Journal of Traditional and Folk Practices
Volume 05(1), June 2017, 25 - 36.
ISSN 2278 - 5906
https://doi.org/10.25173/jtfp.2017.5.1.62

Pharmacognostic standardisation of *Vanya* (wild) and *Gramya* (cultivated) forms of *Amalaki* fruit (*Emblica officinalis* Gaertn.)

R H Amina[1]*, Jollykutty Eapen[1], M S Deepa[1], M Navas[2]
[1]Department of Dravyagunavijnanam, Govt. Ayurveda College,
Thiruvananthapuram- 695001, Kerala, India
[2]Ethnomedicine and Ethnopharmacology Division, Jawaharlal Nehru Tropical Botanic Garden and Research Institute, Palode, Thiruvananthapuram- 695562, Kerala, India
*aami0057@gmail.com

Received : 15 May 2017 Accepted: 14 Jun 2017

Abstract

The *Amalaki* (*Emblica officinalis* Gaertn.,) one of the most popular and highly reputed medicinal plant used in Ayurveda is widely distributed throughout India. Its fruits are said to be the best rejuvenating drug as per Ayurveda. According to the reports of National Medicinal Plant Board, *Amalaki* is the first and most demanded medicinal plant in Kerala.The fruits are rich source of vitamin C and has multifaceted utilities in pharmaceutics, herbal cosmetics and health supplements. The Ayurvedic Pharmacopoeia of India (API) mentioned both fresh and dried fruits of *Emblica officinalis* Gaertn. as the officinal part. The present study is an attempt to standardise the 3 forms of *Amalaki* fruit (wild, under cultivation in homestead and commercial cultivar) by preliminary pharmacognostic evaluation including macroscopic, microscopic studies and powder analysis. Also the study discusses the diagnostic features of each variety.

Keywords: *Amalaki*, Ayurveda, *Emblica officinalis*, Pharmacognosy, Microscopy.

1. Introduction

The fruit of *Amalaki* (*Emblica officinalis* Gaertn.) belonging to the family-Euphorbiaceae is one of the most popular and highly demanded drug used in Ayurveda and is described as the best *vayahsthapana* (anti-aging) drug. It is commonly known as Indian gooseberry. It is one of the main ingredients of many Ayurvedic formulations including the *Chyavanaprasa*, *Amruthotharam kashayam* and *Amalaki rasayanam* to name a few. *Amalaki* fruit has also multifaceted utilities in pharmaceutics, 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, 2005). In Ayurveda, there are references on different forms of *Amalaki* such as *vanya* (wild) and *gramya* (cultivated). *Vanya* forms is said to be small and hard like stone, while *gramyam* is mentioned as big, soft and fleshy (Sharma, 2011). Ayuvedic classics do not mention any substitute for this drug. But *Phyllanthus indofischeri*, a medicinal plant considered as vulnerable now, is known to be the substitute (IUCN, 2015).

Various studies conducted on *E. officinalis* fruit suggest that it has antioxidant (Shukla *et al*., 2009), hypolipidaemic (Mathur *et al.*, 1996), anti-diabetic (Mehta *et al.,* 2009), anti-inflammatory (Middha *et al.,* 2015), anti-ulcerogenic (Sairam *et al.,* 2002), growth promoting (Patel *et al.,* 2016), anti-pyretic and analgesic (Peri-

* Corresponding author

anayagam *et al.,* 2004), chemopreventive (Bhati *et al*., 2016), memory enhancing (Vasudevan *et al.,* 2007), antimicrobial (Ahmad *et al.,*1998), etc.

Based on the demand, *Amalaki* is one of the most important medicinal plants in India. A study to assess the demand and supply of medicinal plants in India reveals that *Amalaki* is the highest consumed botanical raw drug by the domestic herbal industry (Ved & Goraya, 2007). National Medicinal Plant Board (NMPB) reported that the quantity of all Kerala annual consumption of *Amalaki* fruit in the year 2005-06 was 634720 kg approximately. It makes *Amalaki,* the first and most demanding medicinal plant in Kerala (SMPB Kerala, 2012).

Considering the demand for this fruit, the plant is widely cultivated in India. Nowadays many cultivated forms of this fruit are available in the market. But data on standardisation are not yet available. The pharmacognostic characters of *Amalaki* is described in many text books but without any specification of its forms (Tendon & Sharma, 2010; Aiyer & Kolammal, 1978; Anonymous, 2011). Since pharmacognosy plays an important role in the evaluation of identity and quality of crude drugs, the present study is an attempt to standardise the 3 genuine forms of *Amalaki viz* wild (*Vanya*), under cultivation in homestead (*Gramya*) and a commercial cultivar through preliminary pharmacognostic evaluation including macroscopic, microscopic and powder analysis.

2. Materials and Methods

It includes sample collection and pharmacognostic evaluation such as macroscopy, microscopy and organoleptic characterisation.

2.1. Sample collection

In the present study, three forms of *Amalaki* such as cultivated in the homestead (*gramya*), wild (*vanya*) and commercial cultivar were collected. The fresh fruits of *Amalaki* was collected from a plant under cultivation in a homestead (average weight - 11gm each) from Kollam district of Kerala and selected as the *Gramya* (named as G_1) (Fig.1). The fresh *Amalaki* fruits (10gm average weight) from the natural habitat was collected from the forests of Palode range, part of Western Ghats, Thiruvananthapuram district, Kerala and selected as the *Vanya* (wild-named as G_2) (Fig.2). The *Amalaki* fruits of a commercial cultivar (average weight- 25 gm each) available in the Kerala fruit market (named as G_3) was also procured from Thiruvananthapuram district, Kerala (Fig. 3). All the samples were authenticated at Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Thiruvananthapuram and voucher specimens were deposited at JNTBGRI Herbarium (TBGT 84561).

Figures of *Amalaki* samples

❖ 1- *Gramya*, 2- *Vanya*, 3- Cultivar

2.2. Pharmacognostic evaluation

Pharmacognostic studies of crude drugs play a very important role in identifying the quality and purity of the drugs. The present study was carried out through macroscopic and microscopic studies.

2.2.1. Macroscopy

The samples were subjected to macroscopic evaluation by observation with naked eyes and by tactile and other sensory characters. The features like shape, size or dimensions, surface, odour, colour, texture odourandtaste were evaluated.

2.2.2. Microscopy

Microscopic evaluation was done under two phases:

A) Histological evaluation and

B) Powder microscopy

A) Histology

The genuine samples of fresh *Amalaki* fruit rinds were cut into pieces of 10 –15 mm in size and put in water taken in a petri dish. The fine transverse sections were taken and the best 5 sections were taken for the study of anatomical characteristics, stained with safranin, mounted in glycerin on glass slides, covered with cover slip. The specimens were observed under compound microscope of both low and high power magnification of 10x, ocular 2x, 4x, 10x and 40x objectives respectively. Microphotographs of the sections were taken with the digital camera attached with digital microscope (Olympus digital-CS41, Japan, with CCD camera with analysis software (digital image-pro) at Pharmacognosy Lab, Drug Standardization Unit (DSU), Government Ayurveda College, Thiruvananthapuram.

B) Powder microscopy

The dried fruit rind of the *Amalaki* samples were powdered and passed through sieve no. 4 and kept in air tight containers. For examining characters of the powder, sufficient amount of powder was taken randomly and placed over slides, stained with safranin, mounted over glycerine and covered with 1 mm cover slips. The slides were examined under a compound microscope and the diagnostic features were documented and photographed with the digital camera attached with the digital microscope (Olympus digital-cs41, Japan, with CCD camera with analysis software digital-image-pro).

3. Results and Discussion

3.1. Macroscopic and organoleptic characterisation

The macroscopic and organoleptic characters of samples were evaluated and the results were presented in Table 1.

Table 1: Macroscopic evaluation

Characters	G_1	G_2	G_3
Shape	Globose	Globose	Globose
Size	2.4 - 3.6 cm in diameter	2.5-3.5 cm	3.5-4.5cm
Colour	Pinkish (immature fruits) pale green(mature fruit)	Pinkish (immature fruit), green (mature)	Slight pinkish (immature fruit); Light green (mature)
Surface	Shining with presence of whitish specks and 6 prominent longitudinal septae	Dark prominent specks and 6 faint longitudinal septae	Shining surface with whitish specks and 6 longitudinal septae
Texture	Smooth	Smooth	Smooth
Odour	Pleasant	Pleasant	Pleasant
Taste	Sour, slightly bitter and acrid followed by sweetness	Sour, bitter	Sour, bitter

3.2. Histological characters

Histological characters of each sample is described below and the features are concluded in table 2.

3.2.1. Histological characters of G_1 sample of *Amalaki* fruit

The transverse section (T. S.) of the fruit rind was more or less circular in outline. The outermost thin cuticle was followed by a single layer of epidermis with thick walled longitudinally elongated or squarish cells. The epidermis was followed by 2-3 layers of hypodermis with transversely elongated cells. The mesocarp was formed of thin walled polyhedral or polygonal and irregularly thickened parenchymatous cells. The cells at the periphery were smaller and slowly increase in size towards the inner mesocarp. The mesocarp was separated by radial bands or septae. Each septum consists of 1-2 layers of narrow elongated parenchymatous cells. A few calcium oxalate crystals and pitted stone cells were present in the mesocarp. Starch grains are a few, rounded and oval shaped, mainly seen in between vascular strands. A few pitted parenchyma cells and small rounded vascular bundle consisting of xylem and phloem vessels and were seen scattered in the mesocarp. Presenceof helical and annular vessels were also observed (plate 1).

3.2.2. Histologic characters of G_2 sample of *Amalaki* fruit

The T. S. of genuine fruit rind was more or less circular in outline. It consists of outermost brownish thick cuticle, followed by single layer of epidermis. The epidermis was made up of narrow thick walled and almost rectangular cells. Beneath the epidermis, 2 to 3 layers of hypodermis can be seen, made up of compactly arranged, tangentially elongated and rectangular parechymatous cells of the mesocarp. The remaining cells of the mesocarp consist of several layers of polyhedral or polygonal and irregularly thickened parenchymatous cells. The cells at the periphery were smaller and slowly increase in size towards the inner mesocarp. Small vascular bundles of varying size were seen scattered in the mesocarp. Prismatic crystals were also seen in the mesocarp. A few stellate crystals were found embedded in the cells. Numerous thick walled pitted stone cells were present in the mesocarpic cells. Isolated or grouped stone cells were seen, more towards the inner side. Oval or round shaped starch grains devoid of hilum were seen as single, or in groups (diads, triads and tetrads) in the mesocarpic cells. The mesocarp is intercepted by radially arranged septa or radial bands, formed of 2-3 layers of small sized parenchymatous cells . In between the parenchymatous layers of the septum, vascular bundle with inner thick walled xylem and outer phloem elements were clearly visible. Also small stone cells were distributed along the margins of the septum. Tannin cells, colouring matter, white resins, isolated thick walled sclereids, pitted parenchyma etc., were present scattered throughout the mesocarp. Pitted parenchyma was present associated with vascular strands, septa and seen more towards the inner side. Spiral vessels and longitudinally running vascular strands were also observed in T. S. of the fruit rind (plate 2 & 3).

3.2.3. Histological characters of G_3 sample

The T. S. of genuine fruit rind was more or less circular in outline. The outermost layer was covered with thick cuticle followed by a single layer of epidermis with tangentially elongated and thick walled cells. Beneath the epidermis, 2-3 layers of hypodermis with compactly

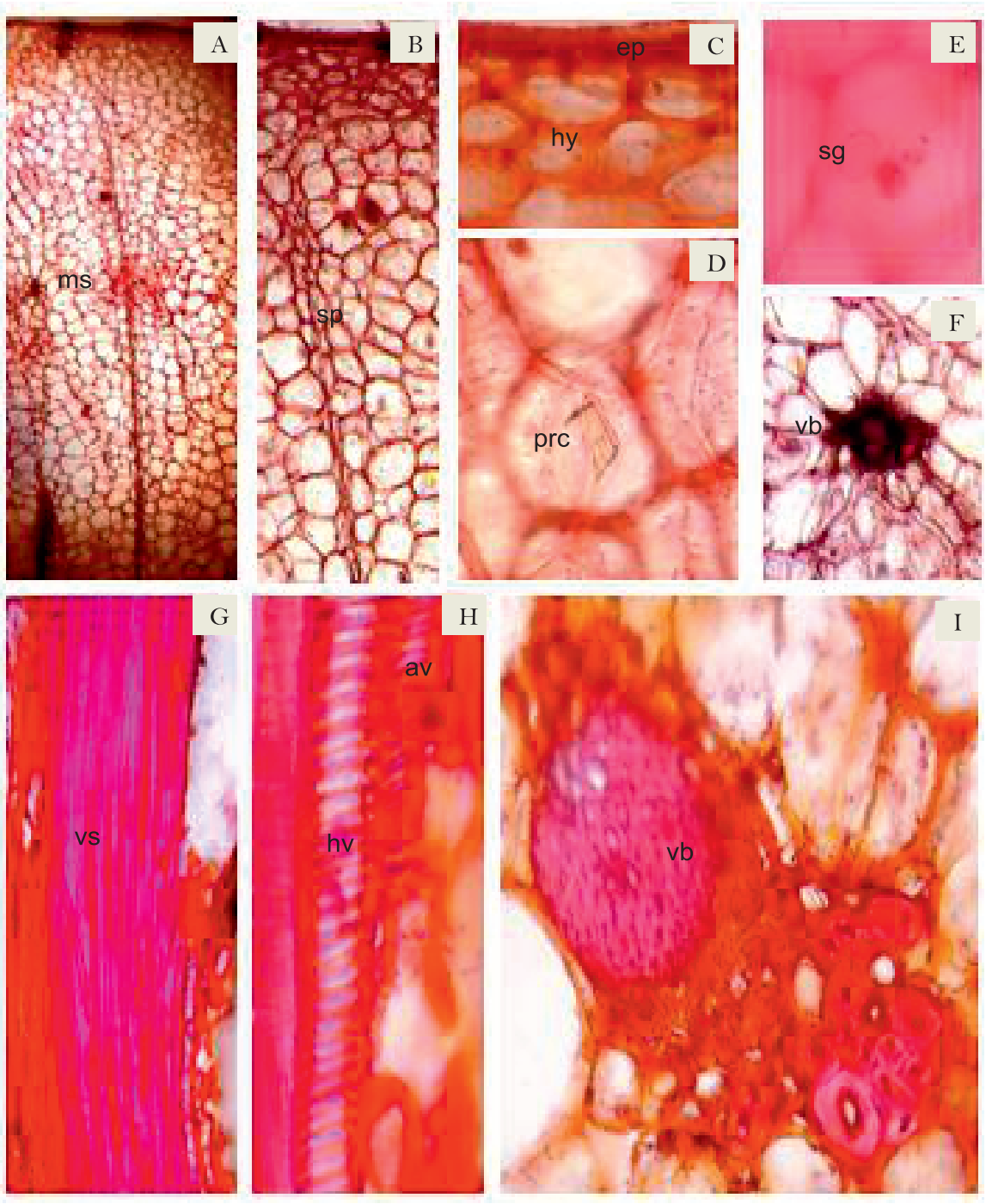

PLATE 1-Histology of G_1 sample

A: Fruit T. S. showing Mesocarp cells -ms; **B**: Septum -se; **C**; Epidermis -ep, Hypodermis - hy; **D**: Prismatic crystal -prc; **E**: Starch grain – sg (single); **F**: Vascular bundle- vb; **G**: Vascular strand- vs (longitudinal); **H**: Helical vessel- hv, Annular vessel- av; **I:** - Vascular bundle- vb

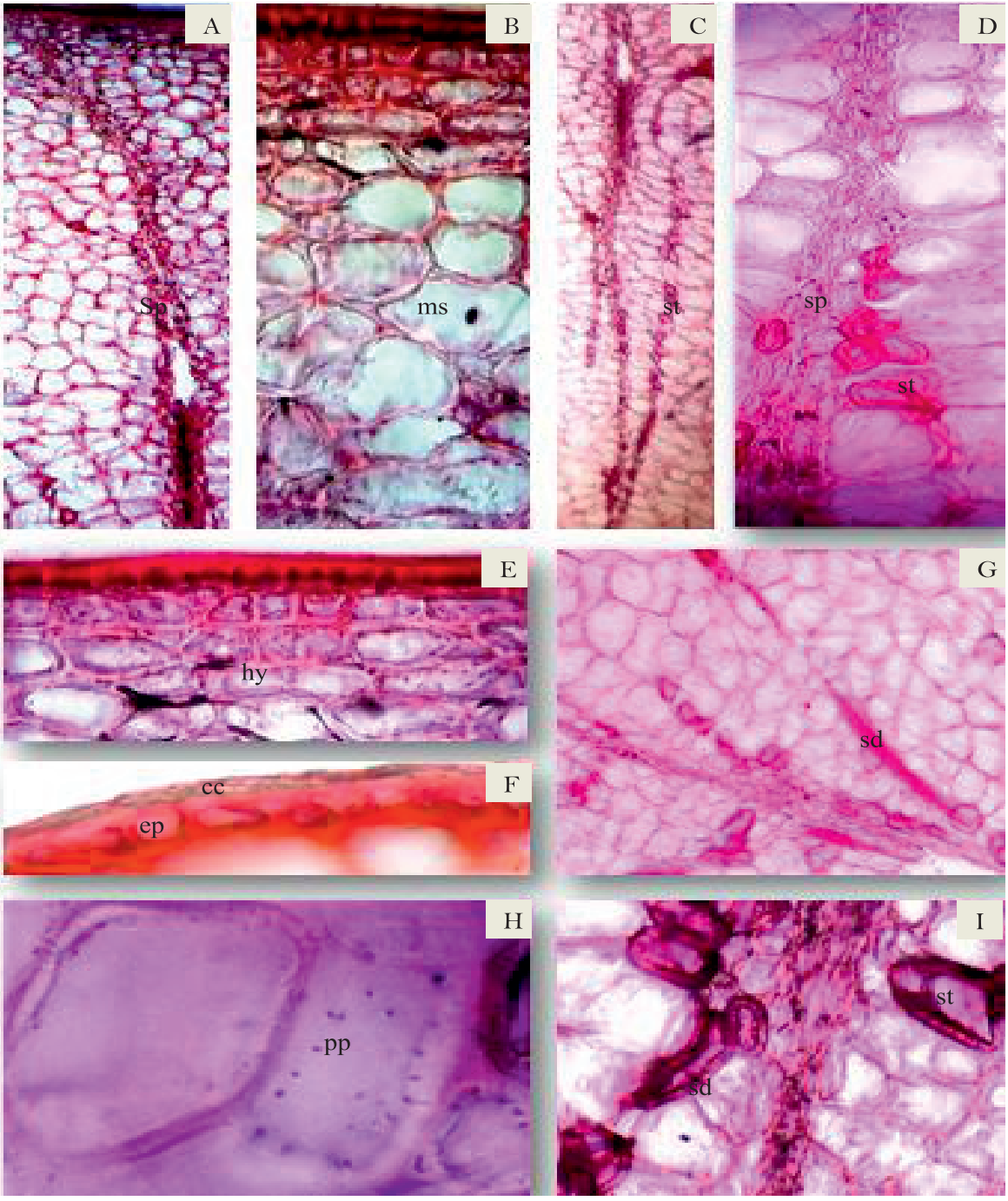

PLATE 2: Histology of G_2 sample

A: T S of the fruit rind showing septum (sp); **B**: Mesocarpic cells- ms; **C**: Stone cells near septum -st; **D**: Stone cells along the septal margins- st**; E**: Hypodermis- hy; **F**: A portion enlarged showing cuticle-cc, Epidermis- Ep; **G**: Sclereids- sd; **H**: Pitted parenchyma-pp; **I**: stone cells-st, Sclereids- sd

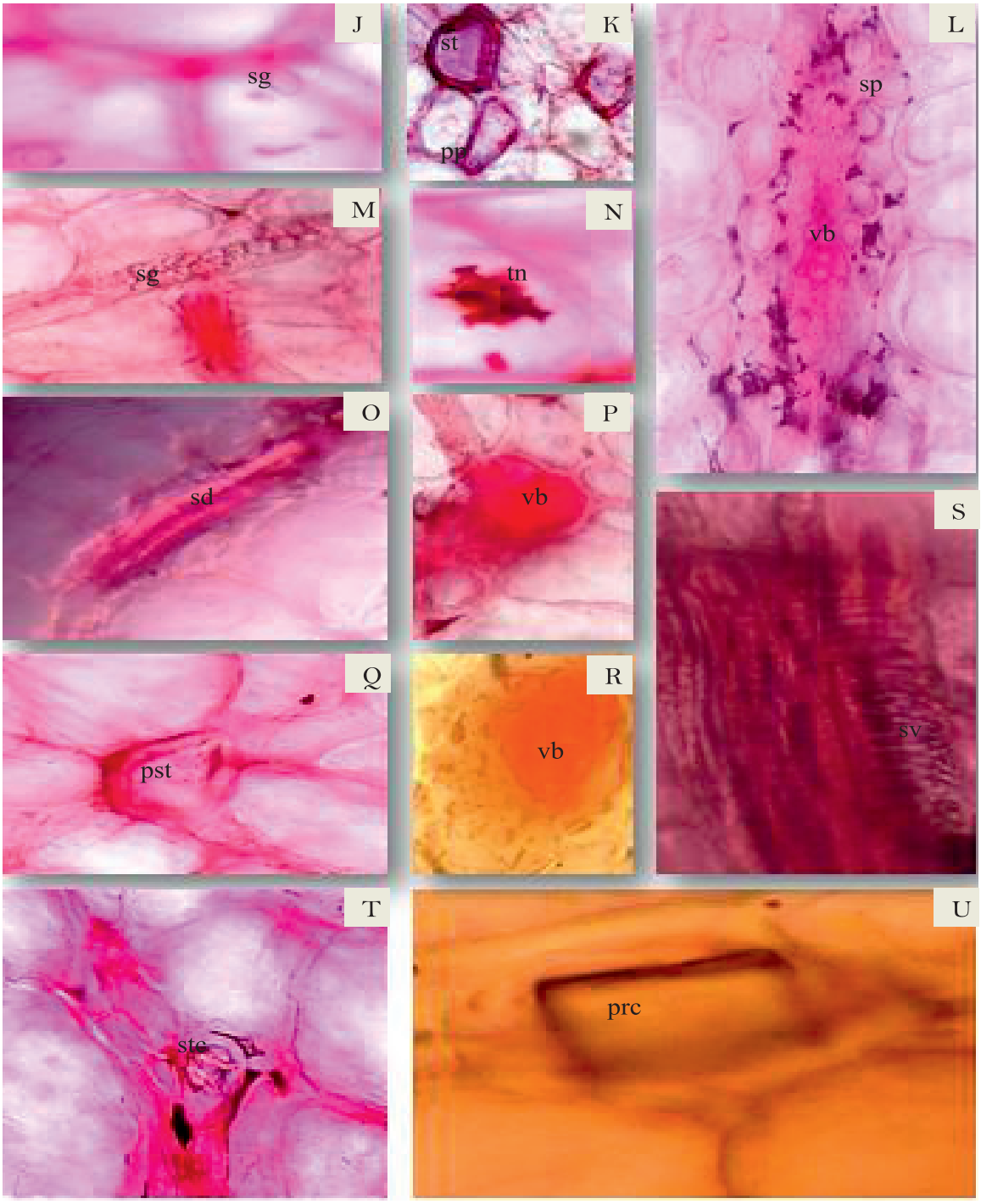

PLATE 3: Histology of G_2 sample

J: Starch grain(single) -sg; **K**: Stone cell -st, Pitted parenchyma -pp; **L**: Vascular bundle (vb) seen in between septal layers; **M**: starch grain group -sg; **N**: Tannins -tn; **O**: Sclereid -sd; **P& R**: Vascular bundle -vb; **Q**: Pitted stone cells -pst; **S**: Spiral vessels- sv, **T**: Stellate crystal- stc; **U:** Prismatic crystal-prc

arranged polygonal cells are seen. Small rounded starch grains were also observed within the cells. The mesocarpic parenchyma consists of thin walled polyhedral and irregularly thickened parenchymatous cells. The cells at the periphery were smaller and became larger towards the inner region. Some of the outer mesocarpic cells contain calcium oxalate crystals and a few pitted parenchyma cells. Mesocarp of the fruit was separated by radial bands or septae. Each septum is made up of 4-7 rows of compactly arranged rounded and polygonal cells, intermingled with thin walled stone cells and pitted parenchyma. Prismatic crystals were also seen in the septal region. Both longitudinal and circular vascular strands were seen scattered in the mesocarpic region. Small rounded vascular bundles consisted of xylem vessels and xylem parenchymas were present. Longitudinal vascular strands constituted helical, annular and spiral vessels. Tannin cells, yellow and white resinous blocks were present scattered throughout the mesocarp (Plate 4).

Table 2: Comparative histological characters of three forms of fresh *Amalaki* fruit

Characters	**Gramya (G_1)**	**Vanya (G_2)**	**Cultivar(G_3)**
Cuticle	Thin	Thick	Thick
Epidermis	Thick walled, longitudinally elongated or squarish	Narrow, thick walled and almost rectangular cells	Thick walled, tangentially elongated
Hypodermis	2-3 layers of transversely elongated	2-3 layers of compactly arranged, tangentially elongated and rectangular parechymatous cells	2-3 layers of compactly arranged polygonal cells
Mesocarpic parenchyma	Thin walled polyhedral or polygonal, irregularly thickened. The cells at the periphery were smaller and became larger towards the inner mesocarp	Thin walled polyhedral or polygonal, irregularly thickened parenchymatous cells. The cells at the periphery were smaller and became larger towards the inner mesocarp	Thin walled polyhedral or polygonal, irregularly thickened. The cells at the periphery were smaller and became larger towards the inner mesocarp
Septum	1-2 layers of narrow elongated parenchymatous cells	2-3 layers of small, polygonal closely packed parenchymatous cells. Stone cells are present along the borders	4-7 layers of compactly arranged rounded and polygonal cells intermingled with thin walled stone cells and pitted parenchyma, prismatic crystals were seen in the septal region
Stone cells	A few	Abundant, more towards the inner side	A few in the mesocarp and septa
Sclereids	Not observed	Thick walled sclereids	Thick walled sclereids
Crystals	Prismatic	Stellate and prismatic crystals	Prismatic crystals present in the mesocarp and more in the septum; tabular crystals were also present
Vascular strands	Longitudinal and circular	Longitudinal and circular	Longitudinal and circular
Vessels	Annular and helical	Spiral	Helical, annular and spiral vessels
Starch grains	Rounded and oval shaped; a few present in mesocarp mainly in between vascular strands	Oval or rounded, without hilum, single or grouped (diads, triads and tetrads) in the mesocarp	A few; rounded;present in the hypodermis
Pitted parenchyma	Very few scattered in the inner mesocarp	Present in the inner mesocarp and along the septum	A few in the mesocarp; mostly in the septum
Resin cells	Present	Present	Present

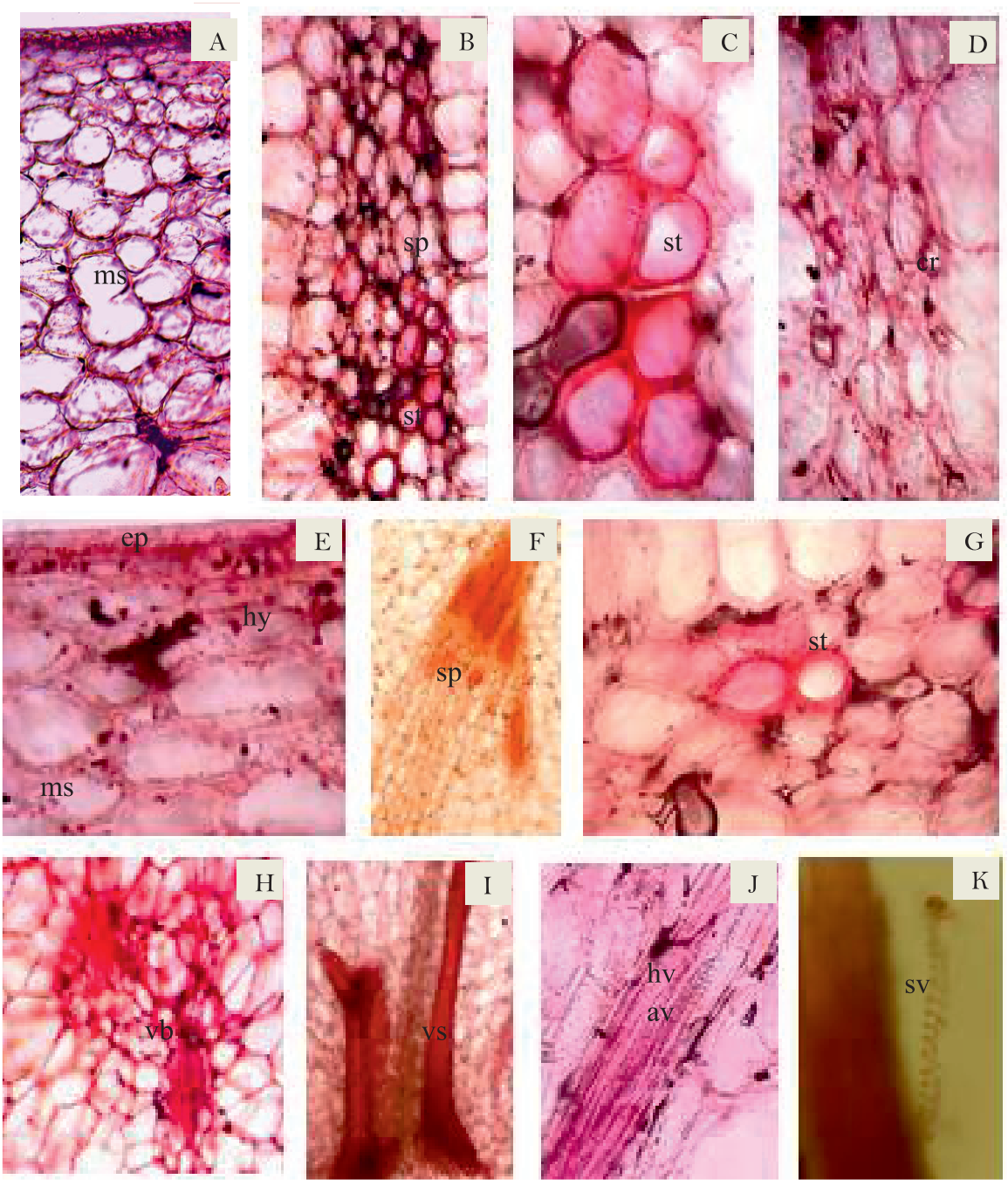

PLATE 4- Histology of G_3 sample

A: T S of the fruit rind showing mesocarp -ms; **B**: Septum- sp, Stone Cell -st; **C**: Stone cells -st; **D**: Crystals -cr; **E**: Epidermis -ep, Hypodermis -hy, Mesocarpic cells -ms; **F**: Multi layered septum -sp; **G**: Stone Cells -st; **H**: Vascular bundle -vb (round); **I**: Vascular strands- vs (longitudinal); **J**: Helical vessel -hv, Annular vessel - av; **K**: Spiral vessel- sv

3.3. Powder microscopy

On examination of powder under microscope all the three samples (of three forms) showed almost similar characters. They showed: presence of epidermal cells in surface view, presence of mesocarpic cells, round or oval starch grains, fragments of pitted parenchyma, prismatic crystals, isolated or group of thin walled stone cells,long fibres and fragments of fibres, single and group of sclereids, white or yellowish resinous blocks and tannin cells (Plate 5).

4. Conclusion

In API, the microscopic features of *Amalaki* were described without any specification of different forms. The present study evaluated the macroscopic and microscopic characters of 3 forms of *Amalaki* fruit. On histological evaluation, the diagnostic features were observed in cuticle thickness, shape of the epidermal and hypodermal cells, number of septal layers, number of stone cells, crystals and vessels. As per API, the cuticle of *Amalaki* is thin (Anonymous, 2001).Where as in the present study, thin cuticle was observed in only in *gramya* sample, while the wild and cultivated forms possess thick cuticle. Epidermal and hypodermal cells also showed slight difference in shape in all the three forms (Table 2). The wild form had a number of stone cells than the other 2 forms (Plate 2- C, D, G). The stellate crystals were seen only in *gramya* and the tabular crystals were found only in the cultivated form. The cutivar (G_3) had 7 septal layers while others have a maximum of 2-3. Annular and helical vessels were present both in *gramya* and cultivar, while the spiral vessels were found in wild and cultivar. In *gramya*, the pitted parenchyma was present in the mesocarp. But in cultivar, it was found mostly near the septum. On powder analysis similar features were found in all the samples. As per reports, thick and straight walled epidermal cells in surface view are embedded with small prismatic crystals of silica (Tendon & Sharma, 2010). But in the present study, no silica crystals were observed in the epidermal cells of any of the forms. The present study reveals that, variations may occur in the pharmacognostic characters of *Amalaki* fruit according to its forms and the present study can help as a very useful botanic tool for identification and authentication of these 3 forms of *Amalaki* fruit.

Acknowledgments

I express my sincere gratitude to the Principal, Govt Ayurveda College, Thiruvananthapuram and Director, JNTBGRI, Palode, Thiruvananthapuram for providing me the necessary facilities. Thanks, are also due to Dept. of Dravyagunavijnanam and Drug Standardisation Unit, Govt Ayurveda College, Thiruvananthapuram. I would like to convey my regards to Dr M A Shajahan, (HOD), Dr Ansary P Y, Dr Shahul Hameed, Dr Indulekha V C of Department of Dravyagunavijnanam and Dr Rajesh, Drug Standardisation Unit, Govt Ayurveda College, Thiruvananthapuram for their support in the successful completion of the work. Finally, I would like to thank Kerala State Council for Science, Technology & Environment (KSCSTE) for providing the financial assistance under the Student Project Scheme.

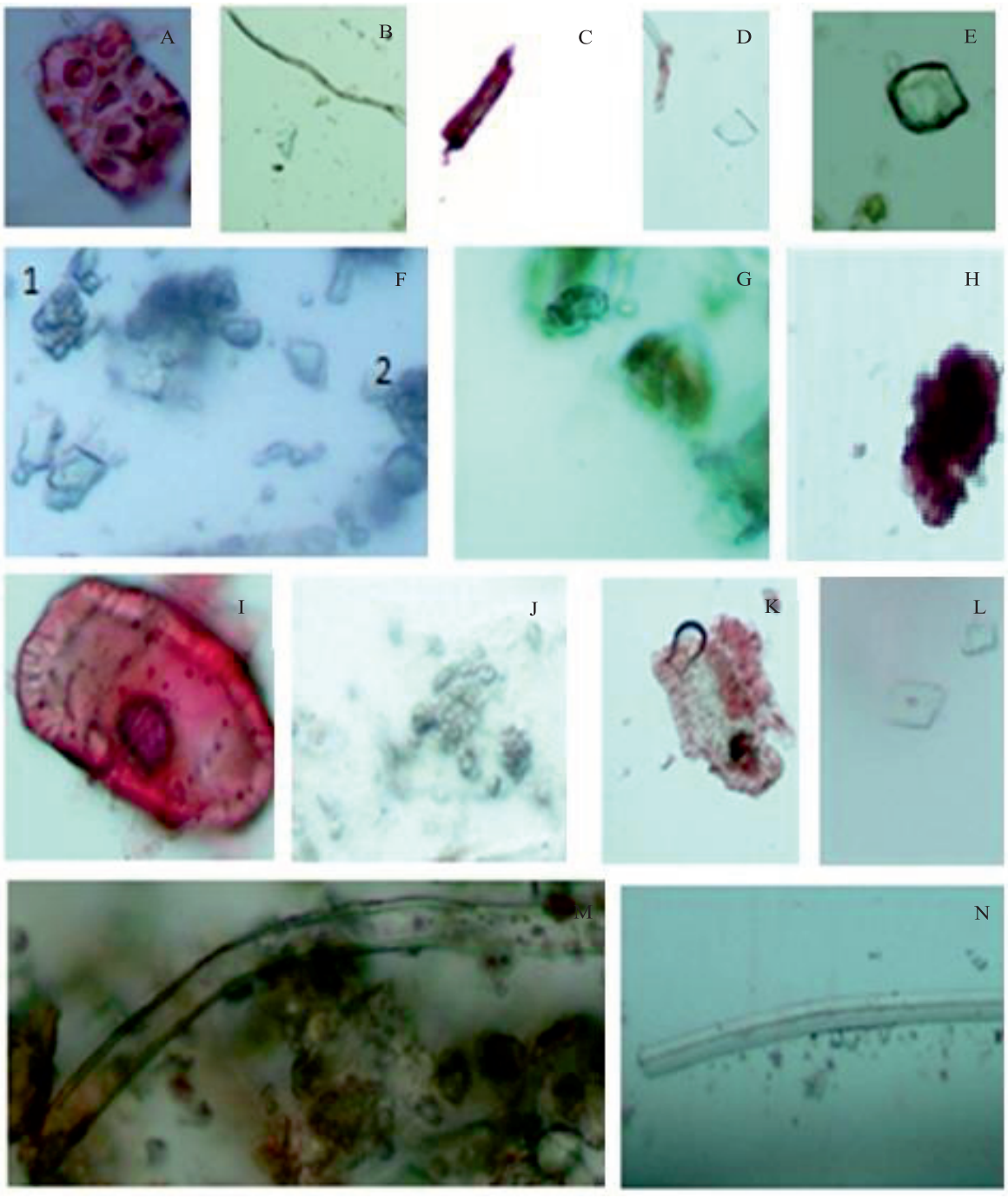

PLATE 5-Powder characters of *Amalaki* fruit

A: Epidermis in surface view; **B**: Long fibre; **C**: sclereid; **D**: Prismatic crystal; **E**: Resinous mass; **F:**1&2-Rosette crystal; **G**: Stone cells; **H:** Tannins; **I**: Stone cell; **J:** Group of starch grains**; K:** Mesocarpic cells; **L**: Pitted parenchyma; **M**: Fibre; **N:** Fragment of fibre

References

1. Ahmad I, Mehmood Z and Mohammad F 1998. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology. 62 (2): 183-193.
2. Aiyer M A, Kolammal M 1978. Pharmacognosy of Ayurvedic Drugs. Vol:7. Revised Ed. Department of Pharmacognosy, Trivandrum. p.118-128
3. Anonymous 2001. Ayurvedic Phamacopoeia of India. Part 1, Vol: 1, 1st Ed. Ministry of Health and Family Welfare, Government of India. p. 6-11
4. Bhati S L, Agarwal M, Chakrawarti A and Purohit R K 2016. Chemopreventive action of *Emblica officinalis* against radiation and cadmium induced hepatic lesions in swiss albino mice. International Journal of Pharmaceutical Sciences and Research. 7(1): 370.
5. Mathur R, Sharma A, Dixit V P and Varma M 1996. Hypolipidaemic effect of fruit juice of Emblica officinalis in cholesterol-fed rabbits. Journal of Ethnopharmacology. 50(2); 61-68.
6. Mehta S, Singh R K, Jaiswal D, Rai P K and Watal G 2009. Anti-diabetic activity of *Emblica officinalis* in animal models. Pharmaceutical Biology. 47(11): 1050-1055.
7. Middha S K, Goyal A K, Lokesh P, Yardi V, Mojamdar L, Keni D S, Babu D and Usha T 2015. Toxicological evaluation of *Emblica officinalis* fruit extract and its anti-inflammatory and free radical scavenging properties. Pharmacognosy Magazine. 11 (Suppl 3): S427.
8. Pandey G 2005. Dravyagunavijnana. Part, 3rd Ed. Choukhamba Krishnadas Academy. Varanasi. p.102
9. Patel A P, Bhagwat S R, Pawar M M, Prajapati K B, Chauhan H D and Makwana R B 2016. Evaluation of *Emblica officinalis* fruit powder as a growth promoter in commercial broiler chickens. Veterinary World. 9(2):207.
10. Perianayagam J B, Sharma S K, Joseph A and Christina A J M 2004. Evaluation of anti-pyretic and analgesic activity of *Emblica officinalis* Gaertn. Journal of Ethnopharmacology. 95(1): 83-85.
11. Sairam K C H V, Rao C V, Babu M D, Kumar K V, Agrawal V K and Goel R K 2002. Antiulcerogenic effect of methanolic extract of *Emblica officinalis*: an experimental study. Journal of Ethnopharmacology. 82(1): 1-9.
12. Sharma P V 2011. Dravyaguna Vijnana. Vol: 2, Reprint Ed. Choukhamba Bharati Academy, Varanasi. pp.760
13. Shukla V, Vashistha M, Singh S N 2009. Evaluation of antioxidant profile and activity of Amalaki (*Emblica officinalis*), Spirulina and wheat grass. Indian Journal of Clinical Biochemistry. 24(1):70-5. Available at Http://Link.Springer.Com/Article/10.1007/S12291-009-0012-3. Accessed on18/3/2016
14. State Medicinal Plant Board Kerala 2012. Raw material consumption [ONLINE]. Available at: http://www.smpbkerala.org/raw-material-consumption.pdf. [Accessed on 7 May 2016].
15. Tandon N and Sharma M (Edts.) 2010. Quality Standards of Indian Medicinal Plants. Vol:8, Indian Council of Medical Research, New Delhi. p.160
16. Vasudevan M and Parle M 2007. Memory enhancing activity of *Anwalachurna* (*Emblica officinalis* Gaertn.): An Ayurvedic preparation. Physiology & Behavior. 91(1): 46-54.
17. Ved D K and Goraya G S 2007. Demand and supply of medicinal plants in India. NMPB, New Delhi & FRLHT, Bangalore, India, 18.
18. www.iucnredlist.org. 2015. *Phyllanthus indofischeri* - IUCN Red List. [ONLINE] Available at: http://www.iucnredlist.org/details/50126631/0. [Accessed on 16 December 2015].