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(RESEARCH ARTICLE)

Development of RP-HPLC method for standardization of *Aegle marmelos* (L.)

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Abstract

In recent times, focus on plant research has increased all over the world and several evidences have been collected to show immense potential of medicinal plants used in various traditional systems. Over the last few years, researches have aimed at identifying and validating plant derived substances for the treatment of various diseases. The bael (*Aegle marmelos*) (L.) is an Indian plant, which has enormous traditional uses against various diseases [1]. The present work aims to compile marmelosin based standardization of *Aegle marmelos,* generated through the research activity using RP-HPLC as a tool. The method developed was found to be accurate, precise and simple for the stated purpose and can be used routinely for standardization of crude fruit extract and herbal formulations containing it.

Keywords: *Aegle marmelos*; Marmelosin; Standardization; RP-HPLC

1. Introduction

Aegle marmelos, also known as bale tree, is a moderate sized, slender, aromatic tree, growing wild throughout the deciduous forests of India. This is generally considered assacred tree by the Hindus, as its leaves are offered to Lord Shiva during worship. Bale fruits are yellowish green, with small dots on the outer surface, oblong to globs, 5.3 cm to 7.2 cm in diameter; weight, 77.2 g; pulp, yellow and mucilaginous, the pulp of dried fruits retains its yellow colour, and also remains intact; rind woody, 4 to 5 mm thick[1].

Various chemical constituents were found in bael such as alkaloids, coumarins, steroids, polysaccharides, tannins, carotenoids etc. Alkaloids: Agelin, aegelenine, marmeline, dictamine, fragrine, O-methylhalfordinine, O-isopentanylhalford iniol, N-4-methoxy styryl cinnamide. Coumarins: Marmelosin, marmesin, imperatorin, marmin, alloimperatorin, methylether, xanthotoxol, scoparone, scopoletin, umbelliferone, psoralen and marmelide. Polysaccharides: Galactose, arabinose, uronic acid and L-rhamnose was obtained on hydrolysis. Tannin: Tannin was also present in leaves and fruit as skimmianine. Carotenoids were also reported, which impart pale color to fruit[2,3].

Aegle marmelos proved various activities such as anti-diabetic activity, hepatoprotective activity, antimicrobial activity, analgesic, anti-inflammatory, ntipyretic activity, antifungal activity and anticancer activity [3].

The present work is focused to develop method for standardization of *Aegle marmelos* (bael) fruit pulp used in many herbal preparations, on the basis of marmelosin - a major chemical constituent present [4,5].

2. Material and methods

Plant Material: Fruits of *Aegle marmelos* were collected from local market. The fruit pulp was dried under shade at room temperature for 30 days and kept in incubator at 35^{0}C for 15 days. Dried fruit pulp was powdered, sieved and stored in

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air tight container until use [4]. Chemicals**:** Reference standard of marmelosin was obtained from UICT, Mumbai (Maharashtra, India). All the chemicals used in this study were AR Grade.

2.1. HPLC Method Development & Optimization

Physicochemical parameters of marmelosin were studied that includes solubility, partition coefficient, absorption maxima and pKa. The optimized method is as follows;

2.1.1. Chromatographic Conditions

Instrument:	Agilent-1220 LC Infinity
Column:	Agilent TC-C18, 4.6×5µm
Detection:	UV-VIS Detector at 247nm
Mobile Phase:	Acetonitrile: Water (70:30)
Flow Rate:	1ml / min
Injection volume:	20µl
Run Time:	20mins

2.1.2. Validation of Method: Preparation of standard Solutions

10mg of marmelosin in 10ml methanol (1mg / ml). From the stock solution different standard solutions (1, 5, 10, 15,20,25,30 µg / ml) were prepared in methanol. The developed method was validated for limit of detection, limit of quantitation, linearity, range, accuracy and precision according to ICH guidelines.

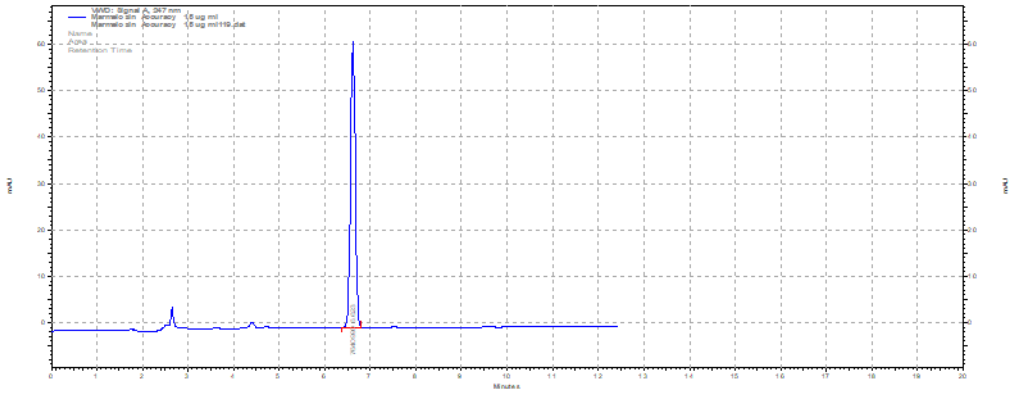

Figure 1 Representative chromatogram of standard marmelosin

2.1.3. Standardization of crude extract

The processed fruit pulp was extracted using soxhlet extraction method. The ethanolic extract was used for estimation of marmelosin content in fruit extract. 1mg of fruit extract was weighed and dissolved in ethanol. Resulting solution was sonicated for 30mins and filtered through whatman filter paper [4, 6]. Finally 10µg/ml of sample solutions were prepared and injected on HPLC (Figure No.02).

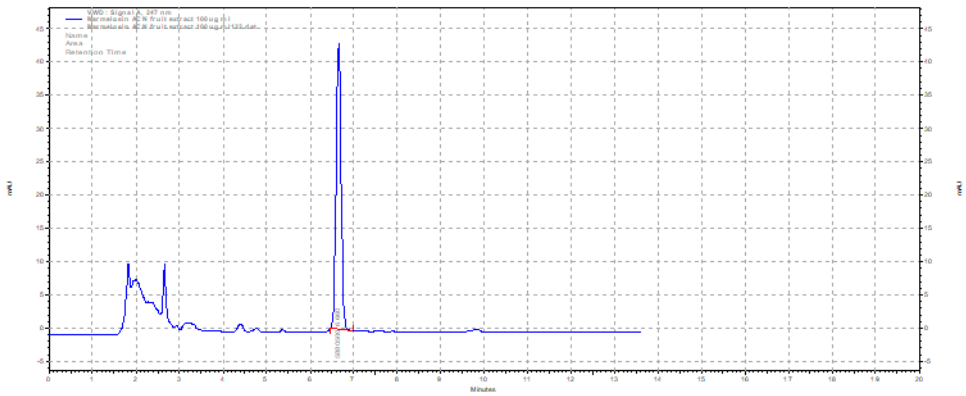

Figure 2 Representative chromatogram of marmelosin from crude extract.

2.1.4. Assay of herbal preparation

20 herbal tablets were taken and weighed; quantity equivalent to 10mg of marmelosin was dissolved in methanol. This solution was sonicated for 30 minutes and filtered using whatmann filter paper. Resulting solution was diluted and injected on HPLC (Figure No.03).

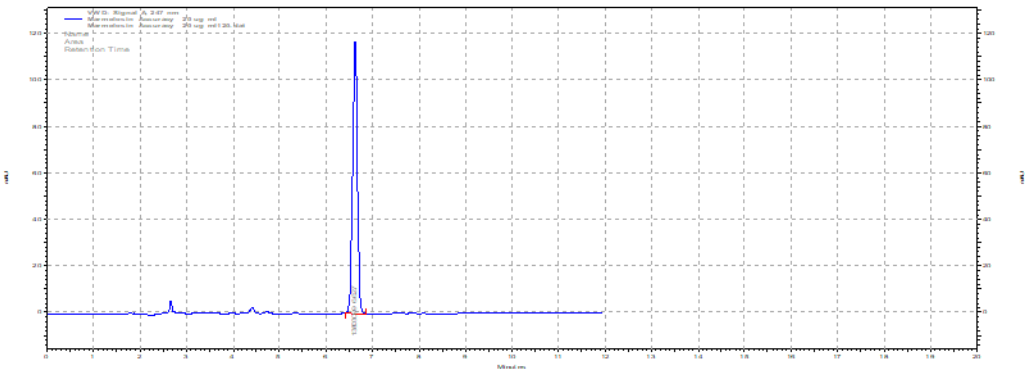

Figure 3 Representative chromatogram of marmelosin of herbal preparation.

3. Results and Discussion

Aegle marmelos (bael) fruit have many pharmacological activities since it is used from the traditional days hence need to be studied. Marmelosin is one of the major chemical constituent of the bael fruit and used as a biomarker. HPLC method was selected for the purpose because chromatographic methods are more accurate and sensitive.

The reported methods [4, 6] has very low sensitivity and required long time for marmelosin to elute. Therefore method was modified and optimized. Acetonitrile: Water (70:30) was used as mobile phase, flow rate was 1ml/min and λ_{max} was 247nm. Method has validated for LOD, LOQ, linearity, precision and accuracy. LOD and LOQ were found to be 0.1mg / ml and 1mg / ml respectively. Method was linear in the range 1-30 µg / ml with r^2=0.998. A precision study shows that coefficient of variance less than 20. This indicates that method was precise. Accuracy study was performed by percentage recovery method. The percent recovery was found to be 95%. The marmelosin content of crude ethanolic extract of fruit pulp of *Aegle marmelos* was found to be 8.1%w/w. The percent purity of herbal formulation of *Aegle marmelos* for marmelosin content was found to be 95%.

4. Conclusion

The developed and validated method was sensitive, accurate, precise and economic. It can be used for standardization of any formulation of *Aegle marmelos*.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The author declares that there is no conflict of interest. If there are potential conflicts of interest, we highly encourage each author to identify and declare clearly to avoid any future investigations by the publisher.

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