International Journal of Research in Engineering, Science and Management
Volume-2, Issue-5, May-2019
www.ijresm.com | ISSN (Online): 2581-5792


# Extraction of Tannic Acid from *Emblica Officinalis* (Avala) by Hot Continuous Extraction Method

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***Abstract*: To extract the active ingredients with the appropri-ate solvents, selected in accordance with the solubility and st-ability of the beneficial substances. Extracts can then be made using different solvents to isolate and purify the active compounds responsible for bioactivity and to identify active ingredient purity of compound it is possible to use high performance liquid chromatographic techniques to separate and purify naturally occurring substances. Advanced techniques like High Pressure Liquid Chromatography (HPLC) accelerate the bioactive molecule purification process. Various varieties spectroscopic method such as UV-visible, Infrared (FTIR), Nuclear Magnetic Resonance (NMR) and mass spectroscopy can identify the purified compounds. Tea leaves, Avala fruits, nettle, wood, berries, Chinese galls and Oak wood is very rich in tannic acid. The objective of this study was to extract tannic acid from fruits of Emblica officinalis (Avala) are containing tannic acid among, it is one of the richest ordinary sources of tannic acid and evaluate the efficiency and quality analysis through different method such as conventional batch extraction and novel extraction technique etc. This paper represents impact on extraction of tannic acid by Soxhlet extraction for 24 hours experimental study for solvent selection such as Distilled water, Ethanol and n-Hexane. Further study the selected solvent though Simple distillation extraction method for 45 min experimental trial and result analysis carried by UV Spectroscopy to optimized raw material & solvent used and to maximize the yield of tannic acid.**

***Keywords*: Simple Distillation, Soxhlet extraction, Tannic acid, Avala.**

## 1. Introduction

Avala (Phyllanthus emblica L.) is a member of the Euphorb iaceae family. It is commonly referred to as Myrobalm Emblic a, IndianGooseberry, Aonla, Amla, Aola, etc. The highest Tannic acid content has been found in the fruit Avala extracts of plants family Phyllanthaceae - in range from 0.782 - 5.078 mg g-1 DW. The comparative extraction study showed significant differences of content of tannic acids in the fruit extracts [10]. Thus Other ingredient present in Avala is vitamin C and vitamin A. This also contains high Quantity of follic acid and tannin and minerals such as calcium, potassium, phosphorus, iron, carotene, and magnesium. According to the USDA National Nutrient Database, Avala are low in calories with 100 grams of fruit containing only 44 calories [14].

Tannic acid is also an essential hydrolysable class gallotann in. The tree is cultivated very widely in India; in fact it is being done at very large scale in some states of the country. Avala fruit is known for many of its medicinal properties for a long time and is reported to be acrid, cooling, refrigerant, diuretic and laxative. Dried fruit is useful in haemorrhage, diarrhoea and dysentery. Avala is an important constituent of triphala, a known ayurvedic formulation used as laxative and treating biliousness. The dried fruit is detergent and is used as shampoo. These are so many known applications of this tree and its products that many companies dealing in traditional medicines use Avala fruit for preparing different formulations for treating several diseases, as health tonic etc.

Tannic acid is an organic compound and mostly used in industries types of tannin, a kind of polyphenol. Its not an strong acid ($pK_a$ around 10), which result uncalculated phenol groups in the structure. These tannic acid chemical formula was generally given as $C_{76}H_{52}O_{46}$, which comparable by means of decagalloyl glucose, but it is a mixture of polygalloyl quinic acid esters or glucoses along with quantity of galloyl moieties per molecule ranges between 2 to 12 which depends on the plant methods those are use on the way to extract the tannic acid. The fruit has comparatively larger concentration of asparagic acid & minerals. Alanine, 2-Aminoglutaric acid, Pyrrolidine-2-carboxylic acid, lysine and asparagic acid are 14.6%,29.6%, 8.1%, 5.3% and 5.4% respectively of the total asparagic acid. The soft delicate part of seed, dried and liberated from the nuts enclose: tannin, tannic corrosive 1.33%, rough cellulose 17.08%; gum 13.75%; c cellulose 17.08% and mineral material 4.12%. Avala natural product fiery debris contains copper, zinc, chromium (3, 4, 2.5 ppm) [1]. *Emblica officinalis* was one of mainly important fruit of tropics and subtropics. It contains large amount of vitamin C and other nutrients like polyphenols, pectin, iron calcium and phosphorus. Many industrial applications also use tannic acid. The best k-nown is leather tanning. Sometimes tannic has been used to cl ear wines. Tannic acids react in wine with proteins to form ins oluble complexes that can be filtered or sedimented.

The main aim of these work is to study solid-liquid extraction of tannic acid. The Soxhlet, batch and Simple distillation

extraction experiment were conducted to find the influence of the Solvent and analyzed by HPLC, FTIR and UV Spectroscopy [2].

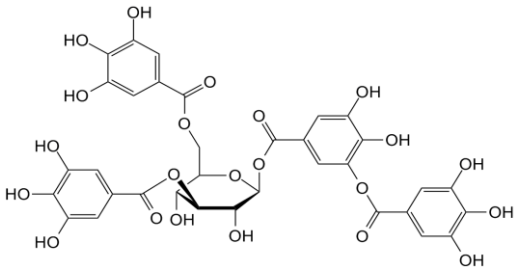

Fig. 1. Tannic acid

## 2. Formulation of tannic acid

*Formation of tannic acid (TA):* The tannic acid was synthesized by slightly modifying the previously reported procedure. [3] The concept of "tannic acid" must first of all be clearly defined. In many plants, tannic acid can be considered primarily those substances of vegetable origin that can only be found in many plants as water-soluble bodies, showing certain chemical behaviour, acquiring astringent characteristics while being able to convert animal conceal into leather. This latter property of the tannic acid, that of converting the animal hide easily decomposable protein into a permanently preserved substance and conveying this well-defined and technically important compounds, has become the criterion of the tannic acid's useful measure. It seems that special substances show the chemical reactions that are unique to the tannic acid and to some of them. [6]

## 3. Materials and methods

The *Emblica Officinalis* used in current work was purchased from herbal store (Akola, India). Standard tannic acid and all solvents such as ethanol, n-Hexane and distilled water purchased from Nashik Dodal Chemical Store and UV-Visible double beam Spectrophotometer (UV-1800, Shimadzu Japan Model No. 2100), HPLC and FTIR was used for analysis purpose. The following relation gives the percentage extraction of tannic acid [3].

$$\% \text{ Extraction of tannic acid at any time t} = \frac{A}{B} \times 100$$

A = tannic acid concentration at any time t in solvent.

B = tannic acid maximum concentration in solvent by Soxhlet extraction method.

## 4. Experimental setup

The investigation of the impact of the solvent (ethanol, n-Hexane and distilled water). The Soxhlet extraction method were done in completely perplexed 500 $cm^2$ borosilicate round bottom flask glass vessel. Using a constant temperature bath, experiments were carried out at 80°C (±1°C).

## 5. Experimental procedure

### A. *Simple Distillation*

The aim of the conventional extraction experiments is used to estimate the percentage extraction of tannic acid and investigation of effect of the working parameters. *Emblica Officinalis* fruits powder grinded were taken 30 g and brought into the extractor, outfitted with a distillation column and it consist of 300 ml of solvent. Solution mixture is heated in a flask until the mixture boils. Pure liquid turns into a vapour and leaves the flask, then vapour is cooled in a condenser which changes back into liquid.Pure liquid collected is called the distillate and is collected in a flask.

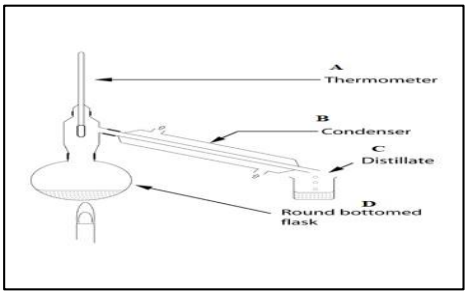


Fig. 2. Schematic diagram view of simple distillation

### B. *Hot Continuous Extraction (Soxhlet)*

The goal of hot continuous extraction (soxhlet) was to decide most extreme recoverable substance of the solute in the crude material. *Emblica officinalis* (Avala) fruit grinded in mixer into an unequivocal size. Soxhlet extraction equipment through 400 $cm^2$ round bottom flash of distilled water and mass of 30 g of raw material (fruit powder) was placed thimble [4], and experimentation performed for 24 h with distilled water. The equilibrium concentration of tannic acid at time 24 h was obtained 672.16 mg/L distilled water.

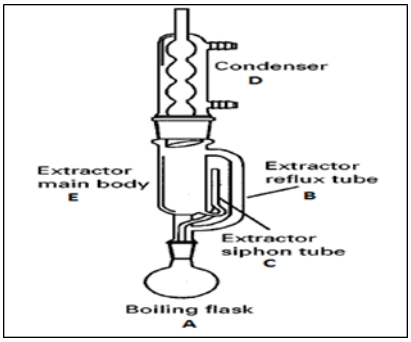


Fig. 3. Schematic diagram view of Soxhlet extraction

### C. *Analytical Methods*

Tannic acid content estimation by high performance liquid chromatography (HPLC) analysis: The Tannic acid content by HPLC was estimated by the method given by Seruga *et al.* (2011) with some modifications. Tannic acid on hydrolysis give Gallic acid. Calibration curves were made by mixing standard tannic acid 0.1 ml with 10 ml acetonitrile to give concentration

of the standards in the range of 1-100 mg $L^{-1}$ of tannic acid. The detection wavelength for tannic acid was 276 nm. The tannic acid was determined from the total area of HPLC 1.0 ml/min and run time of 6.0 min with HPLC column parameter C18 (250 x 4.6 mm, 5um and expressed as TAE $g^{-1}$ of sample. For the sample preparation, 0.1 ml of sample was extracted in 10 ml of mobile phase in different solvent such as Distilled water, ethanol and n-Hexane.

*FTIR Spectral analysis:* To find out the functional characteristics of the samples were scanned by Fourier Transform Infrared (FTIR) spectrophotometer (Perkin Elmer, Spectrum 100) in the range of 4000-600 cm with a resolution of 4 cm. All the samples were analyzed in duplicate. Results are shown as mean ±standard deviation [10]. The significant difference was analyzed by using Analysis of variance.

Table 1
Standard value (wavelength) of compound

| S. No. | Group | Vibration | Approach frequency/ experimental ($cm^{-1}$) |
|---|---|---|---|
| 1. | Arom-CH-OH | v OH | 3400/3381 |
| 2. | Arom-$CH_2$-OH | $v_a$ $CH_2$ | 2920/2925 |
| 3. | Arom-C-O | v C-O | 1630/1614 |
| 4. | Arom-$CH_2$-OH | δ $CH_2$ | 1420/1452 |
| 5. | Arom-O- | $v_a$ C-O | 1050/1165 |
| 6. | Arom-O- | v C-O | 800/646 |

Standard tannic acid, which compound is belongs to the approach frequency

*UV Spectroscopy:* Powdered fruits of *Emblica officinalis* were subjected to analysis under ultra violet light after treatment with various chemical. These parameters were taken into account i.e., observation under maximum wave length U.V (276 nm) UV–Visible double beam Spectrophotometer (UV-1800, Shimadzu Japan) with 4 cm quartz cuvettes.

## 6. Results and discussion

### A. *Influence of Solvent*

The Fig. 2. shows influence the solvent on extraction of tannic acid in different solvents such as ethanol, distilled water, and n-Hexane. Tannic acid concentration increases in solvent with increase in time and polarity of solvent. (Distilled water > ethanol > n-Hexane). Distilled water was an ecofriendly solvent. Hence, distilled water was used throughout this work. Fig. 2. Demonstrates that the introductory rates of extraction of tannic acid are exceptionally quick and get to be consistent after about 45 min.

### B. *Analysis report of HPCL testing*

The rate of extraction of tannic acid is found to increment with retention time of 3 min as shows in Fig. 3. The rate of extraction of tannic acid from fruit of *Emblica Officinalis* with distilled water as solvent which stirred at 500 rpm in an agitated vessel at temperature range from 30-60°C [7]. The initial rate of extraction of tannic acid is fast. The tannic acid yield increased with increasing the area covered by peak

Table 2
Retention time

| S. No. | Retention time | Name | Area |
|---|---|---|---|
| 1 | 3.01 | Tannic acid | 20237 |

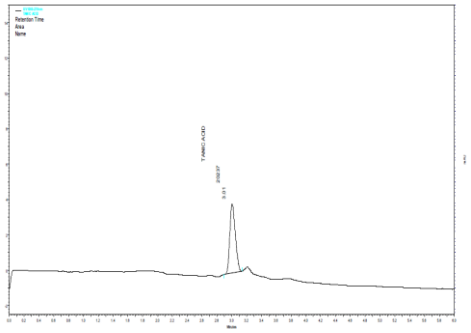

Fig. 4. Graph of Tannic acid standard (10 ppm) spectrum in HPLC

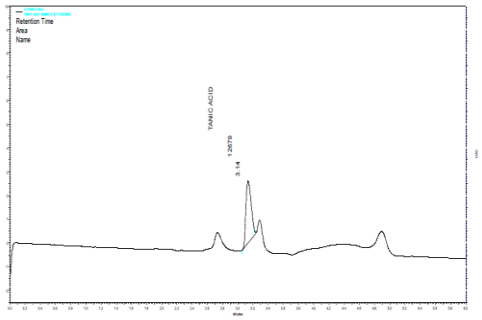

Fig. 5. Graph of sample extracted in Distilled water (10 ppm) in HPLC

Table 3
Retention time

| S. no. | Retention time | Name | Area |
|---|---|---|---|
| 1 | 3.11 | Tannic acid | 12679 |

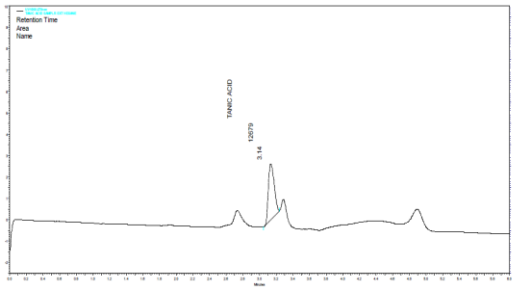

Fig. 6. Graph of sample Extraction in Ethanol (10 ppm) in HPLC

Table 4
Retention time

| S. no. | Retention time | Name | Area |
|---|---|---|---|
| 1 | 3.11 | Tannic acid | 2109 |

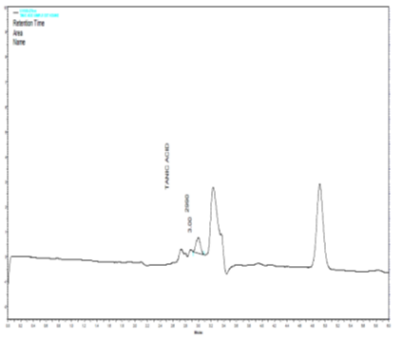

Fig. 7. Graph of sample Extraction in n-Hexane (10 ppm) in HPLC

Table 5
Retention time

| S. no. | Retention time | Name | Area |
|---|---|---|---|
| 1 | 3.00 | Tannic acid | 2990 |

- *Tannic acid HPLC Calculations sheet*

*Standard Preparation*

10 mg tannic acid standard — 10 ml Acetonitrile
0.1 ml — 10 ml Mobile phase

*Sample preparation*

*Distilled water extract*

10 mg Sample — 10 ml Acetonitrile
0.1 ml — 10 ml Mobile phase

*Ethanol extract*

10 mg Sample — 10 ml Acetonitrile
0.1 ml — 10 ml Mobile phase

*n-Hexane extract*

10 mg Sample — 10 ml Acetonitrile
0.1 ml — 10 ml Mobile phase

Formula

$$\% \text{ Tannic acid} = \frac{\text{Area of sample} \times \text{Concentration of standard} \times \text{Purity}}{\text{Area of standard} \times \text{Concentration of sample}}$$

Table 6
Result of HPLC

| | Area | % of Tannic acid |
|---|---|---|
| Standard | 20237 | |
| Distilled Water | 12679 | 62.55 |
| Ethanol | 2109 | 10.42 |
| n-Hexane | 2990 | 14.77 |

## C. Analysis report of FTIR testing

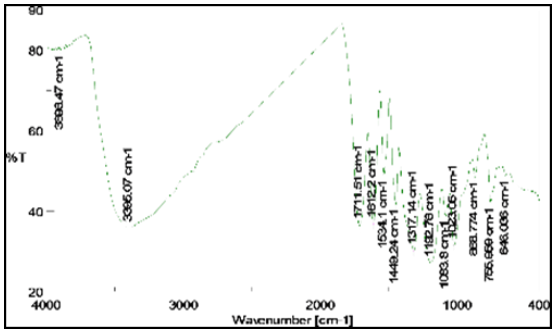


Fig. 8. Graph of Tannic acid standard spectrum in FTIR testing

Tannic acid vibration through standard value of -OH value from 3400 to 3381 $m^{-1}$ as the standard spectrum curve for tannic acid sample as shown in Fig. 8. Thus extraction of tannic acid were conducted with distilled water, ethanol and n-Hexane and the sample result graph as shown below.

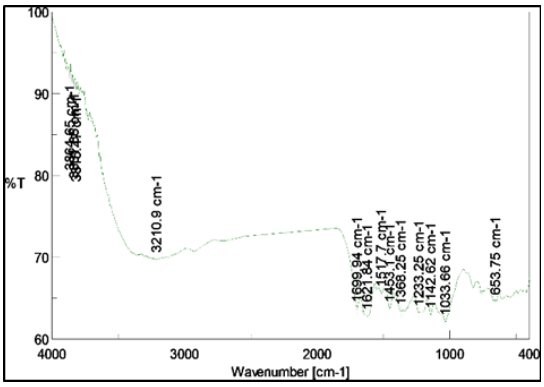


Fig. 9. Graph of Sample in Distilled water extraction in FTIR testing

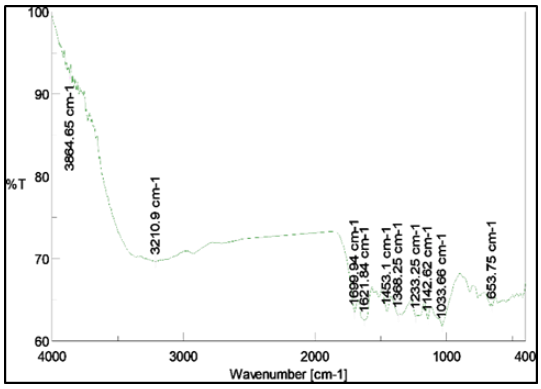


Fig. 10. Graph of Sample in Ethanol Extraction in FTIR testing

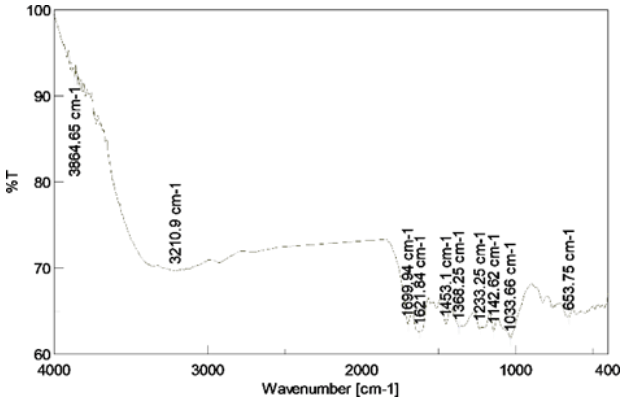


Fig. 11. Graph of Sample in n-Hexane Extraction in FTIR testing

## D. Analysis report of UV Spectroscopy testing

Shimadzu 3600 UV-Vis spectrophotometer furnished with quartz cell 4 cm is utilized for the absorbance considers. The λmax range of tannic acid at 276 nm was recorded on a Bruker spectrometer at 300°K, utilizing Distilled water, ethanol and n-Hexane as a solvent and Tannic acid (TA) as an inner reference compound [4].

Table 7
Result of UV Spectroscopy

| S. no. | P/V | Wavelength (nm) | Abs |
|---|---|---|---|
| Distilled water | | | |
| 1 | Peak | 276.00 | 0.264 |
| 2 | Peak | 208 | 1.352 |
| Ethanol | | | |
| 3 | Peak | 276.00 | 0.212 |
| 4 | Peak | 208.00 | 1.104 |
| n-Hexane | | | |
| 5 | Peak | 276.00 | 0.808 |
| 6 | Peak | 217.00 | 1.913 |

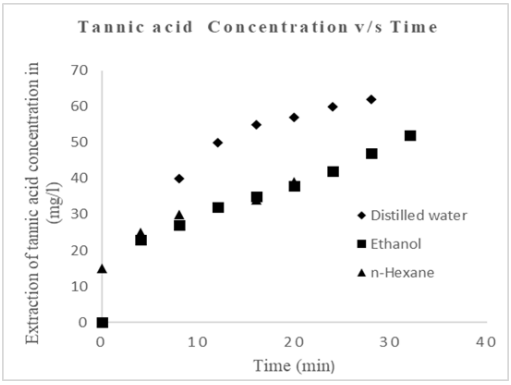


Fig. 12. Graph of Concentration (mg/l) of extracted tannic acid with solvent distilled water, ethanol and n-Hexane v/s in UV Spectroscopy testing

## 7. Conclusion

This paper described impact of three different solvent for recovery of Tannic acid from Embilca (Avala) fruit through Soxhlet extraction (Hot continues extraction) methods out of three different solvent (Distilled water, ethanol, n-Hexane) distilled water is found good which maximum yield as compared with other solvent and the cheapest & easily available solvent.

As shown in the analyzed graph of HPLC, FTIR and UV Spectroscopy result for distilled water solvent maximum extraction yield was obtained with experimental worked with Hot Continues extraction (Soxhlet) and Simple distillation.

The % extraction of tannic acid from Avala fruit powder increases with increases in extraction time at 28 min 62 mg/l concentration of tannic acid. Simple Distillation extraction method was performed & compared with Hot continuous extraction (Soxhlet) solvent extraction methods, high percent extraction of tannic acid at less time of extraction.

Hence in above experimental work of the extraction and analysis report, universal solvent distilled water which is good and cheapest for extraction. 62.55 % of tannic acid is extracted as compare with other solvent (ethanol-10.42% & n-hexane 14.23%).

## Acknowledgement

The authors gratefully thankful for financial support provided by College of Engineering and Technology, Department of Chemical Engineering Akola for providing equipment facilities for current work.

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