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In vitro propagation of Phyllanthus emblica (L.) through different explants

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ABSTRACT

Phyllanthus emblica commonly called Amla is an important medicinal plant that belongs to Euphorbiaceae and is used in Ayurvedic, Unani, and Tibetan medicine systems. Due to its importance in medical systems, amla has a very high commercial value but propagation through conventional methods is difficult due to its highly unresponsive seeds and extended first reproductive phase. The present study propagates amla through micropropagation by direct as well as indirect organogenesis through nodes and leaves respectively. On MS medium, nodes (mature and juvenile) and leaves were inoculated with various concentrations of PGRs. When it came to nodes, mature nodes did not respond, whereas juvenile nodes produced the best results for axillary bud induction on MS medium containing BAP+GA3. For callus induction, MS medium with 2,4-D gave the best results.

Keywords: Phyllanthus emblica, micropropagation, axillary bud induction, callus, callus multiplication.

INTRODUCTION

Phyllanthus emblica (L.) (synonym *Emblica officinalis* Gaertn.), also known as 'Aonla,' 'Amla,' or Indian Gooseberry, is a member of the Euphorbiaceae and Geraniales order. The plant could be referred to as the "Mother of herbs" because of its effective therapeutic properties and significant medicinal and therapeutic demand [1]. As per ancient Indian mythology, the Amla tree was the very first tree to emerge on Earth. [2]. It is a deciduous tree that is indigenous to India, Indonesia, and the Malay Peninsula, as well as China, Myanmar, Sri Lanka, and Pakistan [3, 4]. The trees are found in tropical, subtropical, and coastal areas of India, along with hill cliffs and mountains (up to 200m and 4500ft., respectively), and are farmed in plain and hilly areas of Kashmir valley. [5,3].

Amla is a subtropical woody fruit-bearing tree that can be propagated in a variety of climatic conditions. The tree is grown in tropical and dry subtropical climates for effective large-scale cultivation. The tree requires 630-800nm of annual rainfall and can withstand temperatures as high as 46°C (warm temperature suitable for fruit growth). Because the tree is deep-rooted and

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deciduous, any type of soil is suitable for its growth. The plant can also survive over arid or semi-arid climatic conditions. *E. officinalis* has a huge potential for growth in high saline areas and, to a lesser extent, ravine land. According to Ayurvedic literature, the fruit has the following properties of rasa (flavor), veery (cooling), and vipaka (digestive). It is widely used in treatment of inflammation, fever and general burning sensations in the body [6]. Amla has proven efficacy as anti-diuretic, febrifuge, and in hairloss control in the Tibetan system of treatment [7]. In India, Amla fruits are largely used in anti-pyretic and anti-inflammatory medications [8,9]. The fruit is known to be rich in a wide array of active ingredients like alkaloids, tannins, amino acids, glycosides, phenolic acids and terpenoids[10-18]. The fruits juice of Amla is reported to have maximum content of vitamin C as compared to other fruits like lime, apple, grapes and pomegranate [19,20]. Different plant parts are rich in various phytochemically active ingredients [21-23] (Table 1)

Table 1: Different phytochemicals present in E. officinalis

Plant part	Phytochemical	Reference
Leaf	Amlaic acid, Astragalin, Ellagic acid, Gallo-tannin, Kaempferol, Kampferol-3-*O*-glucoside, Phyllantidine, Phyllantine, Rutin, Tannin	[24], [25]
Whole plant	Ascorbic acid, Lupenone	[26]
Seed	β-sitosterol, Flavonoid, Linolenic acid, Linoleic acid, Myristic acid, Oleic acid, Palmitic acid, Stearic acid, Tannin	[24], [26]
Fruit	3-6-di-*O*-galloyl-glucose, Alanine, Ascorbic acid, Aspartic acid, Arginine, Chibulinic acid, Chebulaginic acid, Chebulic acid, Chloride, Copper, Corilagic acid, Corilagin, Cystine, D-fructose, D- glucose, Ellagic acid, Emblicanin – A,-B, Emblicol, Ethyl gallate, Gallic acid, Gallic acid ethyl ester, Gibberellin A_4, Gibberellin A_7, Gibberellin A_9, Glucogallin, Glucose, Glutamic acid, Glycine, Histidine, Iron, Isoleucine, Leucine, L-malic acid 2-*O*-gallate, Manganese, Magnesium, Methionine, Myo-inositol, Myristic acid, Niacin, Nitrogen, Pectin, Phenylalanine, Phosphorus, Phyllemblinic acid, Phyllemblic acid, Polysaccharide, Potassium, Proline, Protein, Quercetin, Riboflavin, Rutin, Selenium,	[27], [28], [24], [29], [30]

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	Serine, Silica, Sodium, Starch, Sucrose, Sulfur, Tannin, Terchebin, Thiamin, Threonine, Trigalloyl glucose, Tryptophan, Tyrosine, Zinc, Zeatin, Zeatin riboside, Zeatin nucleotide, Phyllantine, Phyllantidine	
Twig	Tannin	[26]
Shoot	3-6-di-*O*-galloyl glucose, β-sitosterol,, Chebulagic acid, Chibulinic acid, Ellagic acid, Gallic acid, Glucogallin, Lupeol	[24]
Root	Ellagic acid, Lupeol	[26]
Bark	β-sitosterol, Leucodelphinidin, Lupeol, Tannin	[24], [25]

Although Amla can be propagated in a wide variety of climatic conditions through seed propagation or budding/grafting [31,32] in tropical and sub-tropical it is a challenge to propagate it for large-scale production[33]. *E. officinalis* cultivated through seeds generally produce an inferior quality of fruits and often show a long juvenile growth period. Trees in forests that are grown through sown seeds do not produce true-to-type plants and show a large variability in the pattern of fruit size, vegetative growth, yield, and size. These trees often require an extended time to attain stage 1 of their reproductive phase and thus are late bearers [34]. Special treatments like scarification, stratification, water-soaking, PGR treatment, and many more as well as favorable climatic conditions are required for fresh seeds to germinate but germination is still not guaranteed because they have hard and thick testa [35]. Methods of budding and grafting are also used for propagating amla plantlets, but these methods are cumbersome and time-consuming.

Consequently, tissue culture-based strategies are important for effective large-scale propagation of the species in a limited space. The present study was undertaken for developing a tissue culture protocol for the culture establishment of Amla.

MATERIALS and METHODS

The mother plant aged 2-3 years was collected from a local nursery in Roorkee, Uttarakhand state of India. Mature nodes (2-3 years old) and juvenile nodes (1-2 months old) were obtained from the mother plant between March and June, and leaves (2-3 months old) were collected between April and June from the mother plant. (Fig 1).

Explant washing and surface sterilization

Nodes were cut into 3-4 cm long segments and were washed with Teepol for 5mins, tween-20 for10 mins, and 1% fungicide(Bavistin) for 15 mins. Nodal segments were treated with varied $HgCl_2$

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concentrations(0.05%-0.1%) for different durations(1-7mins). Leaves were cut into 1-2cm bits, cleaned with Teepol for 5 minutes, then tween-20 for 5 minutes, and finally 1% fungicide (Bavistin) for 5 minutes. Finally, leaves were surface sterilized for varying lengths of time (30 -120secs) with different concentrations of HgCl2 (0.05%-0.1%).

Nodal culture establishment and culture conditions

For culture establishment, thoroughly washed and surface disinfected nodal explants(mature and juvenile) of length approx 3-4 cm containing axillary buds were inoculated on an induction medium. The induction medium constituted of full-strength MS basal medium (liquid and semi-solid) (Murashige and Skoog, 1962) comprising 3% carbon source (Sucrose or sugar), 0.6% (w/v) agar, and various combinations and concentrations of PGRs such as BAP (0.5-4.0mg/l), GA3 (0.5-1.0mg/l), NAA (0.1mg/l), IBA (1mg/l). The media's pH was maintained at 5.8. The media was autoclaved at 120° C and 15psi for 15 minutes after being poured into 250 ml flasks in 100 ml aliquots. The temperature conditions in culture room were kept at 37 ± 2^0C, with 60-65% relative humidity, 16:8(16 hrs light and 8 hrs dark) light period supplied by yellow bulbs (1300+ lumens (100 watts), and white lights (50 µ mol m^{-2} sec^{-1}).

***In-vitro* callus induction and culture conditions**

Leaf segments that were surface disinfected were were cut into leaf bits (approx. 1-2cm) and slight incisions were made in the leaves with a sterile scalpel. The leaf bits were inoculated horizontally on the culture medium. The culture medium for callus induction consisted of a full-strength MS basal medium, 3% carbon source (Sucrose, sugar), and 0.6% w/v agar. The media was supplemented with various concentrations of PGRs viz., 2,4-D (0.5-1.0mg/l), BAP (1.0 mg/l). The pH, autoclaving and incubation conditions were the same as that of nodal explant culture.

After culturing the callus for 2 weeks in a callus induction medium, the callus was subcultured onto the multiplication medium. The medium contained a full-strength MS medium, 3% sucrose, and 0.6% w/v agar used for the purpose. Prior to adding agar to the multiplication medium, the pH was set at 5.8 using 1N NaOH and IN HCl. After adding agar to the medium, PGRs were added. The medium was supplemented with Auxin 2,4-D (1mg/l). The medium was poured in 100ml aliquots into a 250ml flask. For 45 days, the callus was cultured at 372° C with 60-65% relative humidity, a 16:8 (16 hours light and 8 hours dark) light period supplied by cool white fluorescent light at an intensity of 50 µmol m^{-2} sec^{-1} and standard yellow bulbs with 1300+ lumens (100 watts) in the culture room.

RESULTS

Initiation of contamination-free cultures of nodal explant

Mature and juvenile nodes of *P. emblica* were subjected to surface sterilization with surface sterilant $HgCl_2$ at varied concentrations for different durations. Both fungal and bacterial contamination appeared in the cultures and were persistent in both mature and juvenile nodes. Also, the mature nodes did not respond to any kind of treatment, they proved to be recalcitrant in liquid as well as semi-solid medium, and hence the juvenile nodes were selected as the explants for further experiments.

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The juvenile nodes proved to be better choice explants as compared to mature nodes. The fungal and bacterial contamination was also seen in the cultures. To prevent fungal contamination, 1% Bavistin(fungicide)treatment was given to the explants for 15 mins. Explants were subjected to surface sterilization by $HgCl_2$. The best result was found to be in the concentration of 0.1% $HgCl_2$ for 3 mins. The period of fewer than 3 mins showed fungal/ bacterial contamination while the duration of more than 3 mins proved to be lethal for the explants. Also, the addition of amoxicillin (25mg/l) in the culture medium prevented bacterial contamination for all the subsequent experiments and was used for culture establishment(Table 2,3) (Fig 2).

Axillary Bud Induction

As mentioned above, the mature nodes of the Amla plant did not respond in any of the mediums (liquid and semi-solid).

However, in the case of juvenile node, the explants responded to various concentrations of PGRs, and different carbon sources in only a semi-solid medium. Full strength MS medium with 3% sucrose, 0.6% w/v agar supplemented with BAP (1mg/l) and GA3 (1mg/l) yielded the best results for bud primordia formation, with an average bud formation of 95%. Aside from this, bud primordia formation occurred in full strength MS medium with 3% sucrose and 3% sugar supplemented with BAP (4mg/l)+ NAA (0.1mg/l)+ 50 mg/l Ascorbic acid with an average bud formation of 25%, BAP(1mg/l)+GA3 (0.5mg/l) with an average bud formation of 60%, and BAP(2mg/l)+ IBA(1mg/l) with average bud formation of 10% (Table 4) (Fig 3).

The effect of temperature was also observed on the axillary bud induction. It was found that in the presence of white light the induction rate was slow however, in the presence of both white light and bulbs(100 watts) the induction rate was high.

Initiation of contamination-free cultures of leaf explant

Initially, the leaf cultures of Amla showed fungal as well bacterial contamination. To prevent fungal contamination 1% Bavistin (fungicide) treatment was given to the explants for 5 mins and surface sterilization of the explants was done with $HgCl_2$ varied concentrations for different durations. The best result, the viable cultures were found to be in the concentration of 0.1% $HgCl_2$ for 60 secs(1 min). The duration of fewer than 60 secs showed excessive contamination while the period of more than 60 secs turned the explants black and eventually the explants died.

Callus induction

After the establishment of contamination-free cultures, the callus induction took place in 2 weeks. The culture medium contained full strength MS medium, different carbon sources (3%), 0.6% w/v agar, and various PGRs with varied concentrations. The callus induction took place in full strength MS medium, 3% sugar, 0.6% w/v agar, PGRs viz, BAP(1mg/l)+ 2,4-D(0.5 mg/l) with average of callus formation of 70%. The best result of callus induction was found in full strength MS medium, 3% sucrose, 0.6% w/v agar, and 2,4-D (1mg/l) with an average callus formation of 90%, the same medium was used to initiate callus formation with a slight change, sucrose(3%) was replaced with sugar(3%) and the average callus formation was 85%. The experiments showed that the cultures containing sugar showed less phenolic leaching as compared to the medium containing sucrose. The

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effect of temperature was also observed on the callus multiplication. The callus multiplied rapidly when the temperature was increased in the presence of white light and bulbs(100 watts) while a slow multiplication rate was observed when cultured solely in white light (Table 5,6) (Fig 4-6).

Callus multiplication

The callus was multiplied after 2 weeks of formation. The best medium for callus multiplication seemed to be full strength MS medium with 3% sucrose and 0.6% w/v agar, supplemented with 2,4-D (1mg/l). The callus multiplied rapidly in the mentioned medium. Also, initially, the callus was pulpy in texture but after the regular transfer, the callus changed its texture from pulpy on day 0, to friable on day 15, to compact on day 30, and finally hard and compact on day 45 (Table 7) (Fig 7-10).

Table 2: Effect of sterilant ($HgCl_2$) treatment on surface sterilization of nodal explants

Concentration (in%)	**Duration (mins)**	**Explant**	**Observation**	**% Bud induction**
0.05	7	**Mature node**	Nil	0
	5		Nil	0
	3		Nil	0
	1		Nil	0
	7	**Juvenile node**	Nil	0
	5		Nil	0
	3		Nil	0
	1		Nil	0
0.1	7	**Mature node**	Nil	0
	5		Nil	0
	3		Nil	0
	1		Nil	0
	7	**Juvenile node**	Bud induction	5

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	5		Bud induction	10
	3		Bud induction	90
	1		Bud induction	40

Table3: Effect of sterilant ($HgCl_2$) treatment on surface sterilization of leaf explants

Concentration (in%)	**Duration (secs)**	**Observation callus induction**	**% Bud induction**
0.05	30	Nil	0
	60	Nil	0
	90	Nil	0
	120	Nil	0
0.1	30	Callus induction	20
	60	Callus induction	93
	90	Callus induction	15
	120	Callus induction	5

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Table4: Observation of Axillary bud induction

MS Media	**BAP (mg/l)**	**GA_3 (mg/l)**	**NAA (mg/l)**	**Kn (mg/l)**	**IBA (mg/l)**	**Activated charcoal (%)**	**Ascorbic acid (mg/l)**	**Mature nodal explant**	**Juvenile nodal explant**	**% Response**
Liquid- L1	1	-	-	-	-	-	-	-	-	0
L2	1	0.5	-	-	-	-	-	-	-	0
L3	0.5	0.5	-	-	-	-	-	-	-	0
Semi-solid-S1	1	-	-	-	-	0.2	-	-	-	1
S2	2	-	-	-	1	-	-	-	+	10
S3	1	1	-	-	-	-	-	-	+	95
S4	2	0.5	-	-	-	-	-	-	-	5
S5	1	0.5	-	-	-	-	-	-	+	60
S6	4	-	0.1	-	-	-	50	-	+	25

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Table5: Effect of culture media on callus induction

Media	BAP (mg/l)	2,4-D (mg/l)	% Response	Minimum period for callus induction	Observation	Appearance of callus
C1	-	0.5	0	0	No callus induction	-
C2	1	0.5	70	10	Callus induction	Whitish green
C3	1	1	5	15	Callus induction	Light Green
C4	-	1	90	7	Callus induction	Green

Table6: Effect of Carbon source on callus induction in young leaves

Media	Sucrose (gm/l)	Sugar (gm/l)	Degree of Callus induction	Degree of Phenolic leaching
C1	30	-	-	+++
C2	-	30	+	++
C3	30	-	+	+++
C4	30	-	++	+++
C4	-	30	+++	+

Degree of callus induction
- No callus

+ Poor callus induction
++ Fair callus induction
+++ Good callus induction

Degree of Phenolic leaching
+ Low Phenolic leaching

++ High Phenolic leaching
+++ Very high Phenolic leaching

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Table7: Observations on callus multiplication

No. of Days	Degree of callus induction	Texture of callus
0	-	Pulpy
15	+	Friable
30	++	Compact
45	+++	Hard and compact

- Transferred callus

+ Fair callus induction
++ Good callus induction
+++Excellent callus induction

Fig1:Juvenile nodal explant

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Fig2:Fungal contamination

Fig3:Axillary Bud Induction

Fig4:Leaf explant

Fig5:Leaf inoculation on medium

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Fig6:Callus induction

Fig7:Callus multiplication

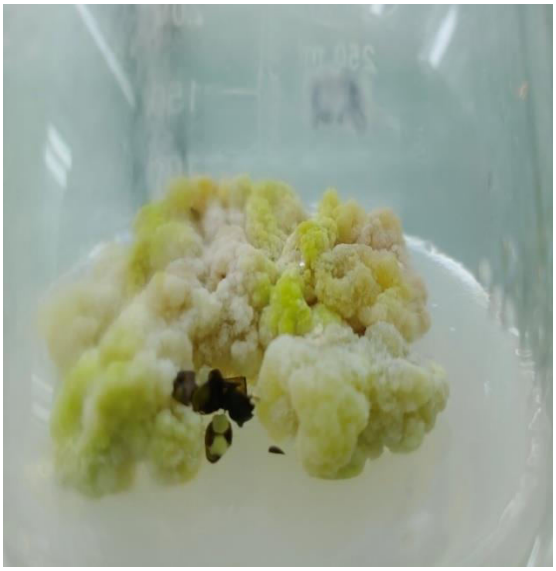

Fig8:Callus on day 15

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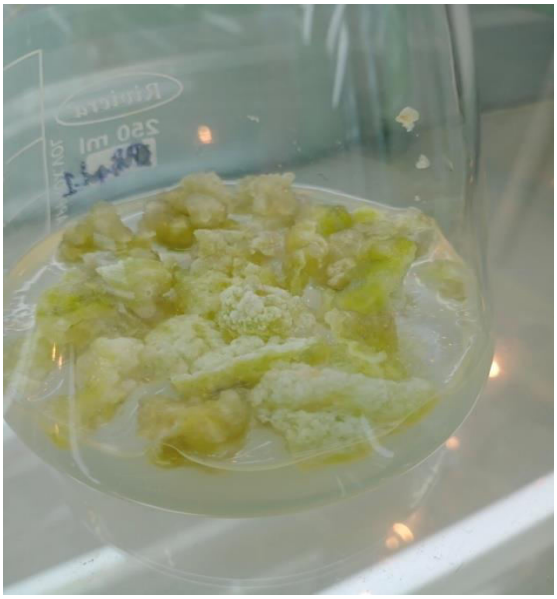

Fig9:Callus on day 30

Fig10:Callus on day 45

CONCLUSION

Phyllanthus emblica (L.) or *Emblica officinalis* (Gaertn.) (Amla) is an extremely valuable medicinal plant that originated in India. Due to the lack of proper resources, the difficulty in cultivation in tropical and sub-tropical regions as well as its not true-to-type new plant generation it is not produced on a large-scale level. These factors have led to the studies of micropropagation of Amla via tissue culture technique. In the present study, the sterilization procedure was standardized for the nodal segments and leaves of the *P. emblica*. For the juvenile nodes, 0.1% $HgCl_2$ treatment for 3 mins was successful to remove contamination and was preceded by 15 mins fungicide treatment. For leaf explants, 0.1% $HgCl_2$ treatment for 1 min preceded by 5mins of fungicide treatment showed the best results. MS medium supplemented with BAP (1mg/l) + GA3 (1mg/l) yielded the best results for the in-shoot bud primordia formation for the juvenile nodal segment. The best results were obtained for callus induction from leaf explants in MS medium supplemented with 2,4-D (1mg/l). The best media for in-vitro callus culture and callus multiplication was observed toward being an MS medium consisting of 2,4-D (1mg/l). The temperature affected the explants as well. It was deduced that both nodal segments and leaf explants responded better to higher temperatures than lower temperatures. The current study's findings can be applied to the mass propagation of *P. emblica* (L.).

REFERENCES

1. Goyal D, Bhadauria S (2008) In vitro shoot proliferation in Emblica officinalis var. Balwant from nodal explants. Indian J Biotechnol 7:394–397.
2. Pria, F.F.; Islam, M.S. Phyllanthus emblica Linn. (Amla)—A Natural Gift to Humans: An Overview. J. Dis. Med. Plants 2019, 5, 1–9.
3. Thilaga S, Largia MJV, Parameswari A, Nair RR, Ganesh D (2013) High frequency somatic embryogenesis from leaf tissue of 'Emblica officinalis' Gaertn.—a high valued tree for non-timber forest products. Aus J Crop Sci 7:1480

Webology, Volume 18, Number 1, 2021
ISSN: 1735-188X
DOI: 10.29121/WEB/V18I1/31

4. Walia, K.; Boolchandani, R.; Dhand, S.; Antony, B. Improving glycemic & lipidemic profile with amla powder (Emblica officinalis) supplementation in adults with type 2 diabetes mellitus. Int. J. Basic Appl. Med. Sci. 2015, 5, 251–258.
5. Rai N, Tiwari L, Sharma RK, Verma AK (2012) Pharmaco-botanical profile on Emblica officinalis Gaertn.—a pharmacopoeial herbal drug. STM J 1:29–41
6. Williamson EM (editor), Major Herbs of Ayurveda, 2002 Churchill- Livingstone, London.
7. Tsarong and Tsewang J. Tibetan Medicinal Plants. 1st ed. Tibetan Medical publications India, 1994.
8. Ihantola-Vormisto A, Summanen J, Kankaanranta H, Vuorela H, Asmawi M, Moilanen E (1998) Anti-inflammatory activity of extracts from leaves of Phyllanthus emblica. Planta Med 63(6):518–524. https://doi.org/10.1055/s-2006-957754
9. Srivasuki K.P. (2012) Nutritional and health care benefits of Amla. Journal of Pharmacognosy, ISSN: 0976-884X & E-ISSN: 0976- 8858, Volume 3, Issue 2, pp.-141-151.
10. L.Z. Zhang, W.H. Zhao, Y.J. Guo, G.Z. Tu, S. Lin, and L.G. Xin. Studies on chemical constituents in fruits of Tibetan medicine Phyllanthus emblica, Zhongguo Zhong Yao Za Zhi. 28 (10) (2003) 940-943.
11. Y.J. Zhang, T. Abe, T. Tanaka, C.R. Yang, and I. Kouno. Phyllanemblinins A-F, new ellagitannins from Phyllanthus emblica, J Nat Prod. 64 (12) (2001) 1527-1532.
12. Y.J. Zhang, T. Abe, T. Tanaka, C.R. Yang, and I. Kouno. Two new acylated flavanone glycosides from the leaves and branches of Phyllanthus emblica, Chem Pharm Bull (Tokyo). 50 (6) (2002) 841-743.
13. Y.J. Zhang, T. Tanaka, C.R. Yang, and I. Kouno. New phenolic constituents from the fruit juice of Phyllanthus emblica, Chem Pharm Bull (Tokyo). 49 (5) (2001) 537-540. 51
14. Y.J. Zhang, T. Tanaka, Y. Iwamoto, C.R. Yang, and I. Kouno. Novel sesquiterpenoids from the roots of Phyllanthus emblica, J Nat Prod. 64 (7) (2001) 870-873.
15. Y.J. Zhang, T. Tanaka, Y. Iwamoto, C.R. Yang, and I. Kouno. Novel norsesquiterpenoids from the roots of Phyllanthus emblica, J Nat Products. 63 (11) (2000) 1507-1510.
16. Y.-J. Zhang, T. Tanaka, Y. Iwamoto, C.-R. Yang, and I. Kouno. Phyllaemblic acid, a novel highly oxygenated norbisabolane from the roots of Phyllanthus emblica, Tetrahedron Lett. 41 (11) (2000) 1781-1784.
17. S.S. Subramanian, S. Nagarajan, and N. Sulochana. Flavonoids of some euphorbiaceous plants, Phytochemistry. 10 (10) (1971) 2548-2549.
18. D. Krishnaiah, T. Devi, A. Bono, and R. Sarbatly. Studies on phytochemical constituents of six Malaysian medicinal plants, J Med Plant Res. 3 (2) (2009) 67-72.
19. S.K. Jain and D.S. Khurdiya. Vitamin C enrichment of fruit juice based ready-to-serve beverages through blending of Indian gooseberry (Emblica officinalis Gaertn.) juice, Plant Foods Hum Nutr. 59 (2) (2004) 63-66.
20. K. Tarwadi and V. Agte. Antioxidant and micronutrient potential of common fruits available in the Indian subcontinent, Int J Food Sci Nutr. 58 (5) (2007) 341-349.
21. Y.J. Zhang, T. Tanaka, Y. Iwamoto, C.R. Yang, and I. Kouno. Novel sesquiterpenoids from the roots of Phyllanthus emblica, J Nat Prod. 64 (7) (2001) 870-873.
22. Y.J. Zhang, T. Tanaka, Y. Iwamoto, C.R. Yang, and I. Kouno. Novel norsesquiterpenoids from the roots of Phyllanthus emblica, J Nat Products. 63 (11) (2000) 1507-1510.

23. Y.-J. Zhang, T. Tanaka, Y. Iwamoto, C.-R. Yang, and I. Kouno. Phyllaemblic acid, a novel highly oxygenated norbisabolane from the roots of Phyllanthus emblica, Tetrahedron Lett. 41 (11) (2000) 1781-1784.
24. Srikumar R, Parthasarathy NJ, Shankar EM, Manikandan S, Vijayakumar R, Thangaraj R, Vijayananth K, Sheela DR, Rao UA (2007) Evaluation of the growth inhibitory activities of triphala against common bacterial isolates from HIV infected patients. Phytother Res 21:476–480
25. Khan KH (2009) Roles of Emblica officinalis in medicine—a review. Bot Res Int 2:218–228
26. Gantait, S., Mahanta, M., Bera, S. *et al.* Advances in biotechnology of *Emblica officinalis* Gaertn. syn. *Phyllanthus emblica* L.: a nutraceuticals-rich fruit tree with multifaceted ethnomedicinal uses. *3 Biotech* **11,** 62 (2021). https://doi.org/10.1007/s13205-020-02615-5
27. Ghosal S, Tripathi VK, Chauhan S (1996) Active constituents of Emblica officinalis: part 1—the chemistry and antioxidative effects of two new hydrolysable tannins, Emblicanin A and B. Indian J Chem B 35:941–948
28. Jagetia GC, Malagi KJ, Baliga MS, Venkatesh P, Veruva RR (2004) Triphala, an ayurvedic rasayana drug, protects mice against radiation-induced lethality by free-radical scavenging. J Altern Complement Med 10:971–978.
29. Singh E, Sharma S, Pareek A, Dwivedi J, Yadav S, Sharma S (2011) Phytochemistry, traditional uses and cancer chemopreventive activity of amla (Phyllanthus emblica): the sustainer. J App Pharm Sci 2:176–183.
30. Srinivasan P, Vijayakumar S, Kothandaraman S, Palani M (2018) Anti-diabetic activity of quercetin extracted from Phyllanthus emblica L. fruit: In silico and in vivo approaches. J Pharm Anal 8:109–118.
31. Wali VK, Bakshi P, Jasrotia A, Bhushan B, Bakshi M (2015) Aonla. SKUAST-Jammu, India, p 30.
32. Jalal A, Tripathi S, Kumar A (2019) Standardization of propagation techniques in different cultivars of aonla (Emblica officinalis Gaertn). Int J Pure Appl Biosci 7:71–77.
33. Sengupta P, Sen S, Mukherjee K, Acharya K (2020) Postharvest diseases of Indian gooseberry and their management: a review. Int J Fruit Sci 20:178–190.
34. Sampath Kumar KP, Bhowmik D, Dutta A, Pd Yadav A, Paswan S, Srivastava S, Deb L (2012) Recent trends in potential traditional Indian herbs Emblica officinalis and its medicinal importance. J Pharmacogn Phytochem 1:18–28.
35. Barathkumar TR (2019) Studies on influence of different seed treatments on dormancy breaking in aonla (Phyllanthus embolic L.). J Pharmacogn Phytochem 2019:131–133.
36. Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497.