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AMALAKI (DRIED POWDER OF *EMBLICA OFFICINALIS* GAERTN) AS FOOD SUPPLEMENT IN DYSLIPIDEMIA - AN ANALYTICAL STUDY

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Abstract

Amalaki (*Emblica officinalis* Gaertn), the super fruit, is well known for its anti-aging, rejuvenating, hypolipidemic, antioxidant, immunomodulatory, anti-inflammatory pharmacological activities. Also, it is the richest source of vitamin C. The active constituent of Amalaki (*Emblica officinalis* Gaertn), is Emblicanin which is different from most other antioxidants as it is a pro-oxidation free cascading antioxidant. This study was aimed at standardization of Amalaki choorna (dried powder of *Emblica officinalis* Gaertn) as food supplement keeping in view of its active constituents responsible for its hypolipidemic action. The powder microscopic study, Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC), Fingerprinting and Densitogram profiling revealed that the test drug Amalaki choorna was as per the standards mentioned as per the Ayurvedic Pharmacopoeial drugs. The powder microscopy showed all the features of dried fruit of *Emblica officinalis*. In HPTLC fingerprinting profile, the R_f value 0.41 Light green under 254 nm is as per the standards indicating presence of Gallic acid, which is a product on hydrolysis of Emblicanin A; and of the spot at 0.36 (0.32±5) is nearest value to the standard R_f value corresponds to ascorbic acid. The results obtained suggest that the study drug Amalaki Choorna can be used as food supplement in dyslipidemia.

Key words : Amalaki (*Emblica officinalis* Gaertn), gallic acid, ascorbic acid, dyslipidemia, TLC, HPTLC.

Introduction

Functional foods and dietary supplements are the two important words commonly witnessed in the branch of nutrition. Ayurveda, an evidence based science, highlights preserving health of healthy and treating the sick. It has a vast description on the concepts and principles of nutrition. It also describes all classes of foods in their raw and processed forms. Amalaki (*Emblica officinalis* Garten) is among the fruits to be consumed on daily basis (Agnivesa, 2010). It can be consumed any time before, during or after the meal (Sushruta, 1997). It is considered the best anti-aging fruit/drug (Vayasthapaka) for all (Sushruta, 2010). Many animal experiments have proved it as an efficient antidyslipidemic/antihyperlipidemic and antioxidant (Takako *et al*., 2007; Anila & Vijayalakshmi, 2002; Mishra, Pathak & Khan, 1981).

The major chemichal constituents of Amalaki (*Emblica officinalis* Garten) are ascorbic acid gallic acid chebulinic acid, chebulagic acid, ellagic acid, 3-ethyl gallic acid, corilagin, emblicanin A and B, Punigluconin, quercetin (Neeraj & Madhu, 2010). The fruits of Amalaki (*Emblica officinalis* Garten) are rich in tannins. The fruits have 28% of the total tannins distributed in the whole plant. The fruit contains two hydrolysable tannins Emblicanin A and B, which have antioxidant properties, one on hydrolysis gives gallic acid, ellagic acid and glucose wherein the other gives ellagic acid and glucose. The fruit also contains Phyllemblin. The active principles are tannins and Gallic acid (Research centre-Natural Remedies, 2009). The antioxidants perform free radical scavenging activity to prevent the oxidative stress leading to dyslipidemia. This study of standardizing Amlaki Choorna (dried fruit powder of *Emblica officinalis* Garten) was a part of a clinical study "A Study on the Effect of Amalaki as Food Supplement in Dyslipidemia". This study was an effort to evaluate the constituents responsible for the antidyslipidemic action of Amalaki

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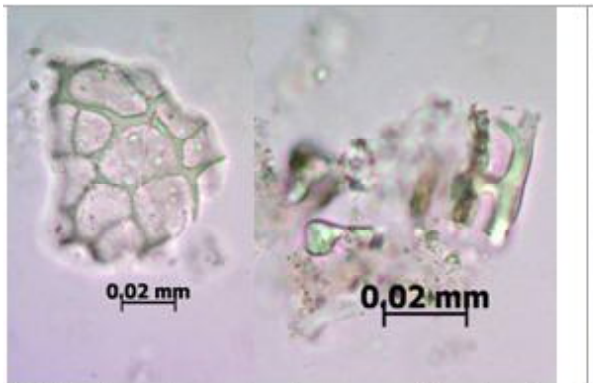
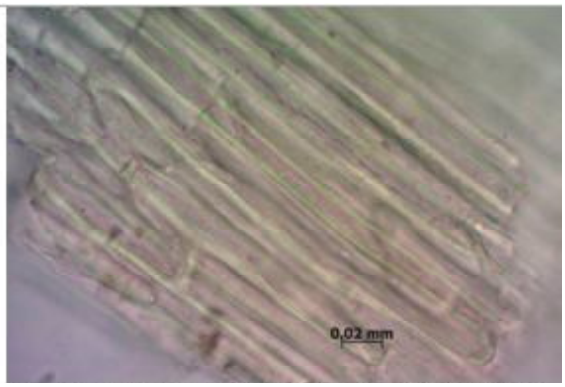
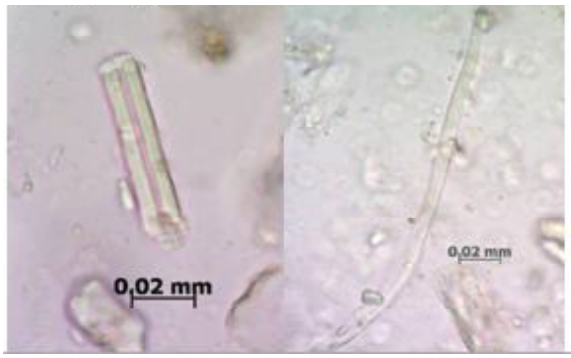


1.1. Epicarp in surface transversely cut view



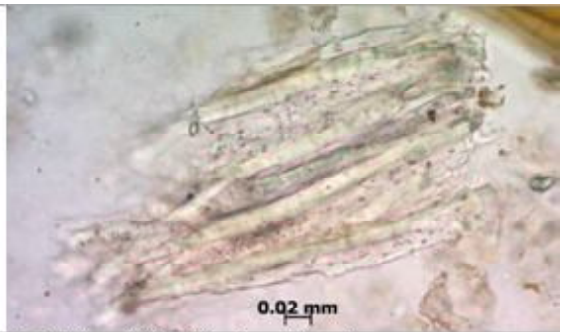
1.2. Longitudinally cut mesocarp parenchyma



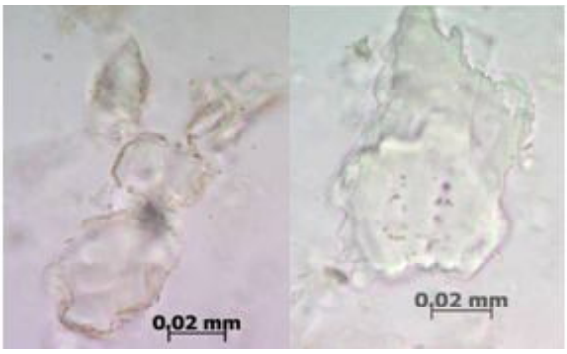
1.3. A fibre and its fragment

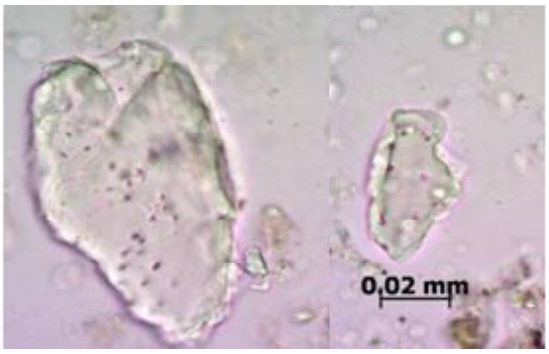
1.4. Sclereidal fibre

1.5. Bundle of xylem elements



1.6. Pitted lignified cells of mesocarp



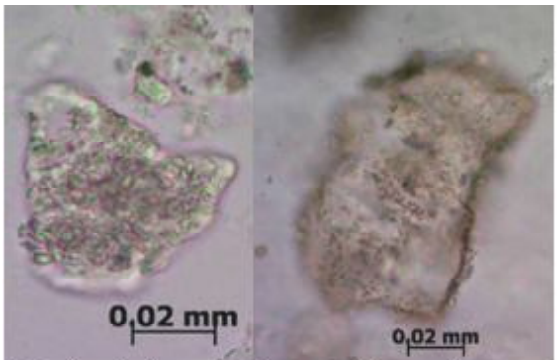


1.7. Pitted mesocarp parenchyma

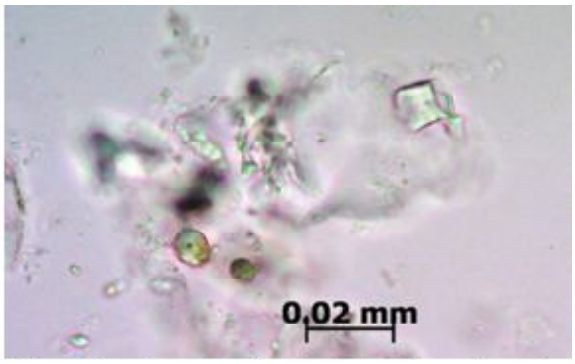
Fig. 1 : Microscopy of Amalaki Choorna.

Fig. 1 continued....

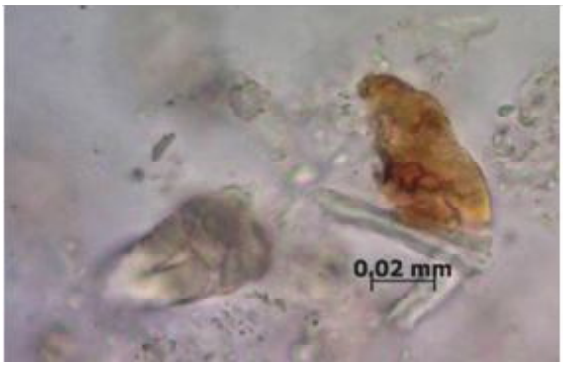
Fig. 1 continued....



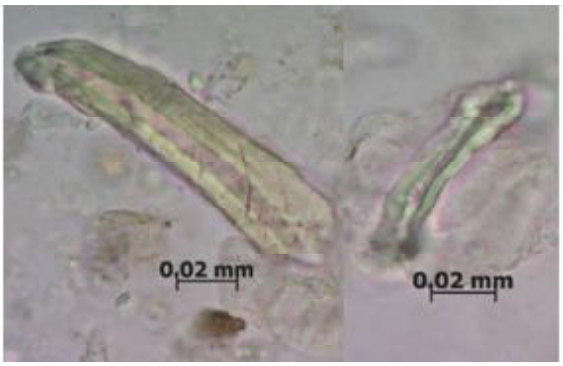
1.8. Cortical parenchyma with starch



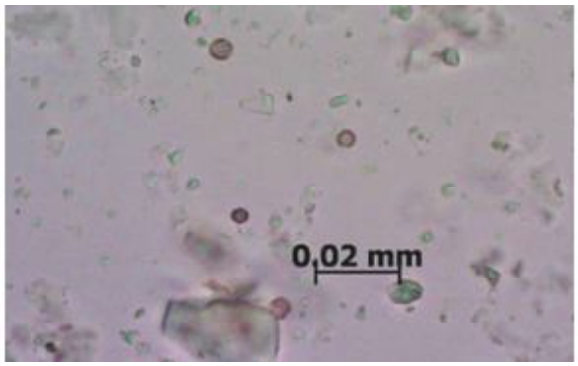
1.9. Simple starch grain and prismatic crystals



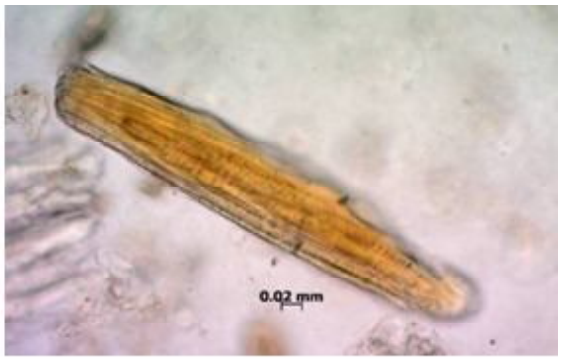
1.10. Fragment of stone cell, fibre and tannin cell



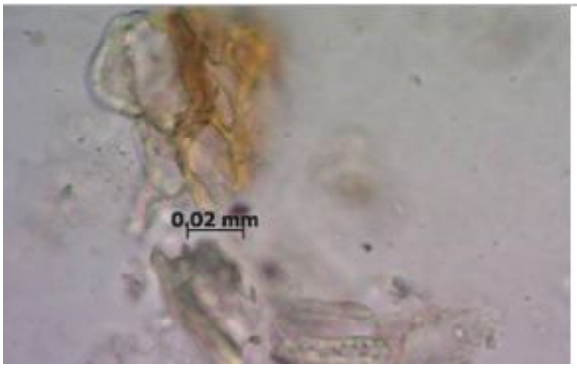
1.11. Sclereidal fibre



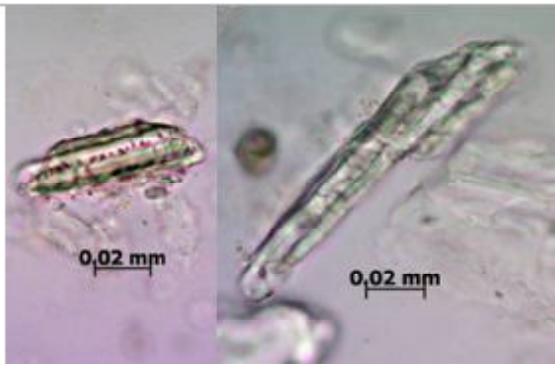
1.12. Oil drop lets



1.13. Sclereidal fibres of endocarp



1.14. Mesocarp parenchyma with tannin

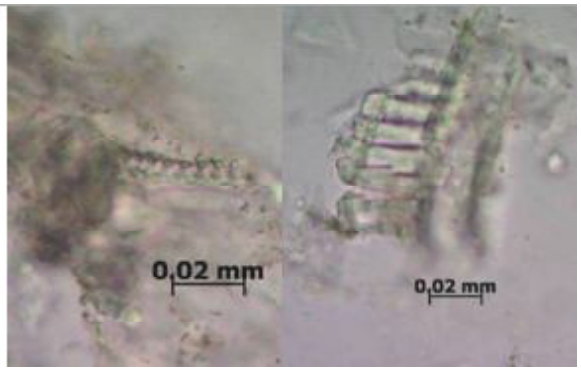


1.15. Fragmnet of sclereidal fibres

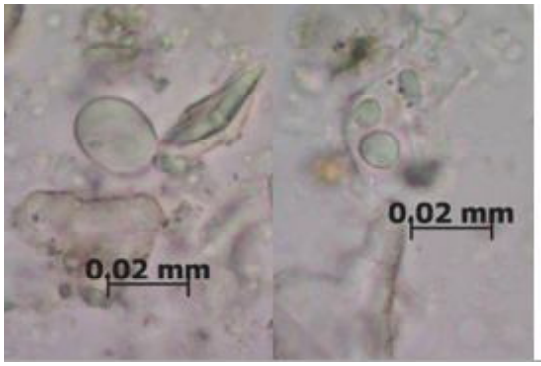
Fig. 1 continued....

Fig. 1 continued....

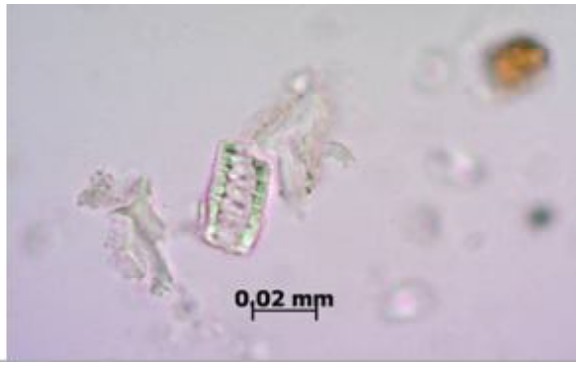
1.16. Tannin cells and starch



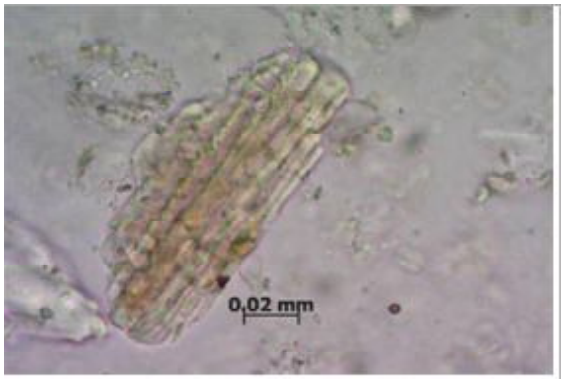
1.17. Fragment of spiral and scalariform vessel



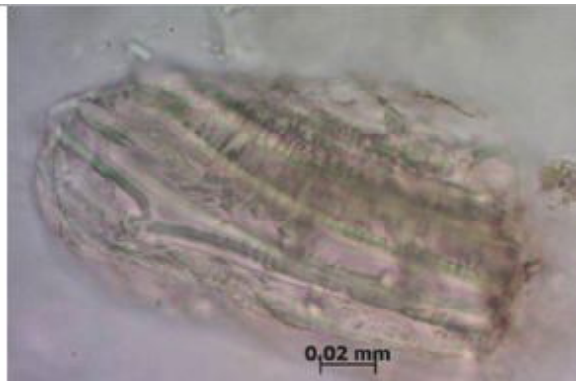
1.18. Starch grains



1.19. Fragment of sclereidal fibre

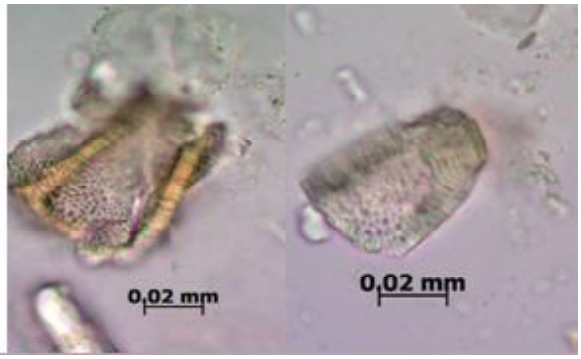


1.20. Phloem parenchyma



1.21. Sclereidal fibre group

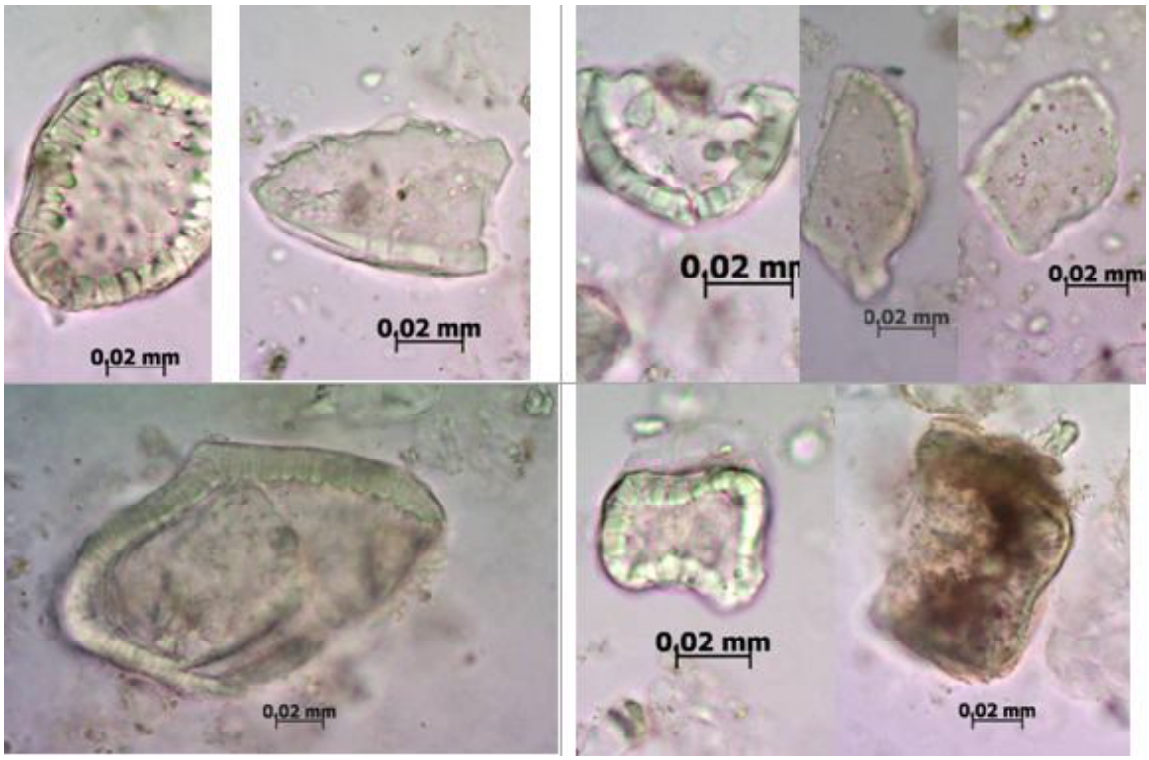
1.22. Sclereid



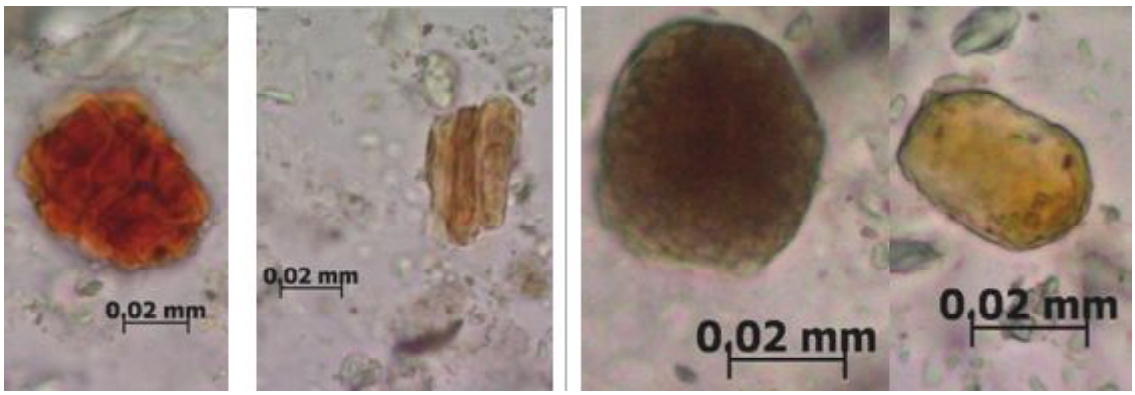
1.23. Sclereids of the stony endocarp

Fig. 1 continued....

Fig. 1 continued....



1.24. Different types of stone cells



1.25. Tannin cells.

Fig. 1 : Microscopy of Amalaki Choorna.

Choorna (dried fruit powder of *Emblica officinalis* Garten).

Materials and Methods

Raw drug collection

The fruit of Amalaki (*Emblica officinalis* Garten) was obtained from a local cultivator in Hassan district of Karnataka State in India and authenticated at Department of Dravyaguna, S. D. M. College of Ayurveda and Hospital, Hassan (Karnataka State), India.

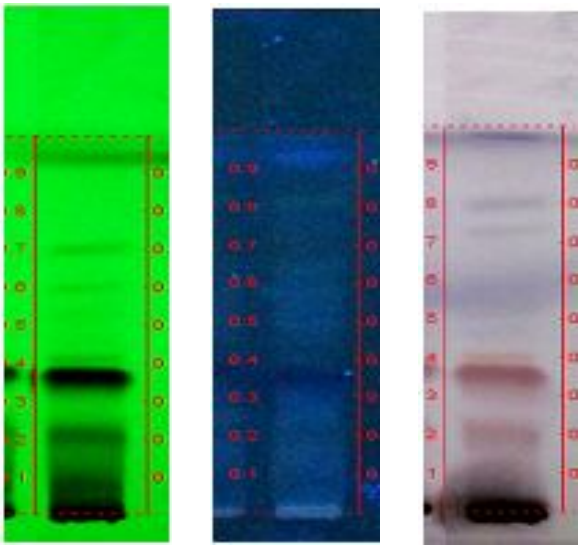
Preperation of Amalaki choorna

The mature fruits of Amalaki (*Emblica officinalis* Garten) bought were cleaned and deseeded initially and later dried under sun. Then the dried Amalaki was powdered and preserved in air tight container.

Instrumentation and techniques

Powder microscopic study and High Performance Thin Layer Chromatography (HPTLC) studies were done at SDM Centre for Research in Ayurveda and Allied Sciences, Kuthpady, Udupi, Karnataka, India as per standard procedure. Following techniques and methods were adopted for the study :

Powder microscopy : Minimum quantity of powder



At UV 254 nm At UV 366 nm Post derivatisation

Fig. 2 : TLC Photodocumentation of Amalaki Choorna.

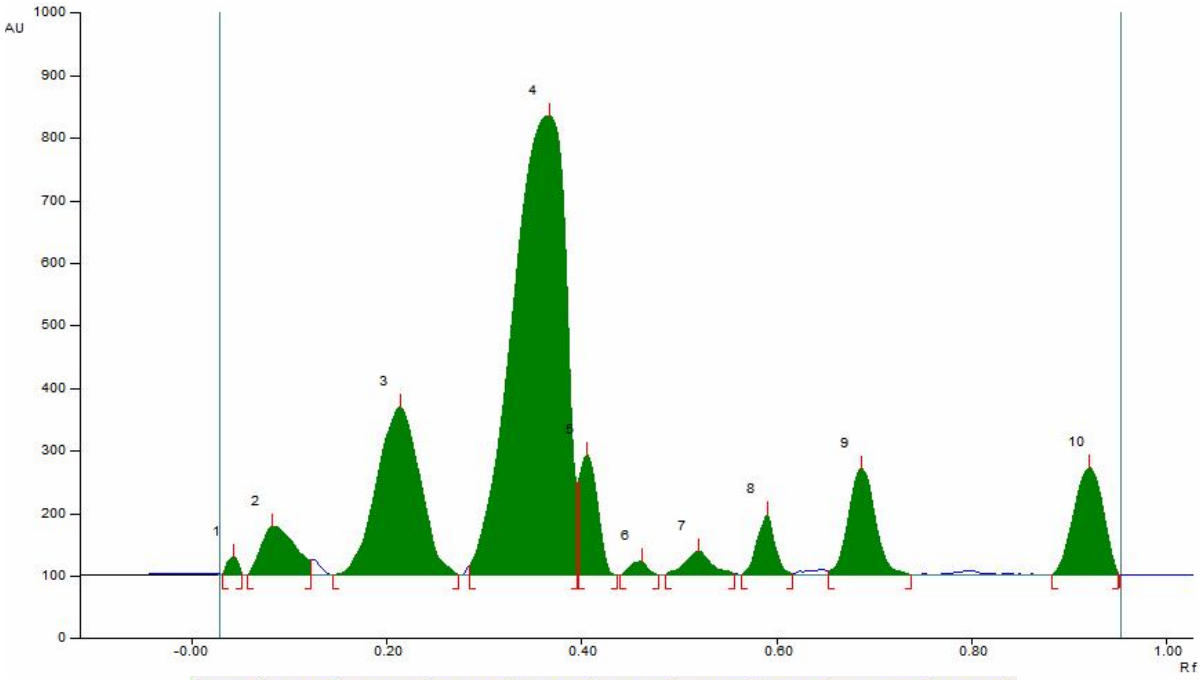
was mounted in a microscopic slide, cleared with chloral hydrate, characters were observed under trinocular microscope (Zeiss AXIO).

Extraction : One gram of powder was dissolved in 5 ml of water and extracted with 10 ml of *n*-butanol. The soluble portion was evaporated to dryness and the residue was dissolved in 5 ml of alcohol.

HPTLC : 15 and 30 µl of the above extract was applied on a precoated silica gel F254 on aluminum plates to a band width of 8 mm using Linomat 5 TLC applicator. The plate was developed in Toluene : Ethyl acetate: Formic acid (1 : 0.7 : 0.1). The developed plates were visualized and scanned under UV 254, 366 and after derivatisation in vanillin-sulphuric acid spray reagent at 620 nm. R_f, colour of the spots and densitometric scan were recorded.

Results and Discussion

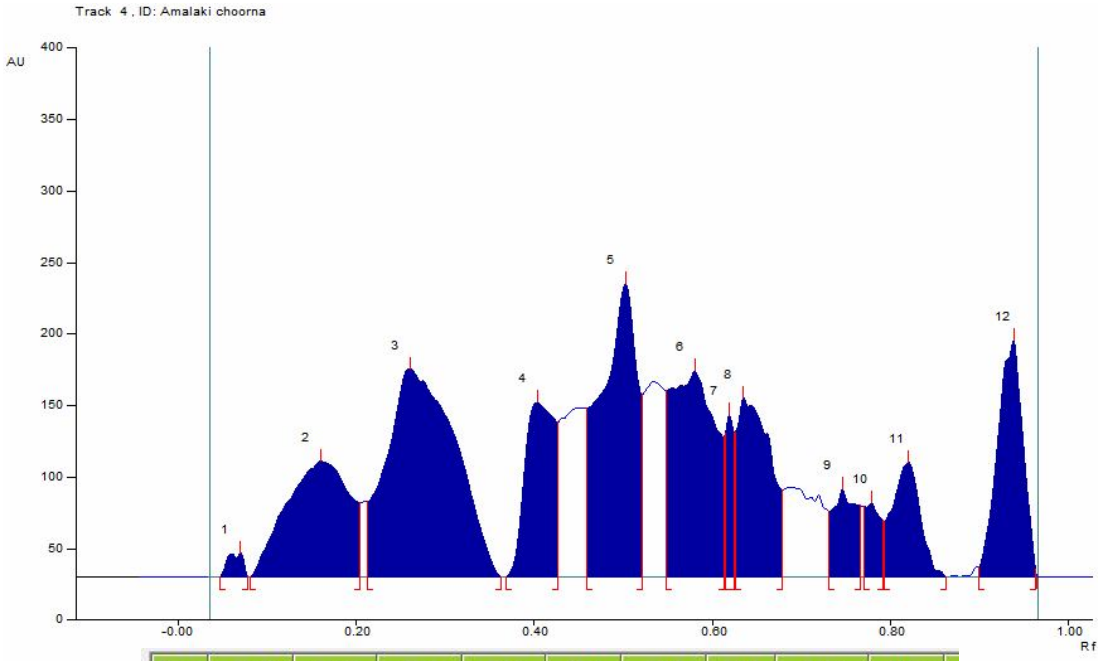
On microscopic examination, Amalaki choorna (dried fruit powder of *Emblica officinalis* Garten) showed the



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.03 Rf	1.2 AU	0.04 Rf	29.1 AU	1.62 %	0.05 Rf	3.0 AU	242.2 AU	0.43 %
2	0.06 Rf	0.7 AU	0.08 Rf	78.2 AU	4.34 %	0.12 Rf	23.6 AU	2204.0 AU	3.88 %
3	0.15 Rf	0.0 AU	0.21 Rf	268.6 AU	14.92 %	0.27 Rf	0.4 AU	9421.9 AU	16.61 %
4	0.29 Rf	15.7 AU	0.37 Rf	733.9 AU	40.76 %	0.40 Rf	46.7 AU	31670.7 AU	55.82 %
5	0.40 Rf	150.7 AU	0.41 Rf	192.2 AU	10.67 %	0.44 Rf	0.0 AU	2843.0 AU	5.01 %
6	0.44 Rf	0.1 AU	0.46 Rf	21.9 AU	1.22 %	0.48 Rf	0.9 AU	322.9 AU	0.57 %
7	0.49 Rf	0.7 AU	0.52 Rf	38.0 AU	2.11 %	0.56 Rf	2.6 AU	816.6 AU	1.44 %
8	0.56 Rf	0.4 AU	0.59 Rf	96.8 AU	5.38 %	0.62 Rf	2.4 AU	1402.0 AU	2.47 %
9	0.65 Rf	5.8 AU	0.69 Rf	170.1 AU	9.45 %	0.74 Rf	0.2 AU	3613.2 AU	6.37 %
10	0.88 Rf	0.8 AU	0.92 Rf	171.8 AU	9.54 %	0.95 Rf	3.7 AU	4201.4 AU	7.40 %

Amalaki Choorna 30µl

Fig. 3 : HPTLC Densitometric scan of Amalaki Choorna at UV 254 nm.



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.05 Rf	0.3 AU	0.07 Rf	17.0 AU	1.30 %	0.08 Rf	0.9 AU	241.9 AU	0.56 %
2	0.08 Rf	0.1 AU	0.16 Rf	81.1 AU	6.18 %	0.20 Rf	51.8 AU	4642.6 AU	10.83 %
3	0.21 Rf	52.9 AU	0.26 Rf	145.5 AU	11.08 %	0.36 Rf	0.3 AU	9279.3 AU	21.64 %
4	0.37 Rf	0.2 AU	0.40 Rf	122.1 AU	9.30 %	0.43 Rf	08.1 AU	3351.8 AU	7.82 %
5	0.46 Rf	118.0 AU	0.50 Rf	205.2 AU	15.63 %	0.52 Rf	27.5 AU	6776.5 AU	15.80 %
6	0.55 Rf	130.4 AU	0.58 Rf	144.4 AU	11.00 %	0.61 Rf	97.8 AU	5912.1 AU	13.79 %
7	0.62 Rf	100.2 AU	0.62 Rf	113.3 AU	8.63 %	0.63 Rf	00.5 AU	851.6 AU	1.99 %
8	0.63 Rf	100.9 AU	0.64 Rf	125.4 AU	9.55 %	0.68 Rf	61.1 AU	3826.0 AU	8.92 %
9	0.73 Rf	46.1 AU	0.75 Rf	61.3 AU	4.67 %	0.77 Rf	49.8 AU	1344.4 AU	3.14 %
10	0.77 Rf	49.3 AU	0.78 Rf	51.9 AU	3.95 %	0.79 Rf	39.8 AU	747.7 AU	1.74 %
11	0.79 Rf	39.0 AU	0.82 Rf	80.2 AU	6.11 %	0.86 Rf	0.3 AU	2057.0 AU	4.80 %
12	0.90 Rf	6.9 AU	0.94 Rf	165.4 AU	12.60 %	0.96 Rf	5.1 AU	3847.0 AU	8.97 %

Amalaki Choorna 30µl

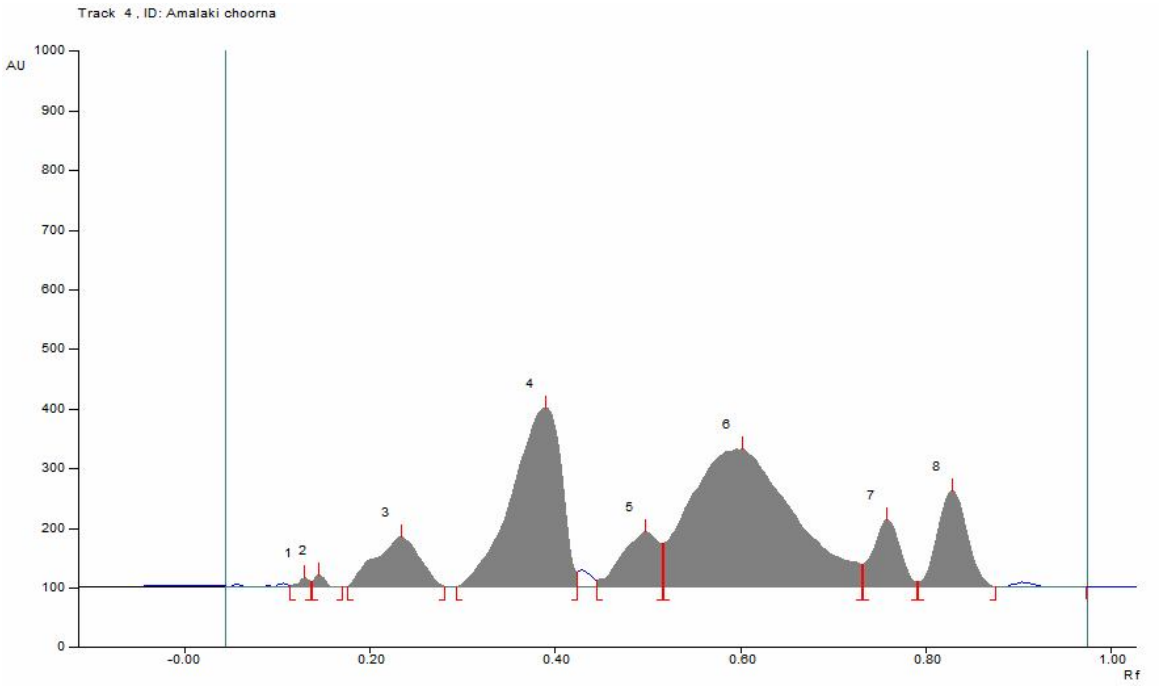
Fig. 4 : HPTLC Densitometric scan of Amalaki Choorna at UV 366 nm.

presence of characters such as epicarp of fruit in surface and in transversely cut view; longitudinally cut thick-walled mesocarp parenchyma; thick-walled fibres and its fragments, often sclereidal in nature; bundle of xylem elements formed by spiral and scalariform vessel; pitted lignified parenchyma cells of mesocarp, often with starch grains and tannin; highly pitted sclereidal fibres of endocarp which are often fragmented; fragment of thin walled phloem parenchyma; thick walled sclereids of the stony endocarp; different types of stone cells and plenty of brown uniformly sized oil drop lets, simple starch grains and tannin cells scattered throughout the powder (fig. 1). The characteristic features showed in standard Emblica officinalis powder are epidermal cells in surface view (fig. 1.1), parenchyma from mesocarp (fig. 1.2), isolated sclereids (fig. 1.22), prismatic crystals (fig. 1.9), pitted vessel attached with parenchyma (fig. 1.6), pitted parenchyma (fig. 1.7), starch grains (fig. 1.18), group of stone cells (fig. 1.24), sclereids from the endocarp (fig. 1.23) and fibres (fig. 1.3) match with the study powder (Neeraj and Madhu, 2010). Tanins, important constituents present in powder microscopic study are known for their antioxidant activity and are responsible for the protection and preservation of the ascorbic acid on drying under sun (Ekta *et al*., 2011).

TLC and HPTLC

TLC fingerprint profile is a systematic representation of all the constitution of samples resolved in the given chromatographic system. TLC photo documentation of extract of Amalaki Choorna (dried fruit powder of *Emblica officinalis* Garten) is presented in fig. 2.

HPTLC fingerprint profile of Amalaki choorna (dried fruit powder of *Emblica officinalis* Garten) was developed. The purity of the bands in the sample extract was confirmed by comparing the absorption spectra



Peak	Start Position	Start Height	Max Position	Max Height	Max %	End Position	End Height	Area	Area %
1	0.11 Rf	2.1 AU	0.13 Rf	14.9 AU	1.46 %	0.14 Rf	9.5 AU	138.7 AU	0.30 %
2	0.14 Rf	9.5 AU	0.15 Rf	19.6 AU	1.93 %	0.17 Rf	0.0 AU	187.8 AU	0.40 %
3	0.18 Rf	0.2 AU	0.23 Rf	83.8 AU	8.25 %	0.28 Rf	0.5 AU	3218.3 AU	6.90 %
4	0.29 Rf	0.7 AU	0.39 Rf	300.5 AU	29.58 %	0.42 Rf	24.8 AU	12669.6 AU	27.15 %
5	0.45 Rf	11.4 AU	0.50 Rf	92.2 AU	9.08 %	0.52 Rf	72.7 AU	2964.1 AU	6.35 %
6	0.52 Rf	73.2 AU	0.60 Rf	231.0 AU	22.75 %	0.73 Rf	37.6 AU	20695.1 AU	44.35 %
7	0.73 Rf	37.9 AU	0.76 Rf	112.6 AU	11.09 %	0.79 Rf	8.1 AU	2650.0 AU	5.68 %
8	0.79 Rf	8.1 AU	0.83 Rf	161.0 AU	15.86 %	0.88 Rf	0.1 AU	4136.9 AU	8.87 %

Amalaki Choorna 30µl

Fig. 5 : HPTLC Densitometric scan of Amalaki Choorna at 620 nm after derivatisation with vanillin sulphuric acid.

Table 1 : R_f values of Amaalaki Choorna (30µl).

UV 254 nm	UV 366 nm	After derivatisation
0.08 Green	0.21 F.White	0.21 Red
0.21 Green	0.36 F.Blue	0.36 Red
0.36 Dark green	0.49 F.Blue	0.41Orange
0.41Light green	0.81 F. Green	0.60 Blue
0.60 Light green	0.93 F.Blue	0.70 Blue
0.70 Light green		

recorded at the start, middle and end position of the band. The HPTLC densitometric scans at UV 254, 366 and after derivatisation are presented in the figs 3, 4 and 5 respectively. The observed R_f values are tabulated in table 1. The R_f values of the spot and their colour by TLC photodocumentation of Amalaki choorna (dried fruit powder of *Emblica officinalis* Garten) extract was developed. *n*-butanol extract of Amalaki Choorna (dried fruit powder of *Emblica officinalis* Garten) at 254 nm showed six spots (0.08 Green, 0.21 Green, 0.36 Dark green, 0.41 Light green, 0.60 Light green, 0.70 Light green), where as under 366 nm, it showed five spots (0.21 F.White, 0.36 F.Blue, 0.49 F.Blue, 0.81 F. Green, 0.93 F.Blue); after derivatisation in vanillin-sulphuric acid spray reagent at 620 nm, it showed five spots (0.21 Red, 0.36 Red, 0.41Orange, 0.60 Blue, 0.70 Blue).

The R_f of the spot at 0.41 Light green under 254 nm is as per the standards (Neeraj and Madhu, 2010), which indicates presence of gallic acid, a product on hydrolysis of Emblicanin A. The presence of gallic acid here, indicates that the Amalaki choorna (dried fruit powder of *Emblica officinalis* Garten) under study contains Emblicanin A which helps prevent oxidative stress leading to dyslipidemia (bhattacharya a, Ghosal and Bhattacharya, 2000). The R_f of the spot at 0.36 (0.32±5) is nearest value to the standard R_f value corresponding to ascorbic acid (Government of India, 2009). Ascorbic acid (Vitamin C) is a dietary antioxidant, helping in reduction of oxidative stress (Jain *et al*., 2013).

Conclusion

Amalaki choorna has shown all the characters of standard dried *Emblica officinalis* fruit. Alcohol extract of amalaki choorna under 254 nm, 366 nm, showed 6 spots and 5 spots, respectively. After derivatization with vanillin sulphuric acid it showed 5 spots. The densitometric scan of amalaki choorna at 254 nm showed 10 peaks; at 366 nm, showed 12 peaks and at 620 nm the densitometric scan showed 8 peaks. The presence of tannins, ascorbic acid and gallic acid (amblicanin) indicates it can act against oxidative stress leading to dyslipidemia and atherosclerosis.

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