



Factors Influencing the Antioxidant Potential of Amla and Its Products

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Authors' contributions

This work was carried out in collaboration by four authors. Author VA managed the literature searches designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors SN, RB and PP managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Emblica officinalis*, locally known as Amla, is a highly nutritious seasonal fruit. The processed fruit could be an important dietary source of natural vitamin C with potent antioxidant activity. The aim of present investigation was to study factors influencing antioxidant potential of Amla and its products.

Methodology: Three Indian varieties of Amla fruits in three ripening states (unripe, semi ripe and fully ripe) were analyzed for total phenolic contents, vitamin C as well as antioxidant potential. Two Amla products were studied over a period of 33 days for these parameters.

Results: In the present data, the fully ripe stage of Amla had the highest value of vitamin C ($804.4 \pm 0.8 \text{ mg}/100 \text{ g}$) and polyphenols ($1300.3 \pm 99.6 \text{ mg}/100 \text{ g}$) than the *unripe*, semi ripe

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stages. The DPPH Scavenging activity was found to be highest in semi ripe stage. ABTS radical scavenging (IC_{50} values) of unripe, semi ripe and ripe were 18.5 ± 0.0071 , 34.7 ± 0.026 , 21.9 ± 0.024 respectively. The stability study for syrup and pickle, processed from Amla fruit, demonstrated sharper decrease of the vitamin C content for pickle than decrease for syrup over the time 33 days but the polyphenols and the free radical scavenging activity remained the same over the time.

Conclusion: The present study suggested that ripening stages influence the antioxidant potential of Amla fruit. The contents of vitamin C showed a significant decrease over time in syrup and pickle but the antioxidant potential remained unaffected.

Keywords: Amla; ripening stages; vitamin C; phenolic contents; antioxidant activity.

1. INTRODUCTION

The Amla tree (*Emblica officinalis* Gaertn.) is native to tropical Southeast Asia, particularly found in central or southern India, Pakistan, Bangladesh, Sri Lanka, Malaya, Southern China and in Mascarene Islands. Amla also called as Indian gooseberry is known for its medicinal and therapeutic properties from the ancient time in India (during 1500 BC-1300 BC) and has been considered as a wonder fruit for health conscious people. It has been grown and known in India for more than 3500 years. In western and eastern hills of India, three species viz. *Phyllanthus emblica*, *Phyllanthus indofisherii* and *Phyllanthus acidus* are of common occurrence [1]. Fruits in North and western India are ready for harvesting in November-December but can remain on the tree till February [2]. There are several varieties of Amla grown throughout different parts of India and the most popular are *Banarasi* and *Chaikiye*. However, these varieties are yet to be researched in terms of physico chemical properties and for value addition [3]. Further the efforts to study effects of maturity on nutraceutical and health related parameters like vitamin C have been limited [4].

Amla (*Emblica officinalis* Gaertn) primarily contains tannins, alkaloids, phenolic compounds, amino acids and carbohydrates. Its fruit juice contains the highest vitamin C ($478.56 \text{mg}/100 \text{mL}$) [5]. It has been considered as one of the super fruits due to high levels of ascorbic acid and antioxidants. Due to rich vitamin C content (L-ascorbic acid) and other phytonutrients in amla fruit, several reports are available attributing its biological and therapeutic effects against diseases like cancer, diabetes etc [6,7]. However, the variability among different amla fruits, depending upon their size, variety, has not been reported. The fruit of *E. officinalis* has been considered to be potent antioxidant source [8,6]. But, amla being a seasonal fruit, the fresh fruit is not available throughout year for consumption. The process/product with higher shelf life as well as higher retention of original antioxidant power needs to be evolved. Vitamin C content increases with fruit growth and attains the maximum levels at maturity [9,4,3] but there is no appreciable change reported in N contents or in reducing and non reducing sugars [1]. Majority of reported studies have been on the nutraceutical and health potential of fruits or the isolated active principles such as tannoids. But considerable diversity exists in between the varieties. Further, efforts are needed to study effects of maturity and storage on antioxidant potential in amla products which has not been much reported.

The objectives of the present paper were: 1) Comparative variability between popular amla varieties and maturation stages for their antioxidant potentials and 2) Process for making

antioxidant rich amla products (pickle or syrup/juice) and study their stability for antioxidant potential

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and solvents used in the study were of analytical grade. 2, 2-diphenyl-1-picryl hydrazyl (DPPH) was purchased from Sigma. 2, 2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was from obtained Aldrich. Ascorbic acid was procured from Hi-media India.

2.2 Raw Materials

Three different varieties viz. *Banarasi*, *Chaikiya* and *Dongri* were available of amla fruits in the market which varied in size and shades of green color and were labeled as C1, C2, C3. The shade of bottle green changes to yellowish green and pale yellow during ripening. For each of the types, a minimum of 5 fresh fruits each of ripe, semi ripe and unripe states were purchased from a local market of Pune, Maharashtra, during the period Jan-Feb 2013 for the analysis in triplicates. Initially, each fresh fruit sample was washed under running tap water followed by washing with distilled water to remove the surface impurities.

2.3 Preparation of Syrup and Pickle

For preparing the juice, unpeeled fruits were weighed (500g) and after removing the seeds, these were minced using a domestic mixer grinder. Juice was filtered and squeezed using muslin cloth. Syrup was prepared by mixing the juice and sugar syrup in 1:2 ratio and stored in a refrigerator in air tight glass bottle without adding any preservative. Pickle was prepared by mixing 200g finely grated unpeeled amla with 30g salt, 70g ready pickle masala (fenugreek seeds roasted on oil, mixed with a powders of mustard seeds, red chili, turmeric and asafetida and ground), 50g jaggery and layered with 30g Soya oil (previously heated and cooled). It was also stored in refrigerator in air tight glass bottle.

2.4 Evaluation of *in vitro* Antioxidant Activity

Estimation of Vitamin C: Determination of vitamin C was done using reduction of 2, 6 dichlorophenol indophenol (DCPIP) as previously reported [10]. Fruit extracts for vitamin C analysis were prepared using 0.1 gram of minced amla tissue (flesh and peel) in case of raw fruits and in case of product 0.1 gram of syrup and pickle and 2 ml of 3% (w/v) meta phosphoric acid (w/v) in water for 3 hours. The homogenates were centrifuged at 15,000 rpm at 4°C for 10 minutes. The supernatant was measured for vitamin C immediately as O.D at 540 nm. Vitamin C was expressed as mg equivalents DCPIP dye reducing potential by comparison with the standard and expressed as mg/100g.

Estimation of total phenolics content: Total phenolic content was determined using the Folin-Ciocalteu's colorimetric assay for total phenol analysis 0.1 gram of amla fruit and product samples were suspended in 2 mL distilled water at room temperature and extracted for 2 hours. In brief, 2 mL of distilled water was pipetted into test tubes followed by 50 µL of test sample extract or 20 to 100 all of standard tannic acid (0.2 mg/mL). 100 µL Folin Ciocalteu reagent (1N) was added to each test tube except sample blank and the solutions were mixed

thoroughly. After 30 seconds, 300 μ L of Sodium carbonate (20% in distilled water) was added. The tubes were incubated at 37°C for 2 h. The absorbance of blue chromospheres was measured at 700nm [11]. The total phenolic concentration was calculated from a calibration curve by plotting (0.2mg/mL) of tannic acid for absorbance at 700 nm and results were expressed as mg of tannic acid equivalents (TAE) per 100 g of sample.

DPPH Scavenging capacity: Measurement of DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity was done [12] with slight modifications. For DPPH Scavenging capacity analysis, 0.1 g of Amla fruit and product sample were taken in methanol and extracted for 2 hours. One mL of DPPH reagent (75 μ M in methanol) and 50 μ L of test samples in methanol were incubated at 37°C for 80 min. Trolox (0.1mg/mL) was used as a standard control and the reduction of the absorbance at 515 nm was monitored. A control reading was obtained using methanol instead of the extract. Free radical scavenging activity was expressed as % Inhibition = $[(A_0 - A_1)/A_0] \times 100$ Where, A_0 is the absorbance of the control (without test samples), A_1 is the absorbance of test samples. Results have also been reported as IC_{50} , which is the amount of antioxidant necessary to decrease the initial DPPH• concentration by 50%.

The Trolox equivalent antioxidant activity (TEAC): The TEAC was estimated using the ABTS⁺ system [13]. The radical scavenging capacity of samples was evaluated against ABTS⁺ generated by oxidizing 5 mM of ABTS (2, 2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt, with manganese dioxide in PBS (pH 7.4) at ambient temperature for 2 hours in dark. For TEAC estimation, 0.1 g of amla fruit and product sample were taken in methanol and extracted for 2 hours. The ABTS antioxidant reaction mixture contained 1.0 mL of ABTS⁺ with an absorbance of 0.85 at 734 nm and 10 μ L of antioxidant testing sample extract in methanol or Trolox (0.1 mg/mL in methanol) as a positive control. Calibration curve of Trolox (0.1mg/mL) was plotted and results were expressed IC_{50} , which is the amount of antioxidant necessary to decrease the initial ABTS⁺ concentration by 50%.

All the assays were done in triplicates for twelve fruit samples and two products and results were expressed as mean \pm SD. Statistical calculations were performed using Microsoft Excel-2007. For the quality control of vitamin C assays and TEAC, the repeatability of OD values for standard curve was compared using one way ANOVA. The results showed non-significant differences between assays ($p > .1$).

3. RESULTS AND DISCUSSION

3.1 Variability in Nutraceutical Potential of Amla Fruit

Estimations of vitamin C, Polyphenols, TEAC and DPPH radical scavenging assay were done for twelve different amla samples based on variety and ripening stages. The values of Vitamin C (mg/100g) are shown in Fig. 1. The ripe state of Amla and semi ripe state showed significantly higher values of vitamin C than the unripe state ($P < .001$, $F = 218$). The values for polyphenols suggested similar pattern (Fig. 2) as that for vitamin C. Further, the two way ANOVA indicated F values to be highly significant indicating differences between varieties ($P = .002$), ripening states and their interactions ($P < .01$). The IC_{50} values for TEAC of unripe Amla (Fig. 3) were lowest for type C1 indicating antioxidant activity to be highest. Also two way ANOVA showed F values to be highly significant ($P < .001$) for variety, ripening states and interaction. From Fig. 4, it can be seen that, among all types, semi ripe stage had the

highest value for DPPH free radical scavenging activity. ANOVA indicated significant F for differences between varieties ($P = .021$) and for ripening stage ($P = .008$).

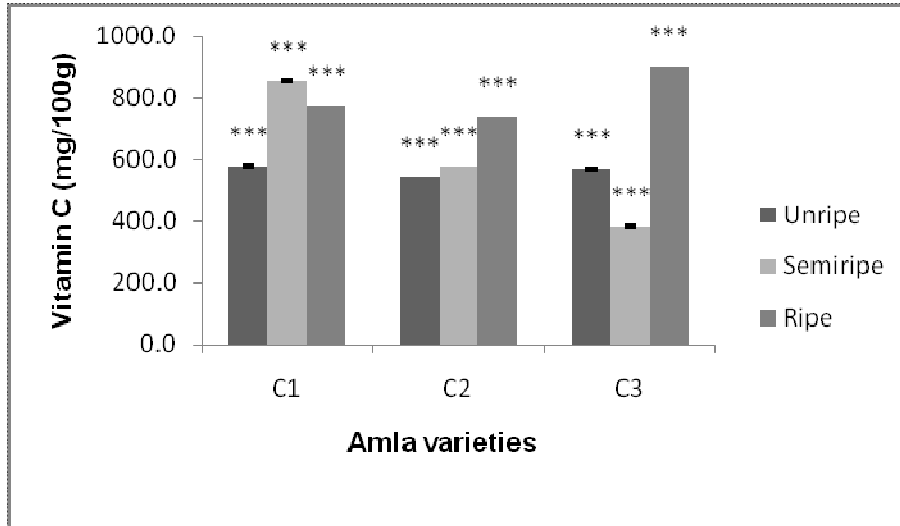


Fig. 1. Vitamin C content of Amla fruit during different ripening stages expressed in mg/100g

Values represent mean of triplicate analyses and differences between varieties and maturation stages were significant ($p < .001$)

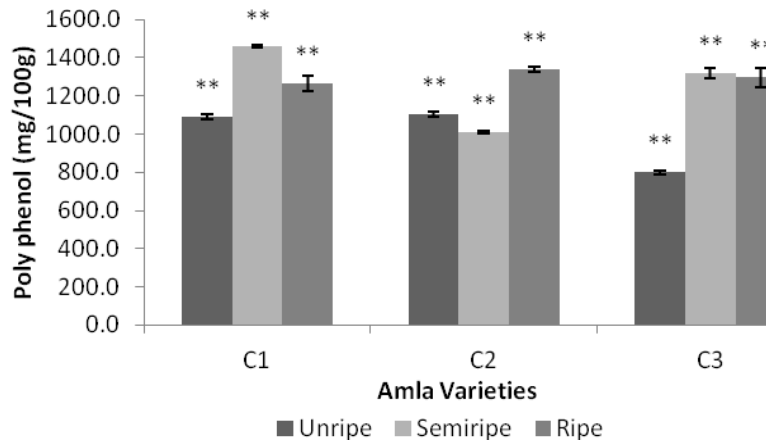


Fig. 2. Total phenol content of Amla fruit during different ripening stages expressed in mg/100g

Values represent mean and S.E. of triplicate analyses and differences between varieties and maturation stages were significant ($p < .005$)

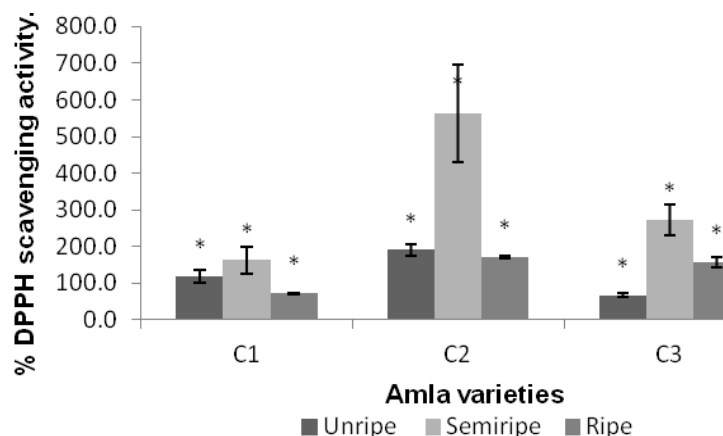


Fig. 3. DPPH radical scavenging activity of Amla fruit during different ripening stages expressed as % value

Values represent mean and S.E of triplicate analyses and differences between varieties and maturation stages were significant ($p < .005$)

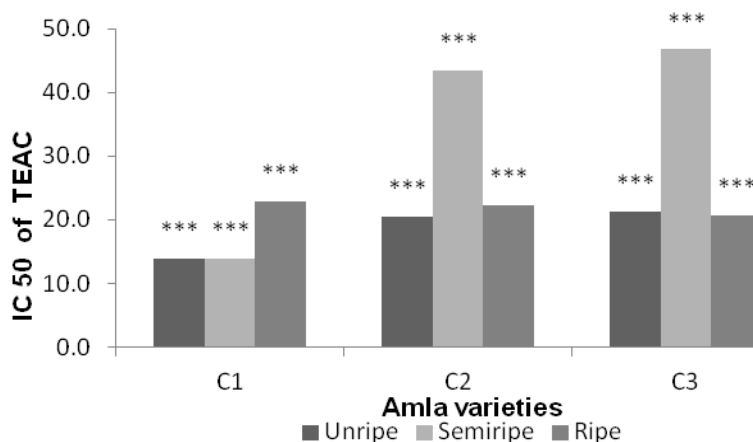


Fig. 4. ABTS radical scavenging activity (TEAC) of Amla fruit during different ripening stages expressed as IC₅₀ value

Values represent mean and SE of triplicate analyses and differences between varieties and maturation stages were significant ($p < .001$)

3.2 Stability Study

Syrup and pickle were freshly made from Amla fruit. These processed products as well as raw Amla stored under refrigerated conditions were analysed over a time period 33 days for the antioxidant potential (Figs. 5-7). As can be seen from the data, over the time of 33 days, vitamin C values for the syrup showed a steady decrease from 64.6 to 32.5mg/100mL. Similar but sharper decreasing trend was observed in case of pickle with a decrease from 60.6 to 14.4 mg/100g over the same time period. This was also reflected in the slope of a linear fit to the data (-0.96 vs. -1.56). However, the polyphenol content of syrup did not show

any decreasing trend over the time and pickle showed a slightly decreasing trend over time. The TEAC of syrup remained unchanged over the time period of 24 days. Similar to syrup, TEAC for pickle was also stable over the time period. DPPH activity also remained stable over the time period like the TEAC activity. On the whole, the antioxidant activity of syrup and pickle remains stable over time.

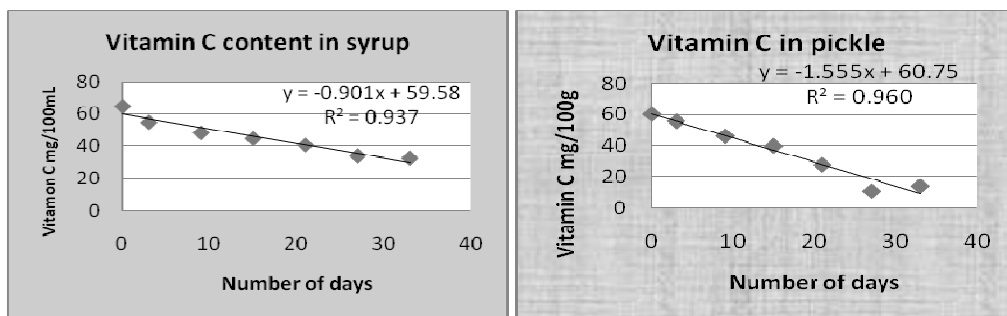


Fig. 5. Stability study of vitamin C of syrup(a) and pickle (b) over a period of 33 days and expressed as mg/100g
Values represent means of triplicate analyses.

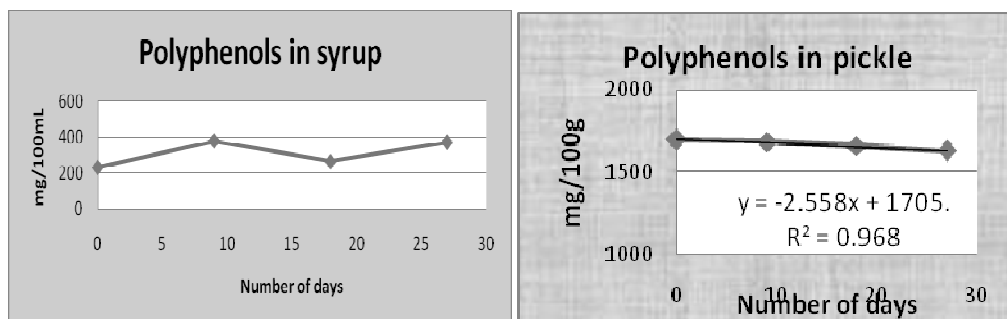


Fig. 6. Stability study of vitamin C of syrup (a) and pickle (b) over a period of 33 days and expressed as mg/100g
Values represent mean and SE of triplicate analyses

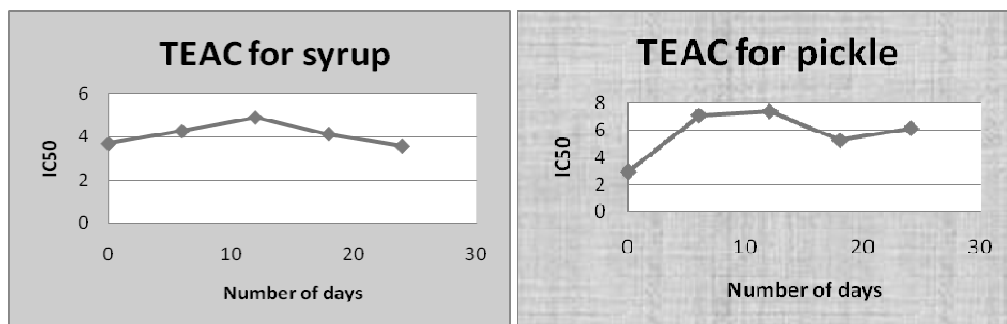


Fig. 7. (a) Stability study of vitamin C of syrup (a) and pickle (b) over a period of 33 days and expressed as IC 50 value
Values represent mean and SE of triplicate analyses

Amla is one of the Indian medicinal plants which are rich sources of antioxidants, apart from other phytochemicals. Amla fruits have many health benefits and are used as a principal ingredient in the preparation of famous ayurvedic preparation known as chyavanprash and now-a-days it is also used as health beverage or Juice [14]. The antioxidant activity of the fruit is due to the presence of low molecular weight tannoids, mainly due to emblicanin A and B [7]. Many papers are published regarding its effects against diseases like cancer, diabetes etc. But the variability among different *Emblica officinalis* fruits, depending upon their size, variety, has not been reported. Secondly, many products have been processed from the fruit. During the processing, there are number of treatments given to make the desired product. These treatments include sun drying, heat treatment, addition of chemical preservatives etc. and due to which the vitamin C content and antioxidant capacity gets reduced.

Amla is a nutritionally and medicinally important fruit due to its high vitamin C content [15]. Fruit has been reported to contain a wide range of ascorbic acid (200-1800mg/100g [2]. While the vitamin C estimations by Agte et al. [16] were found to be 245mg/100g in big amalaki, 275mg/100g in medium amlaki and 350mg/100g in small amlaki. Present results cover wider varieties of fruit and indicated a wide range of vitamin C contents (542.2-902.2mg/100 g) in amla fruits samples. The fruits contained 563mg/100g in unripe stage, 604.4mg/100g in semi-ripe stage and 804.4mg/100g in ripe stage. A mature amla fruit has also been reported to contain more vitamin C as compared to developing and immature fruit [4]. These results are in agreement with present results and indicate that synthesis of vitamin C continues during maturation of fruit with higher levels in ripe and semi ripe state. The polyphenols, which are major group of antioxidants, were also found to be highest at semi ripe stage suggesting use of ripe fruits for their optimum benefit of antioxidant quality.

Amla being a seasonal fruit, development of products that retain the health potential is of utmost importance. Stability study was the other objective. For this, amla syrup and pickle were studied for more than a month's time and assessed for the antioxidant potential over this time period. Among the antioxidants present in amla syrup, vitamin C is more susceptible to oxidation and loss of activity than the polyphenols. The levels of vitamin C showed decreasing trend as expected. It is likely that aqueous conditions are less favorable for its stability unlike polyphenols. Present data indicated that ingestion of diluted amla syrup (100 mL syrup +200 mL water) as a drink would meet the RDA for vitamin C. More efforts to conserve vitamin C contents in Amla syrup are needed. In case of pickle the rate of loss of vitamin C was higher than that of syrup. In comparison to vitamin C, the values for polyphenols and free radicals scavenging assay like DPPH, TEAC remained same over the same time period. That indicated major contribution of antioxidants like polyphenols which scavenge the free radicals and which are more stable give the antioxidant activity to this fruit. The results about loss of vitamin C contents are similar to those reported by Singh et al [3] where ascorbic acid content significantly declined in all the four cultivars during 135 days storage.

Amla is considered as one of the super fruits due to its richness in vitamin C and antioxidant potential. The importance of amla was known to ancient Indians as evident from the use of the fruit in various famous Ayurvedic preparations including Chavanprash. The hard liner is that most of vitamin gets lost during processing and storage. This paper highlights the variability in the contents of vitamin C and the antioxidant activity of marketed amla fruits and their products. The data is useful to develop process/product with higher shelf life as well as higher retention of original antioxidant power.

4. CONCLUSION

The vitamin C thus estimated in *Emblica officinalis* fruit was found to be highest in ripe stage, suggesting that synthesis of vitamin C is continued throughout the ripening stage. While the polyphenol content and the DPPH free radical scavenging activity viz. antioxidant potential was highest in semi ripe stage. The stability study of syrup and pickle, processed from amla fruit, demonstrated that the vitamin C content decreased over the time. In syrup, 49.70% loss of Vitamin C was observed after 33 days whereas in pickle it was about 76.29% but the polyphenols and the free radical scavenging activity remained the same over the time. The amla ripening stage study and stability study of processed product suggested that amla and its products are a rich source of antioxidants including phenolic compounds and offers opportunities for development of value-added products which is having nutraceutical and food applications to enhance health benefits.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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