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Phytochemical screening of an ayurvedic *Rasayana* formulation: *Amlaki rasayana*

Rajesh S MonyDOI: <https://doi.org/10.22271/phyto.2023.v12.i2a.14618>**Abstract**

Amlaki Rasayana is a classical formulation prepared with *Amla* (*Emblica officinalis*), *Madhu* (Honey) and *Ghritha* (Clarified butter) mentioned in the text *Charaka samhita*, having the rejuvenative property called *Rasayana*. This formulation is prepared by a classical procedure called *Bhavana*, where to the starting *Amla* powder is subjected repeated additions of Juice from definite quantity *Amla* with successive drying. After 21 such steps definite quantity of Ghee (Clarified butter) and Honey is mixed to get the final formulation. The analytical methods and protocols developed helps to monitor the entire process operations and finished product Quality assurance.

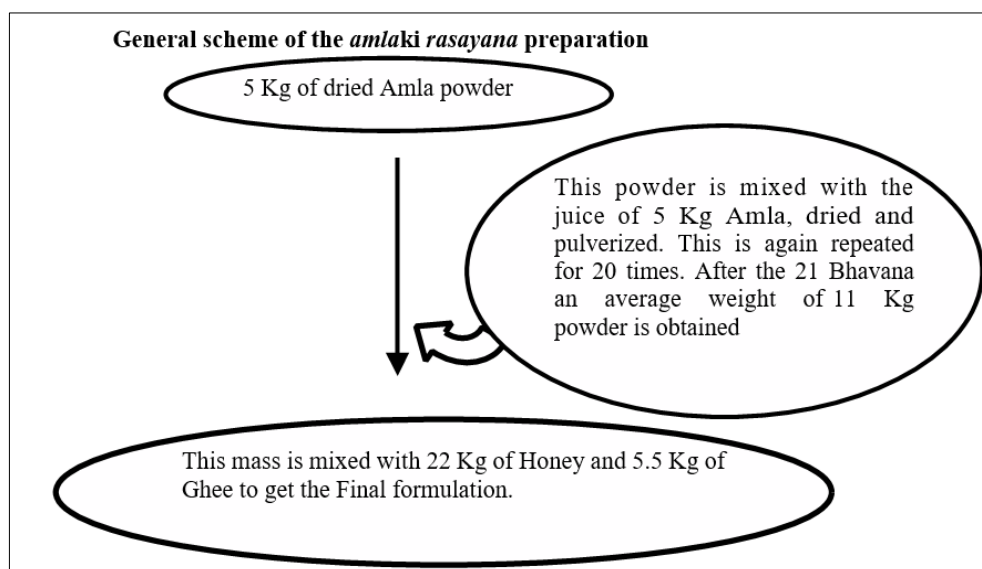
Keywords: *Amlaki rasayana*, *Rasayana* formulation, *Emblica officinalis*

Introduction

A central theme of this work is to point out the chemical complexity of this formulation (Chemical Markers from the ingredient formulation / Secondary metabolites / modified phytoconstituents, etc.) of commercial use, which necessitates considerable ingenuity on behalf of the analyst in harnessing the resolution and senility of the procedures that are applied.

The design of these methods typically necessitates consideration of extraction, evolving the procedures for fractional and differential extraction, solvent partitioning and chromatographic identification and quantification procedures.

Therefore, the formulation and raw and dried *Amla* were subjected to HPTLC analysis by developing a method for the determination of gallic acid and ellagic acid in the respective methanol extracts. The developed method has been validated as per ICH guidelines (ICH Q2A, 1994; Q2B, 1996)

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Materials and Methods

Equipment

HPTLC system of CAMAG (Swiss) with Linomat V, Twin trough chamber, Scanner III, 100 microlitre hamilton syringe and Wincats software. Sartorius high accuracy analytical balance and Water bath.

Chemicals

GR grade methanol, petroleum ether, toluene, ethyl acetate, formic acid, and anhydrous sodium sulfate of Merck, Standard gallic acid and ellagic acid of Sigma Aldrich and precoated silica gel 60 F₂₅₄ TLC aluminum plates (10x10, 0.2 mm thick) of Merck were used.

Samples

1. Raw *Amla*
2. Dried *Amla* powder

3. *Rasayana A* (after 1st *Bhavana*)
4. *Rasayana B* (after 5th *Bhavana*)
5. *Rasayana C* (after 10th *Bhavana*)
6. *Rasayana D* (after 15th *Bhavana*)
7. *Rasayana E* (after 21st *Bhavana*) and *Rasayana F* (final Formulation after mixing with honey and ghee). Each sample is taken from three batches.

Extraction of Samples

Weight equivalents

Raw *Amla* and Dried *Amla* powder contains an average 75.0% w/w and 3.0% w/w moisture respectively. After each *Bhavana* and drying the sample have an average 2.0% w/w moisture. After each *Bhavana* an average of 286 gm solids has been added to the preceding mass. The final formulation has 2.0% w/w moisture and around 71% w/w honey plus ghee.

Table 1: Chemicals

Raw <i>Amla</i>	Dried <i>Amla</i> powder	<i>Rasayana A</i> (after 1 st <i>Bhavana</i>)	<i>Rasayana B</i> (after 5 th <i>Bhavana</i>)	<i>Rasayana C</i> (after 10 th <i>Bhavana</i>)	<i>Rasayana D</i> (after 15 th <i>Bhavana</i>)	<i>Rasayana E</i> (after 21 st <i>Bhavana</i>)	<i>Rasayana F</i> (final Formulation)
10 gm	2.5 gm (2.58g including moisture)	2.5 gm (2.55g including moisture)	2.5 gm (2.55g including moisture)	2.5 gm (2.55g including moisture)	2.5 gm (2.55g including moisture)	2.5 gm (2.55g including moisture)	2.5 gm (9.3g including Honey, Ghee & moisture)

For Raw *Amla*

10gm moisture free macerated mass is extracted completely with (3x 10ml) repeated extraction with methanol after the prior removal of the petroleum ether fraction. Then moisture removed by adding 3.0 gms anhydrous sodium sulfate. The combined extract is then concentrated to 10 ml and 5 microloitre is used for HPTLC.

Dried *Amla* powder

2.58 gm powder is extracted completely with (3x 10ml) repeated extraction with methanol after the prior removal of the petroleum ether fraction. Then moisture is removed by adding 3.0 gms anhydrous sodium sulfate. The combined extract is then concentrated to 10 ml and 5 microloitre is used for HPTLC.

For *Rasayana A, B, C, D, E & F* samples

2.55 gm mass is extracted completely with (3x 10ml) repeated extraction with methanol after the prior removal of the petroleum ether fraction. Then moisture is removed by adding 3.0 gms anhydrous sodium sulfate. The combined extract is then concentrated to 10 ml and 5 microloitre is used for HPTLC.

For *Rasayana F* sample

9.3 gm mass is extracted completely with (3x 10ml) repeated extraction with methanol after the prior removal of the petroleum ether fraction. Then moisture is removed by adding 3.0 gms anhydrous sodium sulfate. The combined extract is then concentrated to 10 ml and 5 microloitre is used for HPTLC.

HPTLC Conditions

Stationary phase: Pre-coated silica gel 60F₂₅₄ TLC plate (10x10 cm, 0.2 mm thickness)

Mobile phase

Toluene: ethyl acetate: formic acid : Methanol (6:6:1.6:0.4 v/v)

Saturation time : 15 min
Scanning wavelength : 280 nm
Lamp : D₂

Calibration graph for Gallic acid

Stock solution of 1 mg/ml is prepared in methanol and further diluted to get the working standard of 200 microgram/ml. The standards are spotted to obtain a concentration gradation at the level of 1.0, 2.0, 3.0, 4.0 & 5.0 microgram concentrations per spot.

Calibration graph for Ellagic acid

Stock solution of 100 microgram/ml is prepared in methanol and further diluted to get the working standard of 25 microgram/ml. The standards are spotted to obtain a concentration gradation at the level of 0.1, 0.2, 0.3, 0.4 & 0.5 microgram concentrations per spot.

HPTLC DATA

1. Fingerprint of Raw *Amla (Emblica officinalis)*

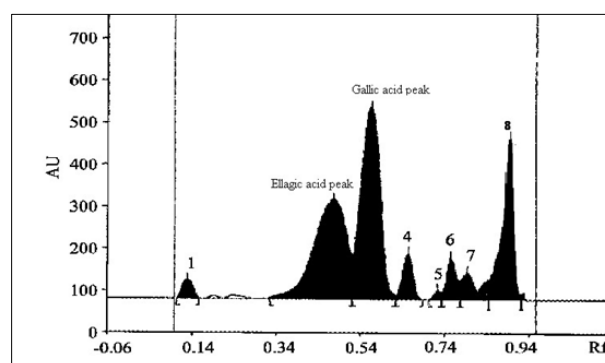


Fig 1: Fingerprint of Raw *Amla (Emblica officinalis)*

2. Fingerprint of Dried *Amla* powder

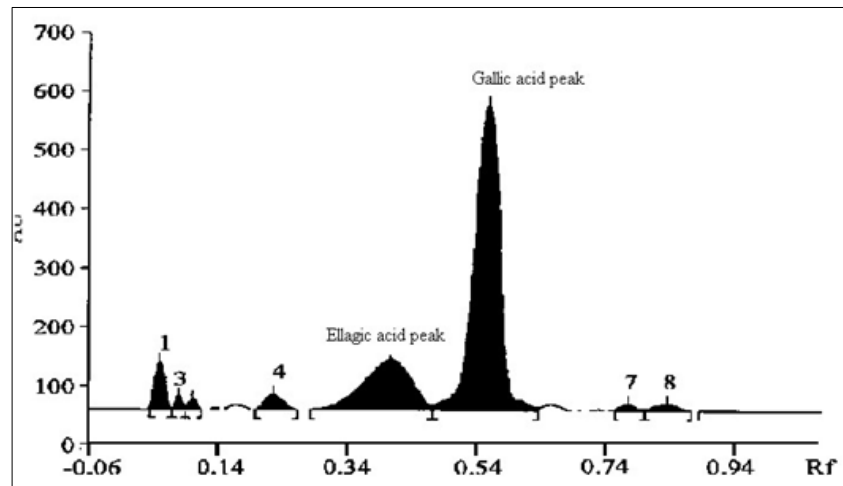


Fig 2: Fingerprint of Dried *Amla* powder

3. Fingerprint of *Rasayana A* (after 1st *Bhavana*)

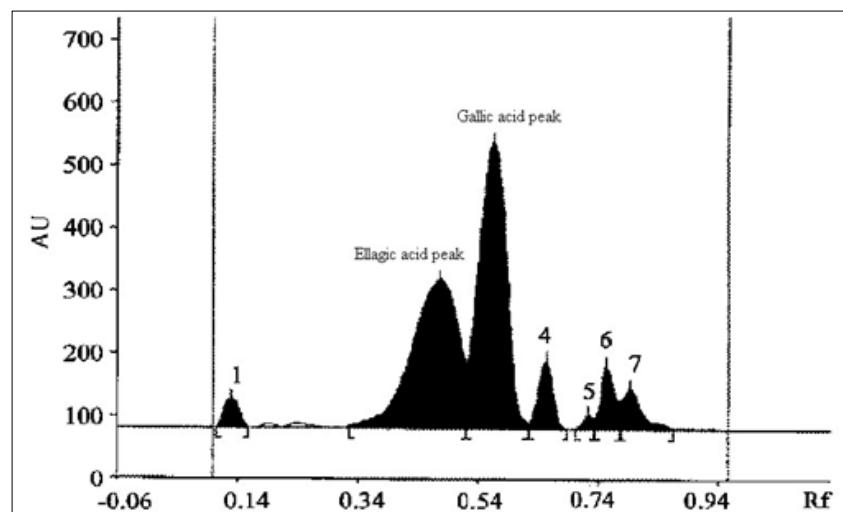


Fig 3: Fingerprint of *Rasayana A* (after 1st *Bhavana*)

4. Fingerprint of *Rasayana B* (after 5th *Bhavana*)

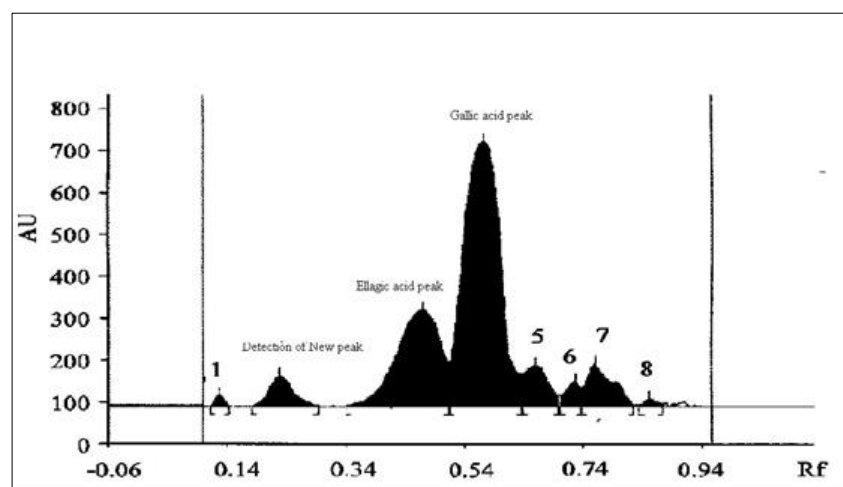


Fig 4: Fingerprint of *Rasayana B* (after 5th *Bhavana*)

5. Fingerprint of *Rasayana C* (after 10th *Bhavana*)

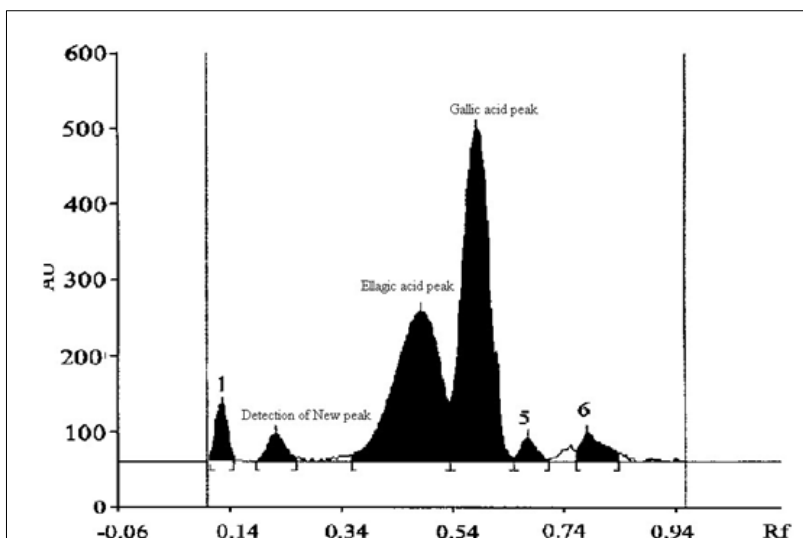


Fig 5: Fingerprint of *Rasayana C* (after 10th *Bhavana*)

6. Fingerprint of *Rasayana D* (after 15th *Bhavana*)

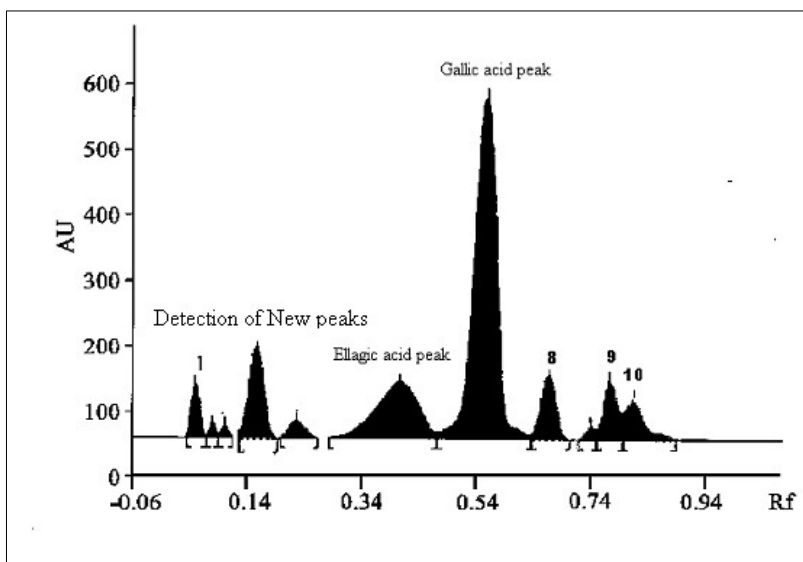


Fig 6: Fingerprint of *Rasayana D* (after 15th *Bhavana*)

7. Fingerprint of *Rasayana E* (after 21st *Bhavana*)

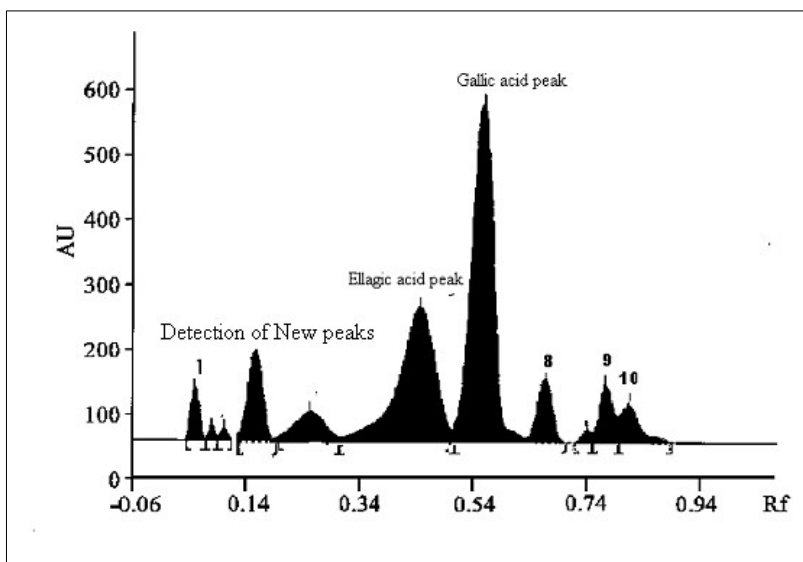


Fig 7: Fingerprint of *Rasayana E* (after 21st *Bhavana*)

8. Fingerprint of *Rasayana F* (final Formulation after mixing with honey and ghee)

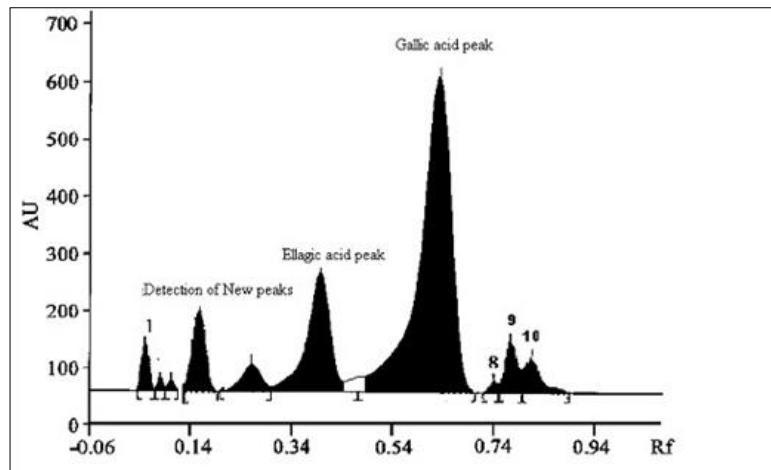


Fig 8: Fingerprint of *Rasayana F* (final Formulation after mixing with honey and ghee)

9. Gallic acid Standard peak

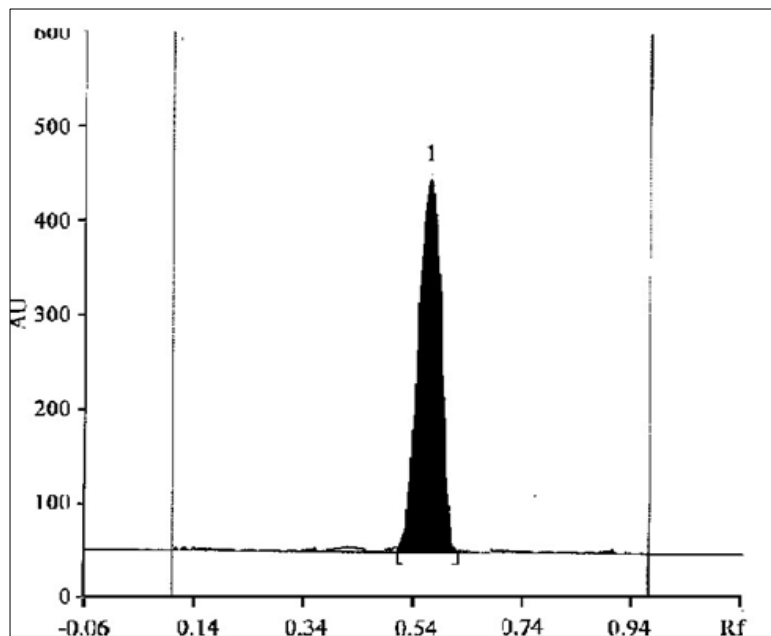


Fig 9: Gallic acid Standard peak

10. Ellagic acid Standard peak

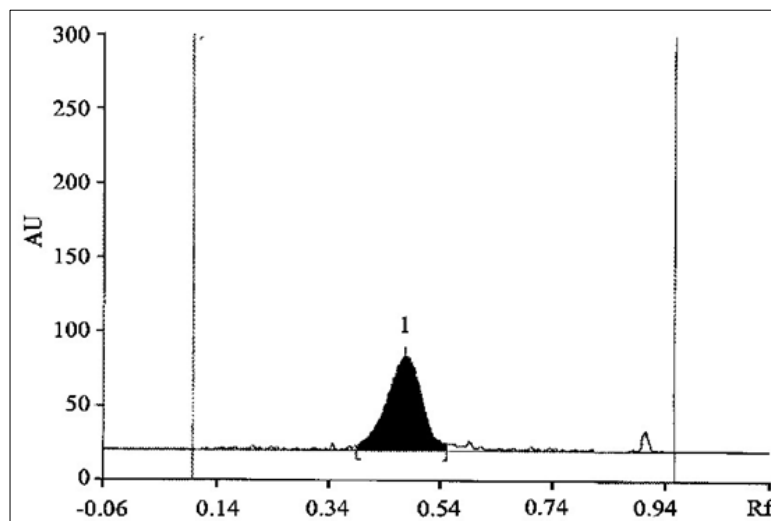


Fig 10: Ellagic acid Standard peak

11. Gallic acid Calibration graph

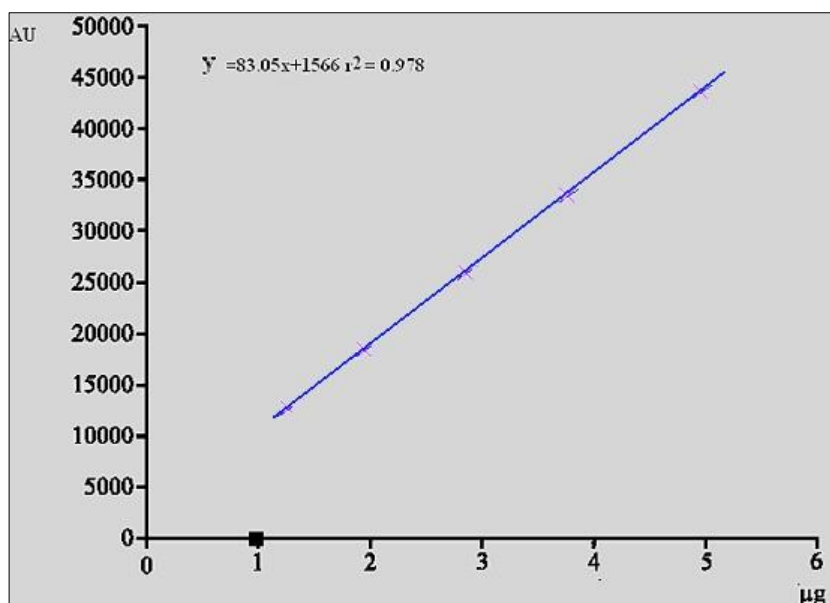


Fig 11: Gallic acid Calibration graph

12. Ellagic acid Calibration graph

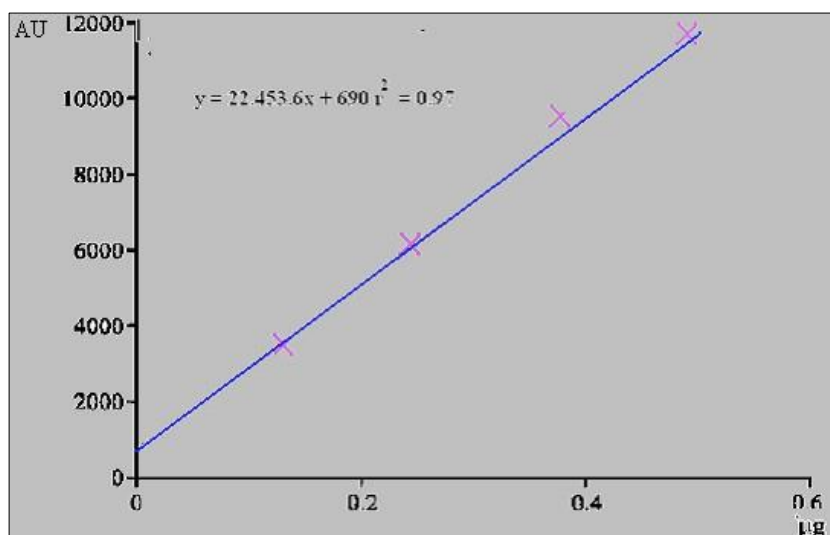


Fig 12: Ellagic acid Calibration graph

HPTLC marker quantification results

The amount of gallic acid and ellagic acid present in the various samples were computed from the above calibration

curves.

The average values are tabulated as follows:

Table 2: The amount of gallic acid and ellagic acid present in the various samples

SN	Sample	Gallic acid (%w/w)	Ellagic acid (%w/w)
1	Raw Amla	0.30 - 0.60	0.09 - 0.30
2	Dried Amla powder	0.90 - 3.50	0.40 - 0.60
3	Rasayana A (after 1 st Bhavana)	4.0 - 6.80	0.67 - 1.50
4	Rasayana B (after 5 th Bhavana)	4.5 - 7.0	0.45 - 0.60
5	Rasayana C (after 10 th Bhavana)	4.5 - 7.0	0.60 - 0.90
6	Rasayana D (after 15 th Bhavana)	4.5 - 7.0	0.40 - 0.58
7	Rasayana E (after 21 st Bhavana)	4.0 - 7.5	0.60 - 0.90
8	Rasayana F (final Formulation after mixing with honey and ghee)		
8A	Assay of markers with respect to the whole mass	1.0 - 3.50	0.38 - 0.60
8B	Assay of markers with respect to the Amla solids only (excluding ghee & honey mass)	5.0 - 7.0	0.58- 0.90

Validation of the HPTLC protocol**Linearity**

The correlation coefficient of Gallic acid and Ellagic acid were found to be 0.978 and 0.970, respectively and thus shows good linearity between concentration and area under the peak.

Accuracy

The percentage recovery of gallic acid and ellagic acid were found to be 97.70 and 97.20 respectively which are satisfactory in a processed herbal formulation.

Table 3: Recovery study of the method for gallic acid

SN	Amount of Rasayana taken (A)	Amount of Gallic acid calculated in A (B)	Amount of Gallic acid added in A (C)	Total theoretical amount of Gallic acid B+C (D)	Total amount of Gallic acid assayed by the HPTLC protocol (E)	Recovery percentage
01	500 mg	12.50 mg	10.0 mg	22.50 mg	21.85 mg	97.10
02	1000 mg	27.82 mg	15.0 mg	42.82 mg	41.79 mg	97.50
03	1500 mg	43.43 mg	20.0 mg	63.43 mg	62.48 mg	98.50

Table 4: Recovery study of the method for ellagic acid

SN	Amount of Rasayana taken (A)	Amount of Ellagic acid calculated in A (B)	Amount of Ellagic acid added in A (C)	Total theoretical amount of Ellagic acid B+C (D)	Total amount of Ellagic acid assayed by the HPTLC protocol (E)	Recovery percentage
01	500 mg	2.10 mg	2.0 mg	4.10 mg	3.97 mg	96.80
02	1000 mg	4.80 mg	5.0 mg	9.80 mg	9.59 mg	97.80
03	1500 mg	7.35 mg	10.0 mg	17.35 mg	16.82 mg	96.90

Specificity

The other peak constituents in the Fingerprints did not interfere either with gallic acid or ellagic acid, which show the protocol is specific. The spectrum of both markers in the formulation are similar to those of standards.

HPTLC Fingerprint observations during progressive bhavana (fortification) processes

After the 5th and 10th Bhavana an additional peak is noticed at Rf 0.14 - 0.15, which is not seen in the Raw Amla and Dried Amla powder. In 15th and 21st Bhavana sample along with the above-mentioned peak another additional three peaks are also observed. i.e. two at Rf 0.07 - 0.10 and one at Rf 0.25 - 0.27.

Conclusion

This HPTLC protocol is rapid, simple and accurate for accessing the genuineness of the formulation through the finger printing of phytochemical nature along with the quantification of major marker constituents in Amlaki Rasayana.

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