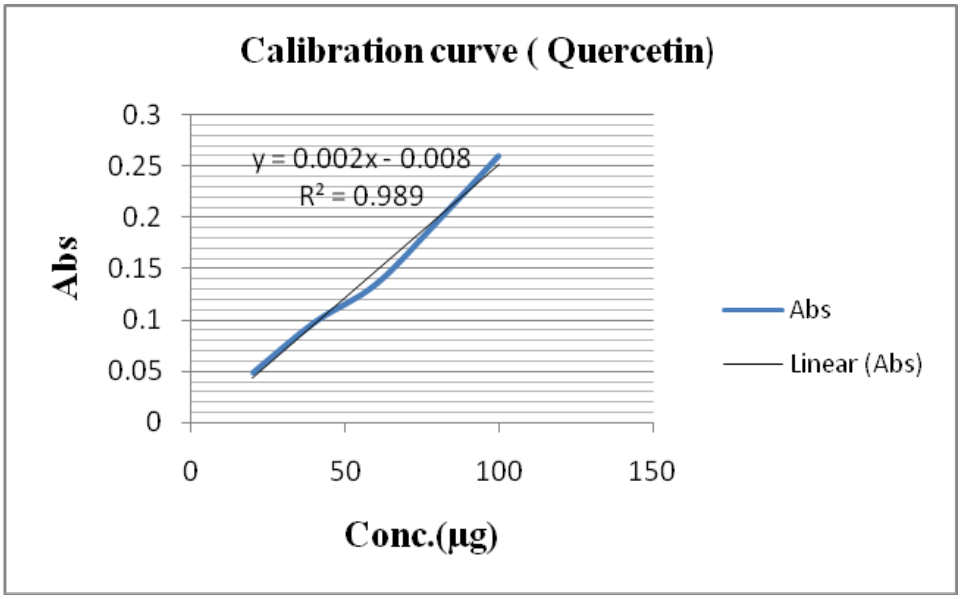

Fig. 3. Calibration (TFC).

Table 2: Total phenolics and total flavonoids in root extracts of *A. marmelos*

Solvent	TPC (mg E GA)	TFC (mg EQ)
Absolute ethanol	26.22 ±1.26	13.66 ±0.18
Absolute methanol	22.94 ±1.06	11.76±1.04
Aqueous ethanol	34.9±1.16	18.26±1.16
Aqueous methanol	29.88±1.06	10.11±0.86
Hydro alcohol	21.35±1.14	15.78±0.16
Water	30.37±1.18	19.88±1.13

Table 3: Total phenolics and total flavonoids in stem extracts of *A. marmelos*

Solvent	TPC (mg E GA)	TFC (mg EQ)
Absolute ethanol	54.54 ±1.08	33.71 ±1.16
Absolute methanol	50.81 ±1.15	31.17±1.26
Aqueous ethanol	42.25±1.16	34.34±1.10
Aqueous methanol	46.10±1.28	24.10±1.14
Hydro alcohol	46.28±1.16	31.34±1.12
Water	58.34±1.24	44.40±1.26

Table 4: Total phenolics and total flavonoids in fruit extracts of A. marmelos

Solvent	TPC (mg E GA)	TFC (mg EQ)
Absolute ethanol	29.20 ±1.25	20.70 ±1.10
Absolute methanol	20.42 ±1.14	15.89±1.20
Aqueous ethanol	66.37±1.20	41.60±1.14
Aqueous methanol	77.62±1.24	50.54±1.18
Hydro alcohol	51.13±1.18	33.06±1.24
Water	50.00±1.16	22.79±1.18

in absolute methanolic extract (77.62) followed by absolute ethanol (66.37), hydro alcohol (51.13), water (50.0), aqueous ethanol (29.2) and aqueous methanol (20.42). TFC follows the order absolute methanol (50.54) > absolute ethanol (41.6) > hydro alcohol (33.06) > water (22.79) > aqueous ethanol (20.7) > aqueous methanol (15.89).

Solvents, such as methanol, ethanol and their combinations with different proportions of water have been used for the extraction of phenolics from plant materials [19]. The present results were also showed that the aqueous based solvents are the most effective extraction solvent for polyphenols.

3.3 Comparative TLC Profiling

The chemical pattern of different extracts was compared using TLC profiling. Variations were observed in terms of number of bands and band intensity which indicate the qualitative and quantitative divergence in chemical constituents.

On visualizing under UV-254 (Fig. 4), a compound with R_f 0.14 was observed in all the extracts of fruit and it was absent in stem and root. The band at 0.17 is specific for root extracts and the intensity is more for absolute ethanol and absolute methanol extracts. The compound with R_f 0.27 is present only in stem extracts. A compound at R_f 0.33 was observed in aqueous ethanolic and aqueous methanolic extracts of stem. A band with R_f 0.38 was common for all the extracts of root and the same was also seen in absolute ethanol and absolute methanol extracts of stem. A compound at 0.61 was observed only in aqueous methanol and methanol extract of fruit.

Under UV 366 (Fig. 5) major compounds were observed at R_f 0.17, 0.26, 0.33, 0.44, 0.48, 0.52, 0.70, 0.78 and 0.81. The differences in the intensity of bands indicate the quantitative variations of phytoconstituents in different extracts. Umbelliferone , the so far reported marker compound, is present in almost all the extracts with varying band intensities, but the same was found to be absent in aqueous extract of fruit. Scopoletin, a known coumarin was found to be present in all the extracts of root and stem.

1: aqueous ethanol extract of root, 2: aqueous methanol extract of root, 3: absolute ethanol extract of root, 4: absolute methanol extract of root, 5: hydro alcohol extract of root, 6: aqueous extract of root.

7: aqueous ethanol extract of stem, 8: aqueous methanol extract of stem, 9: absolute ethanol extract of stem, 10: absolute methanol extract of stem, 11: hydro alcohol extract of stem, 12: aqueous extract of stem.

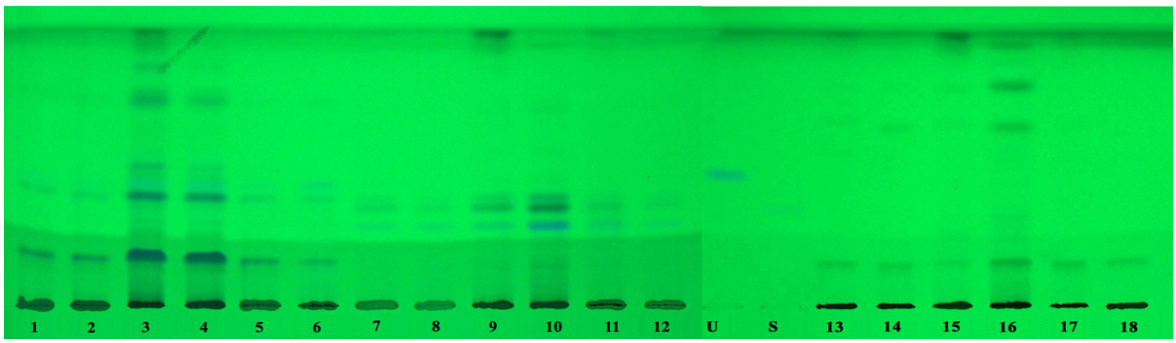

Fig. 4. TLC profiling of different extracts of *A. marmelos*. (254 nm).

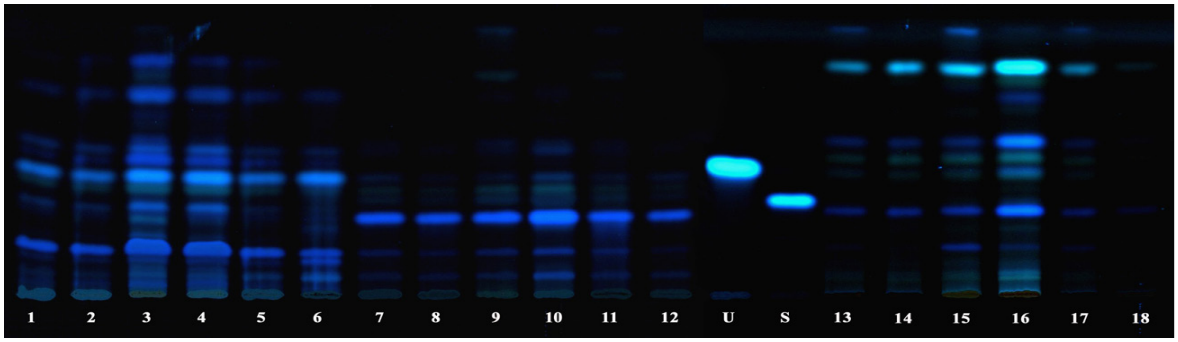

Fig. 5. TLC profile of different extracts of *A. marmelos* (366 nm).

13: aqueous ethanol extract of fruit, 14: aqueous methanol extract of fruit, 15: absolute ethanol extract of fruit, 16: absolute methanol extract of fruit, 17: hydro alcohol extract of fruit, 18: aqueous extract of fruit.

U: Umbelliferone, S: Scopoletin

4. Conclusion

The results of the present studies revealed that hydro alcoholic extract exhibited better yield with comparable chemical constituents with that of aqueous extract. Possibility of reducing the quantity of raw drugs by 40 % can be explored if raw drugs are being extracted with hydro alcohol (ethanol: water 50:50) instead of water. In the case of root, the higher quantities of individual components are found in absolute alcoholic (both ethanol and methanol) extracts. But the banding pattern of aqueous extract is matching with that of hydro alcoholic extract. Two coumarins umbelliferone and scopoletin, so far reported markers in *Aegle marmelos*, were detected in all the extract except aqueous extract of fruit. Among the six solvents used for the extraction, hydro alcohol showed the maximum yield of extract. Contrary to extraction yield, the quantity of individual components was more in aqueous and absolute alcohols, both ethanol and methanol.

5. Acknowledgement

The authors are thankful to the authorities of Arya Vaidya Sala Kottakkal for extending the facilities and TATA Trust, Mumbai for financial assistance.

6. References

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