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### RESEARCH ARTICLE

## PHYSICAL AND PHYTOCHEMICAL EVALUATION OF COMMERCIAL SAMPLES OF DRIED FRUIT RIND OF AMALAKI (*PHYLLANTHUS EMBLICA* LINN.) PROCURED FROM HERBAL DRUG MARKET IN KERALA, INDIA.

R H Amina, Jollykutty Eapen and M S Deepa.

Department of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram, 695001, Kerala, India.

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#### Key words:-

Amalaki, *Phyllanthus emblica* Linn., Physical and phytochemical evaluation, Herbal drug market, Ayurveda.

### Abstract

*Amalaki* (*Phyllanthus emblica* Linn.) is one of the most popular and highly reputed medicinal plant used in Ayurveda. The plant is widely distributed throughout India. Its fruits are said to be the best rejuvenating drug in Ayurveda. The Ayurvedic Pharmacopoeia of India (API) mentions both fresh and dried fruits of *Phyllanthus emblica* Linn. as the officinal part. The reports of National Medicinal Plant Board (NMPB) say that *Amalaki* is the first and most demanding medicinal plant in Kerala. In Kerala, the dried fruit rind is used in a variety of formulations either as single or in combinations. The present study was undertaken to assess the genuinity and quality of dried *Amalaki* fruit rind available in herbal raw drug market in Kerala. 28 commercial samples (1 urban and 1 rural) of dried *Amalaki* fruit rind were collected from each 14 districts of Kerala. Its characters were compared with the genuine dried fruit rind of *Amalaki* and also with the standards mentioned in API. Comparisons were done with the aid of various physical and phytochemical evaluations. Statistical comparison of physical and phytochemical parameters of genuine and market samples with one sample t test was significant at  $p < 0.01$  except for total ash content. On qualitative analysis of market samples, trace amount of flavonoids were detected. All heavy metals screened were under the permissible limits in all samples. The study revealed that the samples of dried *Amalaki* fruit rind available in herbal drug market in Kerala are genuine but possess poor quality and purity.

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### Introduction:-

Ayurveda is gaining tremendous recognition globally on virtue of the guaranteed safety and efficacy of the medicines. The fruit of *Amalaki* (*Phyllanthus emblica* Linn., Family-Euphorbiaceae) is one of the most popular and highly reputed drug used in Ayurveda and is described as the best *vayahsthapana* (anti-aging) drug. It is commonly known as Indian gooseberry. It is the main ingredient of many Ayurvedic formulations including the *Triphala churna*, *Chyavanaprasa* and *Amalaki rasayanam* to name a few. *Amalaki* fruit has also multifaceted utilities in pharmaceuticals, herbal cosmetics and health supplements. Its fruits are rich source of Vitamin C and is said to have the properties like *tridoshahara*, *medhya*, *rochana*, *deepana*, *chaksusya* and *keshya*. (Pandey, 2015)

**Corresponding Author: - R H Amina.**

Address: - Department of Dravyagunavijnanam, Govt. Ayurveda College, Thiruvananthapuram, 695001, Kerala, India.

*Amalaki* is one of the most important medicinal plant in India, as per the demand calculations. A study to assess the demand and supply of medicinal plants in India revealed that *Amalaki* (*P. emblica* L.) is the highest consumed botanical raw drug by the domestic herbal industry. The estimated annual industrial consumption of dried *Amalaki* fruit, by the domestic herbal industries, has been estimated at 17,000 MT that correlates to a quantity of 85,000 MT of green fruits. (Ved and Goraya, 2007). As per the reports of National Medicinal Plant Board (NMPB), the quantity of all Kerala annual consumption of *Amalaki* fruit on the year 2005-06 was 634720 kg approximately (SMPB Kerala, 2012). Also, it was reported that Ayurvedic medicine manufacturer's in Kerala required 4,00000 Kg *Nellikathodu* (dried *Amalaki* fruit rind) at the rate of 70 Rs per kg (NMPB, 2013).

The problems of modern age like deforestation, rapid urbanization, extinction of many plants, uncertainty in identification and profit motives has led to widespread adulteration. As the demand for the Ayurveda has increased, the procurement of genuine drugs with proper identity and purity has become the need of the day. Nowadays Ayurvedic physicians mainly depends pharmaceutical preparations from reputed manufactures for treatment and are rarely involved in direct collection and preparation of medicines. Since the cultivation and production of *Amalaki* fruit, is not sufficient to meet the requirements of herbal drug industries, there are possible chances of adulteration. *Amalaki* fruit extract is widely available in the market for the commercial purpose. So there may be possible chance of exhausted crude drug in Kerala herbal drug market and herbal drug industry.

Dried *Amalaki* fruit rind is a major ingredient of many Ayurvedic preparations. If the processing and storage is not done properly, it may get contaminated with microbial growth; which decrease the quality of the drug. In this situation we cannot ensure the quality of dried *Amalaki* fruit rind available in the market that we use for the medicinal purpose. So it is necessary to study the quality of dried *Amalaki* fruit rind available in the herbal drug market in Kerala. Physical and phytochemical evaluation plays a major role in the quality determination of the medicines. So the present study aimed to assess the quality of dried *Amalaki* fruit available in herbal drug market in Kerala, India through the physical and phytochemical analysis. Finally, it will benefit the herbal drug manufactures and thus the consumers.

## **Materials and Methods:-**

### **Sample collection:-**

#### **Genuine sample collection:-**

The genuine sample of fresh *Amalaki* fruits were procured from Trivandrum district, Kerala and authenticated at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode. Voucher specimens were deposited at JNTBGRI Herbarium (TBGT 84561 dated 7/7/2016). The samples were thoroughly cleaned, cut into small pieces and dried well. It was powdered in a mini pulveriser, sieved through mesh size 80 and kept in labeled air tight container.

#### **Market sample collection:-**

Two market samples (one from urban area and one from rural area) were purchased from each of the 14 districts of Kerala. All the 28 market samples were grouped into Sample A (Urban samples) and Sample B (Rural Samples) and each sample of group A and group B was separately packed in zip locked polythene bags in crude form and in labeled air tight containers in powder form.

#### **Sample Evaluation:-**

In sample evaluation, physico-chemical analysis, qualitative analysis, heavy metal analysis and chromatographic screening were carried out at Drug Standardization Unit (DSU), Government Ayurveda College, Thiruvananthapuram.

#### **Physico-chemical evaluation:-**

Foreign Matter (FM), Total Ash (TA), Acid Insoluble Ash (AIA), Moisture Content (MC), Cold Water Soluble Extractive (WSE), Alcohol Soluble Extractive (ASE), Fibre Content (FC), Reducing Sugar (RS) and Total Sugar (TS) contents were evaluated according to the standard procedures.

#### **Qualitative analysis:-**

The qualitative tests for steroids, flavonoids, alkaloids, tannins, saponin and phenol were conducted in ethanolic extracts of genuine and market samples. The tests conducted for the plant constituents are charted in table 1

**Table 1:-** Qualitative analysis of *P. emblica* L.

| Phytochemicals | Test conducted                   |
|----------------|----------------------------------|
| Steroid        | Liebermann-Burchard's Test       |
| Flavonoid      | Shinoda's test                   |
| Alkaloid       | Mayer's test, Dragendroff's test |
| Tannin         | Lead acetate test                |
| Saponin        | Foam test                        |
| Phenol         | Ferric chloride test             |

**Chromatographic screening:-****Thin Layer Chromatography:-**

The TLC analysis was conducted as per the standard procedures in ethanolic extracts of the dried fruit rind of genuine *Amalaki* (*P. emblica* L.) and the market samples. A number of solvent systems were tried for better results and maximum number of spots were obtained in the solvent system, Toluene: Ethyl Acetate: Formic acid in the proportion of 6:3:1. Best results were obtained after derivatization with Anisaldehyde sulphuric acid reagent and the plate was heated at 110<sup>0</sup> C for 5 minutes. The spots were first visualized in visible light and then in UV (254 nm and 366 nm), Iodine chamber and after derivatization. The Rf values were calculated.

**High Performance Thin Layer Chromatography:-**

2 µl of the Ethanolic extract of the samples of *Amalaki* (*P. emblica* L.) and market samples of the drug were applied as 8mm band length in the 10 × 200 Silica gel 60F<sub>254</sub> TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The plate with the samples applied were kept in TLC twin trough developing chamber (after saturation with solvent paper) with respective mobile phase [Toluene: Ethyl acetate: Formic acid in the ratio 6:3:1 for both HPTLC profiling of *Amalaki* samples and Gallic acid (GA) analysis] up to 70 mm. The plate was dried by hot air to evaporate solvents from the plate. The plate was kept in photo documentation chamber (CAMAG REPROSTAR 3) and the images were captured at white light, UV 366nm, UV 254nm and UV 258 nm. The plate was photo documented in UV 254nm, UV 258nm and UV 366nm using photo documentation (CAMAG REPROSTAR 3) chamber. Before derivatization, the late was fixed in scanner stage (CAMAG TLC SCANNER) and scanning was done at UV 254nm, 258nm and UV 366nm. The peak table and peak densitogram for each profile with spots obtained were noted. The software used was Win CATS 1.3.4 version.

**Heavy metal analysis:-**

Atomic absorption spectroscopy was used to determine the heavy metal elements and some nonmetal elements in atomic state. Determination of Lead, Cadmium, Nickel, Copper, Iron and Zinc in ppm levels in test drugs were carried out using standard procedure at DSU, Government Ayurveda College, Thiruvananthapuram. Thermo Electron Corporation M-series AA Spectrophotometer was used for the purpose.

**Statistical techniques:-**

Both descriptive and inferential statistics were used in the statistical analysis. Significant difference between the genuine sample value and market sample values were tested using one-sample t test with the test value of the difference being set as zero (market sample and genuine sample as equivalent). A calculated P value less than 0.05 was considered to be statistically significant.

**Results:-****Physico-chemical evaluation:-**

Physico-chemical parameters of *Amalaki* samples were evaluated and the observations were presented in Table 2

**Table 2:-** Physico-chemical evaluation of genuine and all market samples.

| Samples | FM       | MC   | VO  | TA      | AIA      | WSE      | ASE      | FC   | RS    | TS    |
|---------|----------|------|-----|---------|----------|----------|----------|------|-------|-------|
| API     | Not > 3% | -    | -   | Not >7% | Not > 2% | Not <50% | Not <40% | -    | -     | -     |
| GENUINE | Nil      | 4.5  | Nil | 3.58    | 0.61     | 59.49    | 41.53    | 9.92 | 14.54 | 18.94 |
| TVM A   | 10.18    | 14.9 | Nil | 3.65    | 0.66     | 15.06    | 32.74    | 8.22 | 4.34  | 6.06  |
| TVM B   | 7.22     | 9.32 | Nil | 2.78    | 0.61     | 43.00    | 35.01    | 8.06 | 5.21  | 7.11  |
| KLM A   | 13.57    | 14.9 | Nil | 5.46    | 2.36     | 32.11    | 26.08    | 8.52 | 5.22  | 8.06  |

|          |       |      |     |       |       |       |       |      |      |      |
|----------|-------|------|-----|-------|-------|-------|-------|------|------|------|
| KLM B    | 18.03 | 14.6 | Nil | 3.13  | 1.37  | 33.58 | 29.82 | 8.91 | 4.01 | 7.22 |
| PTA A    | 8.52  | 4.01 | Nil | 2.04  | 0.54  | 49.2  | 27.34 | 8.04 | 2.11 | 5.30 |
| PTA B    | 6.23  | 9.23 | Nil | 6.90  | 3.68  | 31.61 | 26.85 | 9.54 | 4.15 | 5.39 |
| ALP A    | 9.015 | 4.17 | Nil | 5.75  | 3.70  | 21.38 | 28.00 | 9.14 | 5.22 | 5.22 |
| ALP B    | 7.55  | 4.11 | Nil | 5.08  | 1.55  | 29.09 | 30.17 | 9.11 | 3.17 | 5.90 |
| KTYM A   | 3.92  | 9.94 | Nil | 1.99  | 0.89  | 39.28 | 22.47 | 9.02 | 5.04 | 6.25 |
| KTYM B   | 10.11 | 4.5  | Nil | 3.27  | 0.25  | 32.80 | 29.33 | 9.33 | 4.11 | 7.22 |
| IDUKKI A | 14.61 | 4.42 | Nil | 1.54  | 0.09  | 42.48 | 20.47 | 9.17 | 6.04 | 8.13 |
| IDUKKI B | 8.12  | 4.06 | Nil | 2.55  | 1.952 | 39.05 | 27.43 | 9.62 | 4.31 | 6.10 |
| EKM A    | 8.37  | 9.08 | Nil | 5.6   | 2.763 | 40.27 | 38.55 | 8.81 | 3.09 | 5.82 |
| EKM B    | 6.08  | 14.9 | Nil | 5.17  | 2.76  | 41.52 | 32.14 | 8.74 | 3.41 | 4.99 |
| THSR A   | 14.06 | 14.9 | Nil | 2.34  | 0.785 | 39.14 | 22.51 | 8.62 | 4.24 | 5.32 |
| THSR B   | 19.37 | 14.9 | Nil | 3.28  | 0.048 | 34.02 | 27.11 | 9.16 | 3.05 | 5.11 |
| PKD A    | 9.62  | 9.92 | Nil | 3.06  | 0.795 | 38.62 | 37.21 | 8.59 | 5.09 | 6.80 |
| PKD B    | 11.55 | 14.6 | Nil | 1.69  | 0.538 | 32.55 | 35.51 | 9.12 | 2.52 | 5.91 |
| MLP A    | 9.30  | 4.93 | Nil | 6.578 | 4.55  | 41.70 | 28.30 | 9.30 | 3.22 | 6.22 |
| MLP B    | 12.85 | 4.41 | Nil | 3.31  | 2.27  | 42.77 | 30.86 | 9.55 | 4.61 | 7.05 |
| KZKD A   | 17.03 | 4.09 | Nil | 2.34  | 3.98  | 47.00 | 23.20 | 8.62 | 2.22 | 4.51 |
| KZKD B   | 14.27 | 9.38 | Nil | 1.67  | 1.066 | 46.88 | 32.21 | 9.21 | 3.90 | 5.76 |
| WYND A   | 11.60 | 4.95 | Nil | 2.43  | 1.79  | 42.2  | 21.61 | 8.21 | 5.81 | 7.23 |
| WYND B   | 7.41  | 4.8  | Nil | 3.861 | 1.731 | 37.01 | 23.51 | 9.22 | 5.52 | 5.52 |
| KANR A   | 18.32 | 14.9 | Nil | 2.88  | 0.099 | 38.01 | 21.18 | 9.46 | 4.27 | 6.23 |
| KANR B   | 12.53 | 9.82 | Nil | 6.44  | 1.671 | 35.13 | 22.64 | 9.06 | 3.91 | 6.01 |
| KSGD A   | 18.14 | 14.3 | Nil | 7.74  | 4.568 | 48.12 | 32.13 | 9.45 | 3.61 | 5.72 |
| KSGD B   | 12.53 | 9.19 | Nil | 1.04  | 0.841 | 34.67 | 31.58 | 8.66 | 2.26 | 4.41 |

TVM- Trivandrum, KLM- Kollam, PTA- Pathanamthitta, ALP-Alappuzha, KTYM- Kottayam, EKM-Ernakulam, THSR- Thrissur, PKD- Palakkad, MLP- Malappuram, KZKD- Kozhikode, WYND - Wayanad, KANR - Kannur, KSGD -Kasarkode; FM- Foreign Matter, TA- Total Ash, AIA- Acid Insoluble Ash, MC- Moisture Content, WSE- Water soluble Extractive, ASE- Alcohol Soluble Extractive, FC- Fibre Content, RS- Reducing Sugar and TS-Total Sugar.

#### Qualitative analysis:-

The results of qualitative analysis are shown in table 3.

**Table 3:-** Qualitative analysis of genuine and market samples of dried *Amalaki* (*P. emblica* L.) fruit rind.

| Samples  | Steroid | Flavonoid | Alkaloid | Tannin | Saponin | Phenol |
|----------|---------|-----------|----------|--------|---------|--------|
| Genuine  | +       | +++       | +        | +++    | -       | ++     |
| TVM A    | +       | +         | +        | +++    | -       | ++     |
| TVM B    | +       | +         | +        | +++    | -       | ++     |
| KLM A    | +       | +         | +        | +++    | -       | ++     |
| KLM B    | +       | +         | +        | +++    | -       | ++     |
| PTA A    | +       | +         | +        | +++    | -       | ++     |
| PTA B    | +       | +         | +        | +++    | -       | ++     |
| ALP A    | +       | ++        | +        | +++    | -       | ++     |
| ALP B    | +       | +         | +        | +++    | -       | ++     |
| KTYM A   | +       | +         | +        | +++    | -       | ++     |
| KTYM B   | +       | +         | +        | +++    | -       | ++     |
| IDUKKI A | +       | +         | +        | +++    | -       | ++     |
| IDUKKI B | +       | +         | +        | +++    | -       | ++     |
| EKM A    | +       | +         | +        | +++    | -       | ++     |
| EKM B    | +       | +         | +        | +++    | -       | ++     |
| THSR A   | +       | +         | +        | +++    | -       | ++     |
| THSR B   | +       | +         | +        | +++    | -       | ++     |
| PKD A    | +       | +         | +        | +++    | -       | ++     |

|        |   |   |   |     |   |    |
|--------|---|---|---|-----|---|----|
| PKD B  | + | + | + | +++ | - | ++ |
| MLP A  | + | + | + | +++ | - | ++ |
| MLP B  | + | + | + | +++ | - | ++ |
| KZKD A | + | + | + | +++ | - | ++ |
| KZKD B | + | + | + | +++ | - | ++ |
| WYND A | + | + | + | +++ | - | ++ |
| WYND B | + | + | + | +++ | - | ++ |
| KANR A | + | + | + | +++ | - | ++ |
| KANR B | + | + | + | +++ | - | ++ |
| KSGD A | + | + | + | +++ | - | ++ |
| KSGD B | + | + | + | +++ | - | ++ |

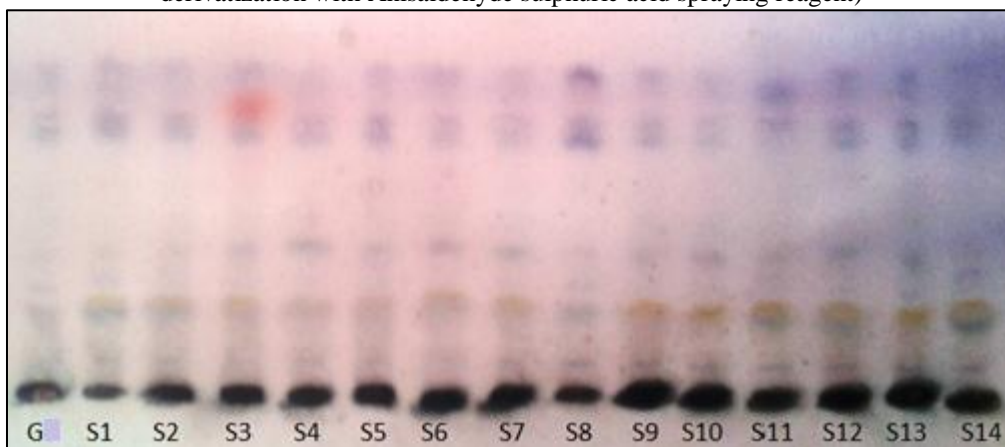
+++ (Appreciable amount), ++ (Moderate amount), + (Traces)

### Chromatographic screening:-

#### Thin layer chromatography:-

In TLC, maximum numbers of spots were obtained after derivatization and are shown in figure.1. TLC results of spot detection and Rf values are represented in table 4.

**Fig 1:-** TLC profile of ethanolic extracts of genuine and market samples of dried *Amalaki* fruit rind (after derivatization with Anisaldehyde sulphuric acid spraying reagent)



G-Genuine, S<sub>1</sub>-Thiruvananthapuram, S<sub>2</sub>- Kollam, S<sub>3</sub>- Pathanamthitta, S<sub>4</sub>- Alappuzha, S<sub>5</sub>- Kottayam, S<sub>6</sub>- Idukki, S<sub>7</sub>- Ernakulam, S<sub>8</sub>-Thrissur, S<sub>9</sub>- Palakkad, S<sub>10</sub>- Malappuram, S<sub>11</sub>- Kozhikode, S<sub>12</sub>-Wayanad, S<sub>13</sub>-Kannur, S<sub>14</sub>-Kasargod samples

**Table 4:-**Spots and Rf values in TLC of genuine and market samples of *Amalaki* samples.

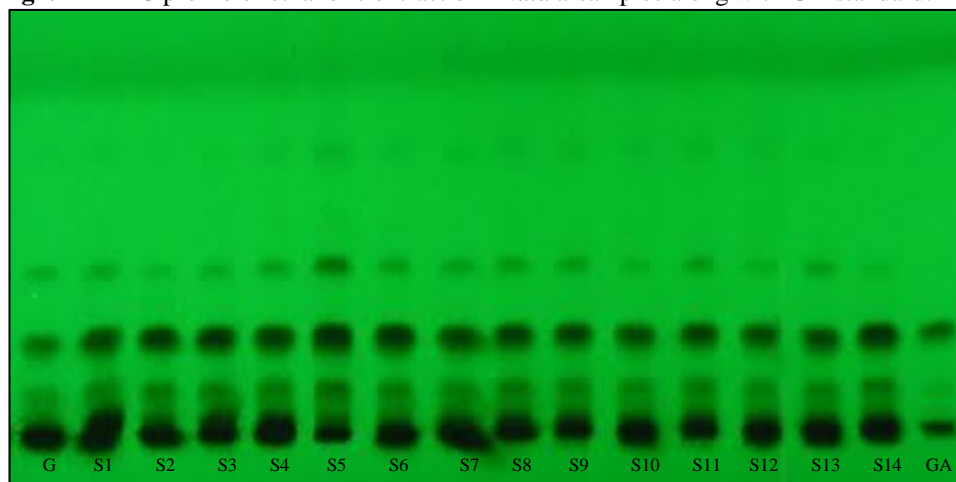
| Alcoholic Extract of samples | Spot detection | No of spots          | Rf value |      |
|------------------------------|----------------|----------------------|----------|------|
| Genuine sample               | UV(254nm)      | 2                    | 0.75     |      |
|                              |                |                      | 0.82     |      |
|                              | Iodine         | 2                    | 0.53     |      |
|                              |                | After derivatization | 5        | 0.11 |
|                              |                |                      |          | 0.18 |
|                              |                | 0.31                 |          |      |
|                              |                |                      | 0.57     |      |
|                              |                |                      | 0.62     |      |
| Market samples of group 1    | UV(254nm)      | 2                    | 0.75     |      |
|                              |                |                      | 0.82     |      |
|                              | Iodine         | 2                    | 0.53     |      |
|                              |                |                      | 0.68     |      |

|                           |                      |   |                                      |
|---------------------------|----------------------|---|--------------------------------------|
|                           | After derivatization | 5 | 0.11<br>0.18<br>0.31<br>0.57<br>0.62 |
| Market samples of group 2 | UV(254nm)            | 2 | 0.75<br>0.82                         |
|                           | Iodine               | 2 | 0.53<br>0.68                         |
|                           | After derivatization | 5 | 0.11<br>0.18<br>0.31<br>0.57<br>0.62 |

#### High Performance Thin Layer Chromatography:-

In HPTLC analysis, genuine as well as the market samples (A samples) were spotted in track 1 to track 15(G-S<sub>14</sub>). To find out the presence of the marker compound- GA in the samples, GA standard was also spotted in the 16<sup>th</sup> track with mobile phase as Toluene: Ethyl acetate: Formic acid (6: 3:1). The spots were visualized under UV (258nm). The number of peaks as well as the Rf values were noted. HPTLC profile, 3D display and peak tables are shown in figures 2-18.

**Fig2:-** HPTLC profile of ethanolic extract of *Amalaki* samples along with GA standard.



**G**-Genuine, **S<sub>1</sub>**-Thiruvananthapuram, **S<sub>2</sub>**- Kollam, **S<sub>3</sub>**- Pathanamthitta, **S<sub>4</sub>**- Alappuzha, **S<sub>5</sub>**- Kottayam, **S<sub>6</sub>**- Idukki, **S<sub>7</sub>**- Ernakulam, **S<sub>8</sub>**-Thrissur, **S<sub>9</sub>**- Palakkad, **S<sub>10</sub>**- Malappuram, **S<sub>11</sub>**- Kozhikode, **S<sub>12</sub>**-Wayanad, **S<sub>13</sub>** -Kannur, **S<sub>14</sub>**-Kasargod samples **GA**-Gallic acid standard

Fig 3:- 3D display of HPTLC peaks of Amalaki samples along with GA standard.

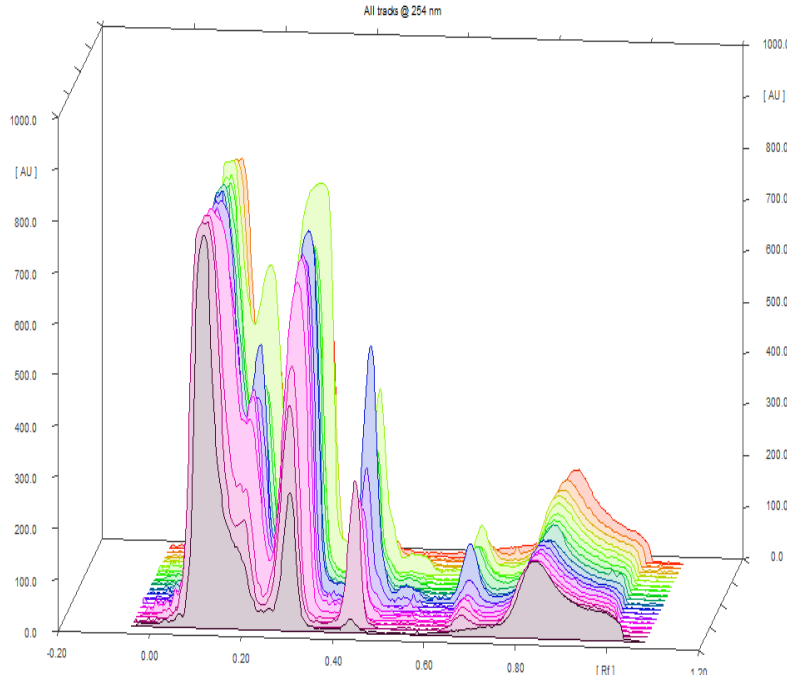
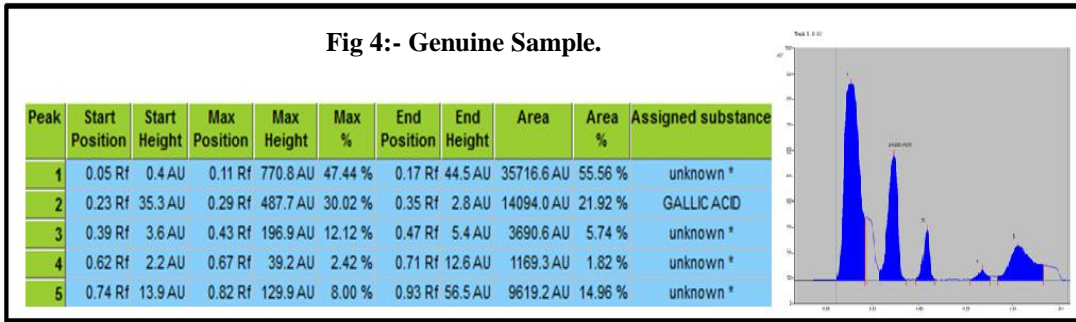
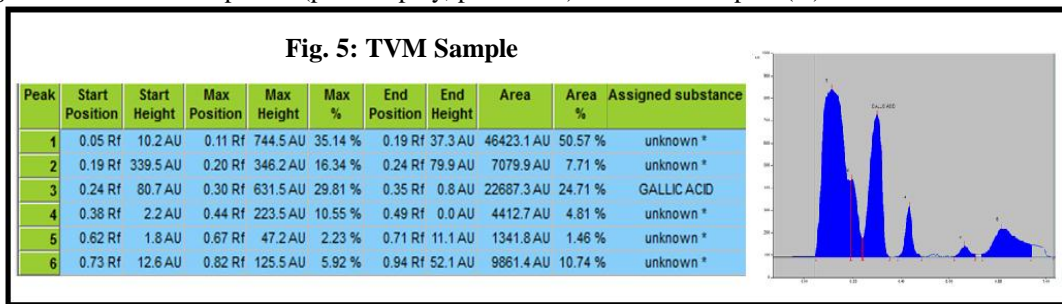


Fig.4:-HPTLC profile (peak display, peak table) of genuine sample



Figures 5-18:- HPTLC profile (peak display, peak table) of market samples (A) from 14 districts of Kerala.



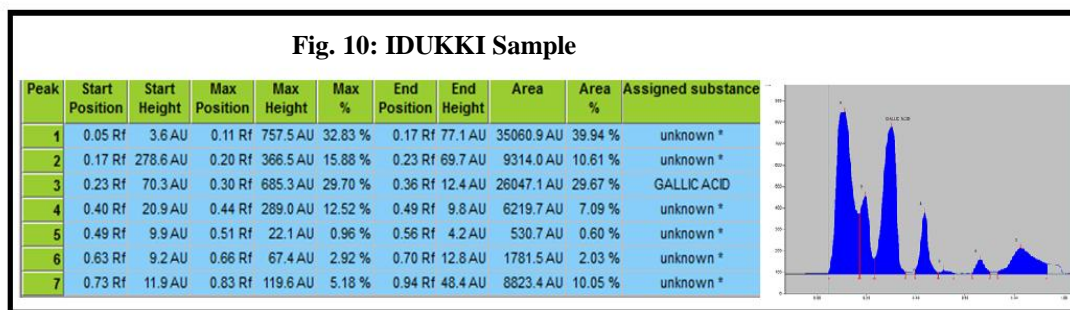
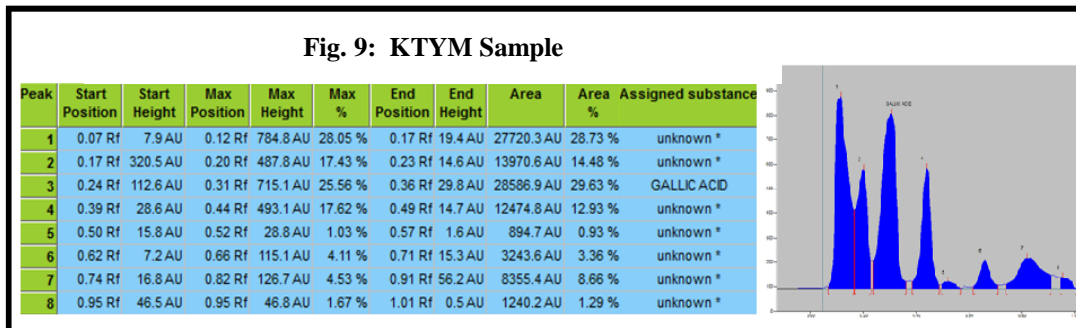
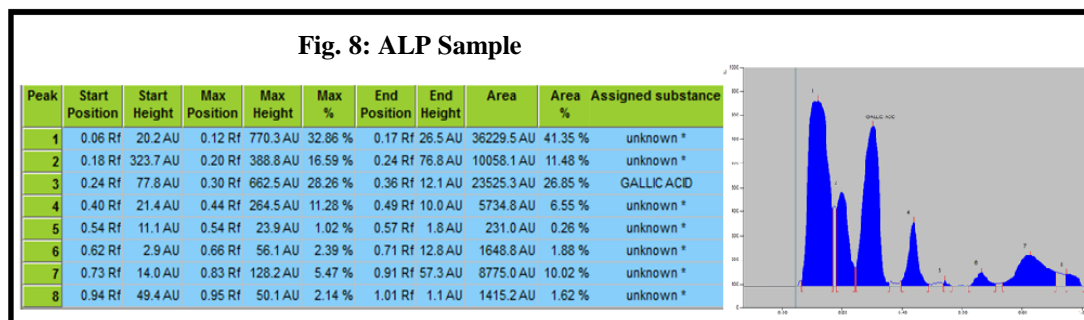
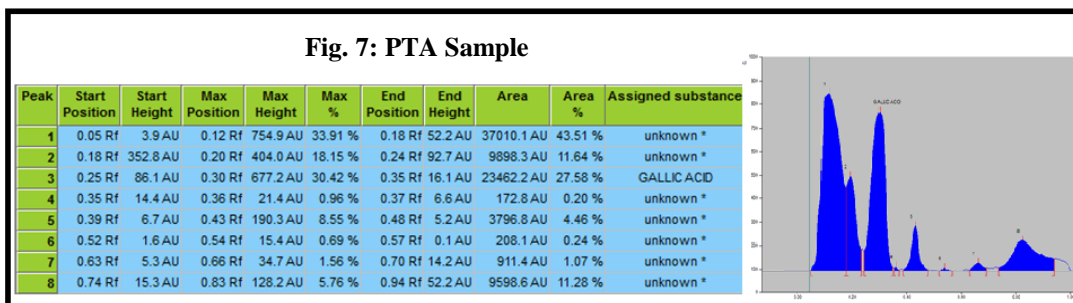
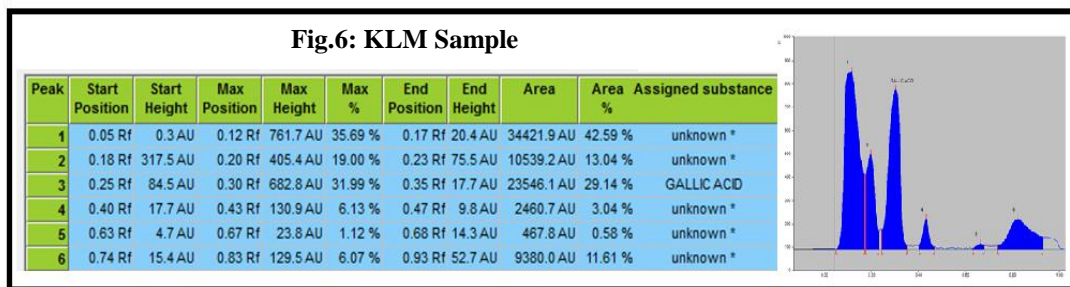




Fig.11: EKM Sample

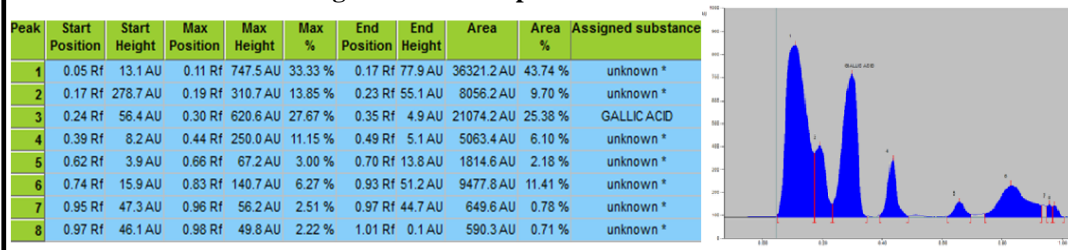


Fig.12: THSR Sample

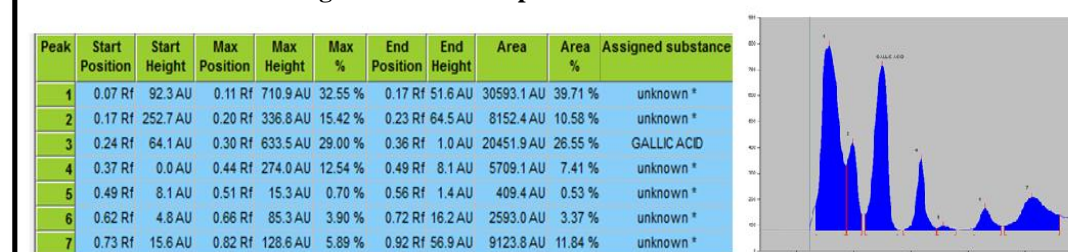


Fig. 13: PKD Sample

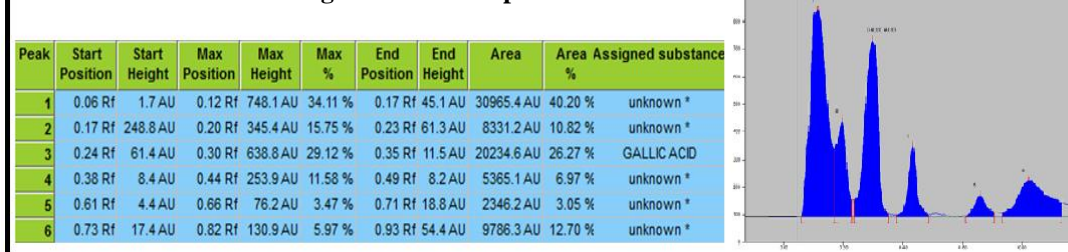
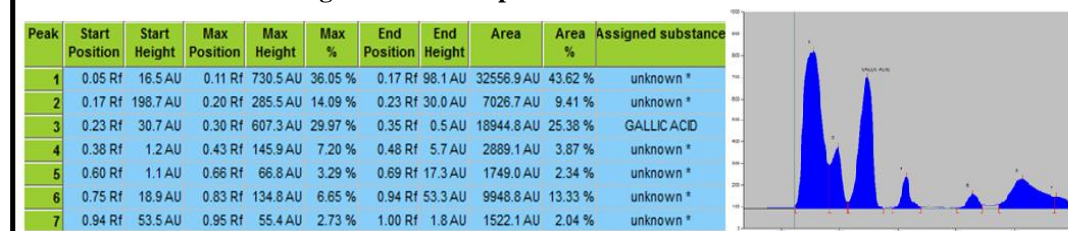
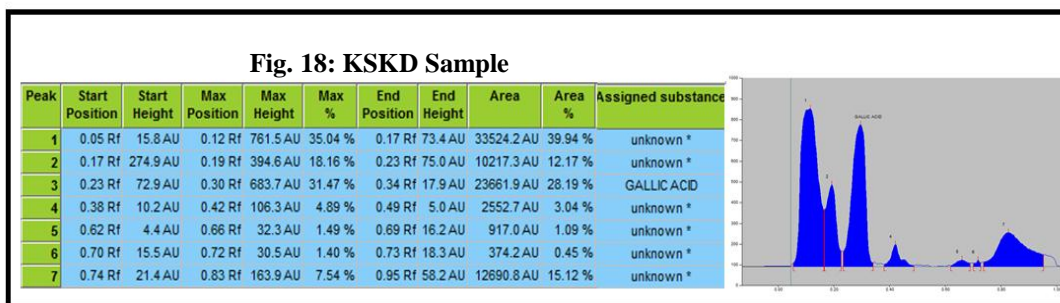
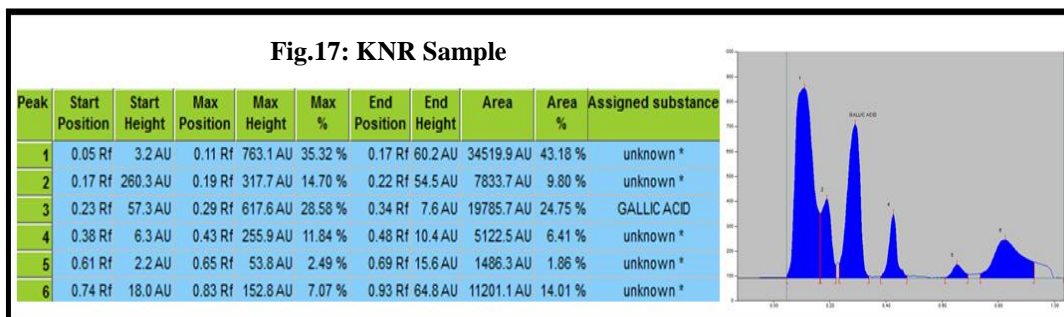
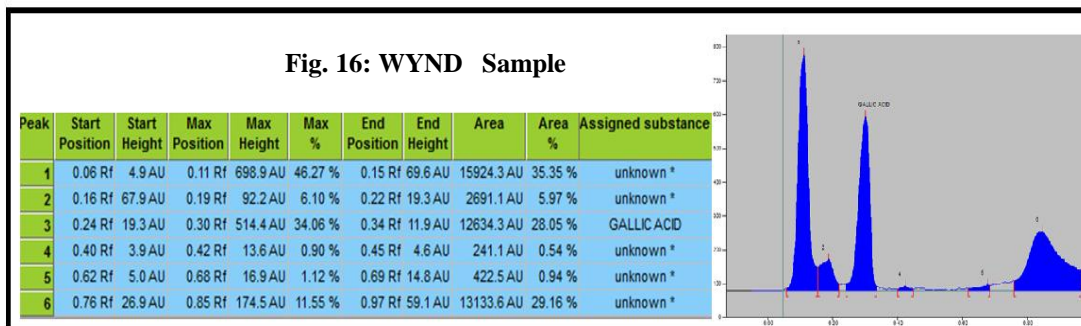
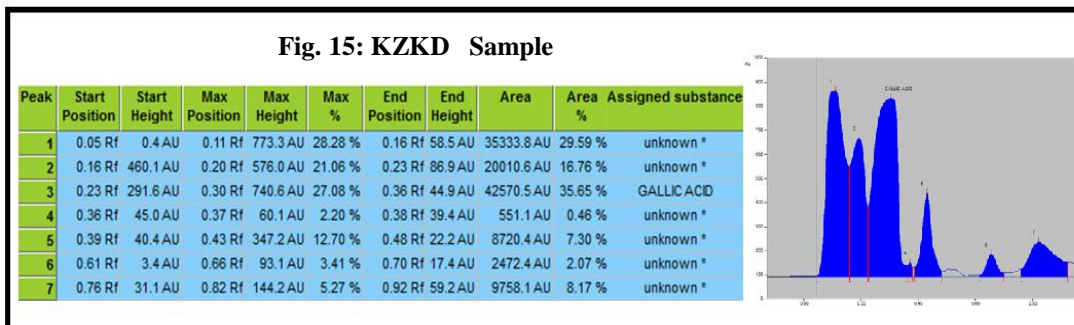


Fig.14: MLP Sample





**Heavy Metal Analysis:-**

Heavy metal content was screened in the market samples as well as in the dried samples of genuine *Amalaki* (*P. emblica* L.) fruit rind. The results are presented in table 5

**Table 5:-** Heavy metal content in genuine and all market samples of dried *Amalaki* fruit rind.

| Samples  | Heavy metal Concentration in ppm |        |        |        |        |        |
|----------|----------------------------------|--------|--------|--------|--------|--------|
|          | Cadmium                          | Zinc   | Lead   | Nickel | Iron   | Copper |
| Genuine  | 0.0012                           | 0.0013 | 0.0342 | 0.0024 | 1.9431 | 0.0015 |
| TVM A    | 0.0009                           | 0.0061 | 0.0561 | 0.0052 | 0.9913 | 0.0001 |
| TVM B    | 0.0020                           | 0.0032 | 0.0021 | 0.0024 | 0.5244 | 0.0011 |
| KLM A    | 0.0011                           | 0.0051 | 0.0030 | 0.0033 | 1.2281 | 0.0008 |
| KLM B    | 0.0021                           | 0.0062 | 0.0012 | 0.0019 | 0.5226 | 0.0051 |
| PTA A    | 0.0003                           | 0.0145 | 0.0041 | 0.0038 | 1.8381 | 0.0013 |
| PTA B    | 0.0054                           | 0.0081 | 0.0214 | 0.0019 | 0.4917 | 0.0044 |
| ALP A    | 0.0021                           | 0.0321 | 0.0104 | 0.0041 | 1.7148 | 0.0016 |
| ALP B    | 0.0023                           | 0.0171 | 0.0211 | 0.0068 | 0.9155 | 0.0031 |
| KTYM A   | 0.0013                           | 0.0072 | 0.0024 | 0.0051 | 0.8442 | 0.0020 |
| KTYM B   | 0.0024                           | 0.0051 | 0.0014 | 0.0033 | 1.0429 | 0.0018 |
| IDUKKI A | 0.0021                           | 0.0011 | 0.0081 | 0.0311 | 0.7826 | 0.0014 |
| IDUKKI B | 0.0032                           | 0.0016 | 0.0024 | 0.0412 | 0.4144 | 0.0072 |
| EKM A    | 0.0004                           | 0.0014 | 0.0462 | 0.0049 | 0.6426 | 0.0006 |
| EKM B    | 0.0011                           | 0.0021 | 0.0112 | 0.0010 | 0.5153 | 0.0015 |
| THSR A   | 0.0014                           | 0.0014 | 0.0056 | 0.0042 | 1.0185 | 0.0011 |
| THSR B   | 0.0009                           | 0.0022 | 0.0048 | 0.0031 | 0.8155 | 0.0011 |
| PKD A    | 0.0008                           | 0.0054 | 0.0083 | 0.0028 | 1.3134 | 0.0016 |
| PKD B    | 0.0010                           | 0.0022 | 0.0024 | 0.0018 | 0.7127 | 0.0011 |
| MLP A    | 0.0054                           | 0.0031 | 0.0531 | 0.0044 | 0.5144 | 0.0021 |
| MLP B    | 0.0003                           | 0.0024 | 0.0511 | 0.0022 | 1.0164 | 0.0071 |
| KZKD A   | 0.0022                           | 0.0022 | 0.0082 | 0.0036 | 0.9211 | 0.0015 |
| KZKD B   | 0.0026                           | 0.0024 | 0.0044 | 0.0015 | 0.6117 | 0.0026 |
| WYND A   | 0.0047                           | 0.0012 | 0.0741 | 0.0032 | 0.8328 | 0.0012 |
| WYND B   | 0.0004                           | 0.0041 | 0.0150 | 0.0011 | 1.0204 | 0.0031 |
| KANR A   | 0.0006                           | 0.0018 | 0.0841 | 0.0017 | 0.3421 | 0.0008 |
| KANR B   | 0.0009                           | 0.0017 | 0.0419 | 0.0024 | 0.5117 | 0.0010 |
| KSGD A   | 0.0015                           | 0.0011 | 0.0472 | 0.0128 | 0.5143 | 0.0018 |
| KSGD B   | 0.0021                           | 0.0014 | 0.0018 | 0.0016 | 0.4188 | 0.0013 |

**Statistical analysis:-**

Statistical comparison of physico-chemical parameters of genuine and market samples with one sample t test was done and it was significant at  $p < 0.01$  except for total ash content.

**Discussion:-**

The present study assessed the genuineness and quality of dried *Amalaki* fruit rind (*P. emblica* L.) available in the herbal raw drug market in Kerala with the aid of various physical and phytochemical evaluations. The implications made from the results are discussed here. Both fresh and dried fruit of *P. emblica* L. are mentioned as the officinal part of drug *Amalaki* in API. The dried *Amalaki* fruit rind is widely used in many Ayurvedic formulations such as Amruthotharam kashayam, Triphala choornam to name a few. In order to assess the genuinity and quality of the dried *Amalaki* fruit rind available in the raw drug market in Kerala, market samples were collected and were compared with genuine dried fruit of *P. emblica* L.

The drug, *Amalaki* fruit rind was sold in the name of “*Nellikathodu* or *Kattunellika*” in various raw drug market in Kerala. The price of raw drug purchased varied from market to market. The market price of the dried fruit rind varied from 120 to 240 per kilogram. Surveys have indicated that the price of a raw drug depends on the quality and extent of adulteration; the relationship between demand and supply also determine the day to day variation in price. The status and reputation of the trader and buyer, mode of payment (cash/credit) and quantity ordered also determine the price of the drug (ENVIS FRLHT, 2015).

On physical and phytochemical evaluation, the mean value of foreign matter, moisture content and acid insoluble ash in market samples was higher than that of genuine sample. Whereas the mean value of the alcohol soluble extractive, water soluble extractive, fibre content, total sugar, reducing sugar were lower than that of genuine sample. But the mean value of the total ash content was more or less same in the market samples and genuine sample. The variation in physico-chemical and phytochemical results may be due to several factors such as different geographical conditions, edaphic factors, environmental conditions, period of cultivation, harvesting, method of collection, source of irrigation and fertilizers, age of the plant, powdering method (Santhosh *et al.*, 2005).

AS per API, seed and endocarp are considered as the foreign matter. In the present study, market samples had many broken pieces of endocarp seen separately and adhered with the cut pieces of fruit rind. Presence of impurities like mud, stones, mould, insects etc were noted in the samples. All these observations reveal the unhygienic and careless processing, transportation and prolonged storage of the material. The increased value of foreign matter implies reduced purity of raw drugs. Herbal drugs should compose of only the officinal part of the plant and be devoid of other parts of the same plant or other plants. They should be entirely free from moulds or insects, including excreta and visible contaminant such as sand and stones, poisonous and harmful foreign matter and chemical residues. Animal matter such as insects and “invisible” microbial contaminants, which can produce toxins, are also among the potential contaminants of all medicines (Folashade *et al.*, 2012). Such drugs should be rejected even though the percentage of other foreign matter is less in them.

The mean value of the moisture content in the market samples was significantly high (9.20%) in comparison with the genuine sample (4.5%). Moisture is one of the major factors responsible for the deterioration of drugs and products. Low moisture content is always desirable for higher stability of drugs (Chandel *et al.*, 2011). The reduction in moisture content is not the only desired factor, sometimes moisture laden air may enter inside and damage the material, if it is not stored in the properly sealed container. Presence of high amount of moisture in any drug may facilitate enzyme hydrolysis or enhance the growth of microbes which leads to deterioration (Sarvesh *et al.*, 2014). As *Amalaki* fruit contains more than 80% water content, any improper management during the process of drying and storage may cause retention of moisture. It affects the quality of the raw drug and thus the medicinal preparations.

Total Ash value represents the presence of inorganic salts like calcium oxalate crystals found naturally in the drug as well as inorganic matter derived from external sources like sand. Incineration of herbal drugs produces ash which constitutes inorganic matter. The mean value of total ash content in the genuine sample (3.58%) and the market samples (3.69%) were more or less in the same level.

Treatment of ash with hydrochloric acid results in acid-insoluble ash. During which most of the natural ash is soluble leaving the silica as acid insoluble ash which represents most of the ash from the contaminated soil. Here the mean value of the acid insoluble ash in the market samples (1.71%) was higher than the genuine sample (0.614%) but lower than the API range.

The extractive values play an important role in evaluation of crude drug. Extractive value indicates the nature of chemical constituents present in the drug. Water soluble extractive value is applied for the drugs which contain constituents such as sugars, and mucilage etc. Alcohol soluble extractive value is applied for the drugs which contain alcohol soluble constituents such as tannins, resins and alkaloids. (Kokate *et al.*, 2014). In the present study, the water soluble extractive value is proved to be higher than the alcohol soluble extractive value in genuine as well as the market samples. This shows that it contain more water soluble principles than the alcohol soluble principles.

The mean value of both the alcohol soluble extractive value (28.427%) and water soluble extractive value (37.44%) in market samples were found to be lower than genuine (ASE-41.435%, WSE-59.49%) sample. Less extractive values indicate the presence of exhausted or deteriorated material or incorrect processing or storage.

Fibre content estimates the residue that remains after treatment with acid and alkali. The crude fibre contains cellulose, hemicelluloses, lignin etc. The fibre content of market samples was comparatively lower than genuine sample.

The percentage of sugar values found in different varieties of *Amalaki* showed variations (Teaotia *et al.*, 1968). In the present study, significant decrease of sugar values was observed in the market samples compared to the genuine sample. This may be due to the admixture of different varieties of *Amalaki* in the commercial samples.

Qualitative analysis of genuine and market samples were done to determine the physiologically active chemical constituents. The study has got supreme importance since the therapeutic properties of the drug mainly depends on the chemical constituents. The result of qualitative analysis showed the presence of alkaloids, tannins, flavonoids, steroids, phenols etc. Saponins were absent in genuine and market samples. Alkaloids and steroids were present in fewer amounts in genuine sample and all the market samples.

Flavonoids were present in appreciable amount in genuine sample, but traces in all the market samples except Alappuzha A sample (moderate amount). The prolonged period of storage and the storage conditions of the market samples may be a cause for decreased content of flavonoids. Individuals in different populations exposed to varying environmental conditions usually show variable accumulation of flavonoids (Jakoola *et al.*, 2004). Environmental factors (temperature, precipitation, solar radiation etc.) in populations throughout the species distribution area are often subjected to latitudinal, longitudinal or altitudinal gradients (Narbona *et al.*, 2010). Thus, flavonoid accumulation may show geographical clines. The plant can react to elevated and low temperatures by altering flavonoid synthesis in a species specific way. In general too high temperature can inhibit biosynthesis and cause degradation of flavonoids. A low temperature can increase flavonoid production, although the accumulation of flavonoids in cold temperatures is light dependent. There appears to be some evidence that cooler temperatures favour the production of flavonoids with a higher hydroxylation level (Jaakola and Hohtola, 2010). The flavonoid synthesis also increases in plant during fruiting and flowering (Lattanzio *et al.*, 2006)

Phenol compounds are secreted to improve resistance when plant undergoes any type of external stress or response (Andersen and Markham, 2016). The studies conducted on 2 varieties of *Amalaki* revealed that Chakiya variety had a lower phenolic content as compared to wild variety (Mishra *et al.*, 2009). In the present study moderate amount of phenolic content was observed in all samples.

Studies suggest ecological conditions like insect feeding and microbial infections may affect secondary metabolite and in turn chemical composition of the plant. Also different parts of same plant contain different concentration of chemical constituents. At the same time, diurnal variations and seasonal changes also account for variability in herbal medicines. The therapeutic or toxic components of plants may vary depending on the part of plant used as well as stages of ripening. (Drew and Myers, 1997) Shelf-life also has obvious impact on availability of active principle. All these suggest that the concentration of secondary metabolites in dried *Amalaki* fruit rind could be varied according to the varying environmental conditions especially stress, temperature, geographical variations, physiological stage of the plant, stages of ripening, collection time, method of processing, storage conditions, shelf life etc.

The plant *A m a l a k i* belongs to Euphorbiaceae family and it was reported that Euphorbiaceae is one among 45 families that have been identified to have hyper accumulation of heavy metals. (Ghosh and Singh, 2005). The present study also screened various heavy metals in the genuine and market samples of dried fruit rind of *Amalaki* and it was found to be within the permissible limits.

For further authentication and identification of chemical constituents TLC and HPTLC analysis of genuine sample as well as the market samples were carried out. On HPTLC analysis, the genuine sample had 5 peaks. The number of peaks in the market samples showed variations comparing to the genuine sample (Fig 4-18). Although slight variations were seen in HPTLC profile of *Amalaki* fruit, the similarities suggest the presence of similar chemical constituents. Moreover, Gallic acid-the marker compound present in the fruit of *Amalaki* was found in all the samples. The chemical constituents in a plant may vary depending on the stage of collection, parts of plant collected, harvest seasons, plant origin (regional status), drying processes, storage conditions and other factors (Kamboj, 2012)

### **Conclusion:-**

Quality is the sum of all the factors which contribute directly or indirectly to the safety, efficacy and acceptability of the product. Hence, medicines prepared with impure and contaminated commercial samples of dried *Amalaki* fruit rind, which does not follow the quality standards can never bring the desired pharmacological actions. The present study reveals that the status of commercially available crude drug *Amalaki* fruit rind is having poor quality and purity. So adequate care should be taken in the processing and storage of herbal drugs to avoid contamination and also to get maximum concentration of the active principles. Also care should be taken to harvest *Amalaki* fruit at proper time in order to get complete therapeutic effect. Although, standardization of herbal products is not an easy

task, manufactures must ensure proper testing of raw materials for better product development and apex institutions like AYUSH should prescribe minimum standards for the finished Ayurvedic product.

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### Conflict of Interest:-

The authors have no conflict of interest.

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