

# Estimation of Amount of Vitamin-C in Various Products of Amla (*Emblica officinalis* Linn.) and Lemon (*Citrus limon* Linn.) to be Consumed as a Natural Immunity Booster

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**Abstract:** Vitamin-C chemically known as L-ascorbic acid is an essential micronutrient for the human body, required for the growth and repair of tissues in all parts of our body. It is used to produce skin, ligaments, blood vessels and form scar tissues, repair and maintain bones and teeth. It contains many antioxidants which removes free radicals and stimulates the immune system. Deficiency of ascorbic acid in human causes bruising, scurvy and oral problems. Moreover, lack of adequate Vitamin-C weakens our immune system which makes our body more vulnerable to infectious diseases. Our body neither makes Vitamin-C on its own, nor stores it. To compensate that, we consume various citrus fruits rich in Vitamin-C such as Amla (*Emblica officinalis* Linn.) and Lemon (*Citrus Limon* Linn.). Various products of Amla and Lemon are used to boost immunity such as pickle, sharbat, canned juice, candies etc. In the present study, we have compared these products using DCPIP titration to suggest which product is best for enhancement of the immune system. The experiment aims at estimation of amount of vitamin C in various products of Amla and Lemon to be consumed as a natural immunity booster.

**Keywords:** Vitamin-C, Amla, Lemon, DCPIP

## 1. Introduction

Vitamin C, also known as Ascorbate is a water-soluble vitamin, synthesized by all plants & most of the animals, one that cannot be stored by the body nor can produce it. It must be replenished daily. Vitamin C helps produce collagen, a protein needed to develop and maintain healthy teeth, bones, gums, cartilage, joint linings and blood vessels. It is a vitamin for humans because the gene for gulonolactone, the terminal enzyme in the AA synthesis pathway has undergone mutation that makes it non-functional. Sebastian J Padayatty and Mark Levine (2016).

For years, various fruits are used to boost immunity most commonly the citrus fruits such as Amla and Lemon. Amla (Botanical name- *Emblica officinalis* Linn.) a source of Vitamin C is a deciduous tree of the family Euphorbiaceae which grows in India, the Middle East & some South East Asian countries. Swetha Dasaroju and Krishna Mohan Gottumukkala (2014).

It is also called Indian gooseberry, native to India. Amla is a huge source of many amino acids and other minerals. It also contains several other phyto-constituents like tannins, alkaloids and phenols. Kaushik Vilas Kulkarni and Shrishail M Ghurghure (2018). Among all hydrolysable tannins, Emblicanin A and B; Gallic acid, Ellagic acid are reported to possess biological activity. Swetha Dasaroju and Krishna Mohan Gottumukkala (2014).

In Ayurveda, Amla fruit is used for the treatment of diarrhea, jaundice and inflammation. Various other parts are used as antibacterial, gastro protective properties etc. S. Mirunalini, and M. Krishnaveni (2010).

Lemon also contains vitamin C and its juice has traditionally been used as a remedy for scurvy before the discovery of vitamin C.

*Citrus limon* (L.) is a tree with evergreen leaves and yellow edible fruits from the family Rutaceae native to Southeast Asia. From there it spread worldwide, especially to Northeast India, Burma and China. NN Al-Jabri and MA Hossain (2016).

Since ancient times, the citrus fruit, especially lemon has been cultivated all over the world and its juice is known to treat high blood pressure, common cold, and irregular menstruation. Moreover, the essential oils of *C. limon* is a known remedy for coughs. The pharmacological potential of *C. limon* is determined by its rich phytoconstituents. The most important group of secondary metabolites in the fruit includes flavonoids and also other compounds, such as phenolic acids and carboxylic acids. The main compounds of essential oil are monoterpenoids, especially D-limonene. These valuable chemical components are the reason for the important position of *C. limon* in the food and cosmetics industries. Marta Klimek-Szczykutowicz *et al.* (2020). As Vitamin-C cannot be produced by our body and is an essential component of our immune system, it is important to consume products rich in ascorbic acid. The present study deals with comparing and contrasting Amla and Lemon and its products namely Raw, Candy, Sharbat, Pickle, Canned juice and also in frozen form using DCPIP titration and suggest products which has higher amount of Vitamin-C between them.

## 2. Materials and Methods

Celin tablet (500mg), 1gm amla pulp, 1gm lemon pulp, 1ml amla sharbat, 1ml lemon sharbat, 1ml amla canned juice, 1ml lemon canned juice, 1gm amla pickle, 1gm lemon pickle, 1gm amla candy, 1gm lemon candy, 1gm frozen amla, 1gm frozen lemon, Distilled water, DCPIP solution (0.01%), pestle, mortar, burette, measuring cylinder, conical flasks, beakers, funnels, Whatman filter paper 1, Laboratory weighing machine.

**Chemical Preparation:** 0.01% of DCPIP solution was prepared by dissolving 0.01 gm of DCPIP powder in 100 ml of distilled water at 25 °C.

In burette: Solution of Vitamin C unknown.

In conical flask: DCPIP solution.

End point: Blue to pink and then colourless.

## 3. Procedure

**Standard Estimation:** V.M. Kumar (2010)

A Celin tablet (500mg) was dissolved in 100 ml of water. This solution was filtered using a Whatman Filter Paper no 1 and collected in a beaker. The solution was transferred to the burette using a funnel adjusting zero mark. 5ml of 0.01% DCPIP solution was taken in a conical flask and titrated against Celin Tablet solution till the colour changed from blue to pink and then ultimately colourless. The reading was noted down as the standard reading.

### For Raw materials:

1gm of amla pulp was taken and crushed in a mortar with the help of a pestle. 100 ml of distilled water was added and mixed well. The solution was filtered using a Whatman Paper no 1. This solution was taken in a burette and titrated against 5 ml DCPIP solution in conical flask till the colour changed to pink and then colourless. The reading was noted and the process was repeated for raw lemon.

### For Candy:

1gm of amla candy powder (by crushing or grinding the candy to convert it into powder form) was dissolved in 100 ml of water. The solution was filtered using a Whatman Paper no 1. This solution was taken in a burette and titrated against 5 ml DCPIP solution in conical flask till the colour changed to pink and then colourless. The reading was noted and the process was repeated for lemon candy.

### For Sharbat:

1ml of amla sharbat was taken and dissolved in 100 ml of water. The solution was filtered using a Whatman Paper no 1. This solution was taken in a burette and titrated against 5 ml DCPIP solution in conical flask till the colour changed to pink and then colourless. The reading was noted and the process was repeated for lemon sharbat.

### For Canned juice:

1ml of amla canned juice was taken and dissolved in 100 ml of water. The solution was filtered using a Whatman Paper

no 1. This solution was taken in a burette and titrated against 5 ml DCPIP solution till the colour changed to pink and then colourless. The reading was noted and the process was repeated for lemon canned juice.

### For Pickle:

1gm of water washed amla pickle was taken and crushed in a mortar using a pestle. 100 ml of distilled water was added and mixed well. This solution was filtered using a Whatman Paper no 1. The solution was taken in a burette and titrated against 5 ml DCPIP solution in conical flask till the colour changed to pink and then colourless. The reading was noted and the process was repeated for lemon pickle.

### For Frozen materials:

1gm of frozen amla (by keeping it in the refrigerator for 1 day) was crushed in a mortar using a pestle. 100 ml of distilled water was added and mixed well. This solution was filtered using a Whatman Paper no 1. The solution was taken in a burette and titrated against 5 ml DCPIP solution in conical flask till the colour changed to pink and then colourless. The reading was noted and the process was repeated for frozen lemon.

### Readings

Standard

(Celin tablet) = 1ml

**Table 1:** Readings from the burette when 5ml of DCPIP solution was titrated with unknown Vitamin C solution

Product	Amla	Lemon
Raw	5.0ml	3.3ml
Candy	4.8ml	2.5ml
Frozen	1.5ml	5.5ml
Sharbat	2.5ml	2.0ml
Canned Juice	3.0ml	4.5ml
Pickle	2.1ml	2.0ml

### Calculations:

#### Control:

1ml of Celin tablet solution = 5mg of Vitamin C.

5ml of DCPIP solution = 1ml of Celin tablet solution

But 1ml Celin tablet solution = 5mg of Vitamin C.

Hence 5ml DCPIP = 5mg of Vitamin C. V.M Kumar (2010)

#### Experiment:

5ml of DCPIP solution required Y ml of (unknown) Vitamin C solution to change the colour.

Therefore, Y ml of unknown contains 5mg of Vitamin C.

One gm of amla/lemon raw and varieties were diluted to 100 ml water.

Therefore, one gm of amla/lemon raw and varieties contains =  $5 * 100 / Y$  mg of Vitamin C.

**Table 2:** Amount of Vitamin C (in mg) present in 1 gm of Amla/Lemon and its products

Product	Amla	Lemon
Raw	100.00 mg	151.50 mg
Candy	104.16 mg	200.00 mg
Frozen	333.33 mg	90.90 mg
Sharbat	200.00 mg	250.00 mg
Canned Juice	166.66 mg	111.11 mg
Pickle	238.09 mg	250.00 mg

We further used **two-way ANOVA** to check if there is any significant variation between Amla/Lemon and its products regarding Vitamin C content.

There are three sets of hypotheses with the two-way ANOVA. Aung Pyae (2019)

The null hypotheses for each of the sets are given below.

The means of the first factor are equal.

The means of the second factor are equal.

There is no interaction between the two factors.

$F > F\text{-critical}$  = There is significant difference between the factors.

$F < F\text{-critical}$  = There is no significant difference between the factors.

$F = F\text{-critical}$  = The factors are equal.

**Table 3:** Readings of various products of Amla and Lemon to be checked if there is any significant difference

Products	Raw	Candy	Frozen	Sharbat	Canned Juice	Pickle
Amla	100	104.16	333.33	200	166.66	238.09
Lemon	151.5	200	90.9	250	11.11	250

**Table 4**

Source of variation	SS	df	MSS	F	F - crit
Rows	656.0844	1	656.0844	0.087448	6.607891
Columns	25035.83	5	5007.166	0.667397	5.050329
Error	37512.67	5	7502.534		
Total	63204.59	11			

Where:

SS = sum of squares of rows/columns

df = degree of freedom = No of observations - 1

MS = Mean square = SS/df

F = F values = MS/MS error

F-critical = Critical statistics F

For Rows:

F-value – 0.087

Significance level – 0.05

F crit – 6.60

Since F-value < F crit

We do not reject the null hypothesis and there is no significant difference.

For Columns:

F-value – 0.667

Significance level – 0.05

F crit – 5.05

Since F-value < F crit

We do not reject the null hypothesis and there is no significant difference.

## 4. Results and Discussion

The present study was done to find Vitamin-C content in Amla, Lemon and its products namely candies, raw, frozen, pickle, sharbat and canned juice and suggest which product is best suitable for boosting immunity naturally. From Table No 1 and 2, it was quite evident that amla frozen contained the highest amount of Vitamin-C at an astonishing 333.33mg per gram of amla while frozen lemon contained the least amount of Vitamin-C i.e., around 90.9mg per gram of lemon.

Table No 3 and 4 used two-way ANOVA which helped us to find that there was not any significant variation between these products.

## 5. Conclusion

Vitamin-C is a much-required micronutrient needed for various functions in our body. It plays an important role in immunity function. Since it cannot be synthesized in our body, we consume outside market Vitamin-C supplements to satisfy our need. However, markets made Vitamin-C products are not only synthetic in nature and can induce many side effects such as diarrhea, nausea, vomiting, etc. but also are costly. To conquer this issue, we suggest natural citrus fruits such as amla and lemon rich in Vitamin-C to boost immunity without any side effects. The optimum Vitamin-C intake is around 200 mg/day and by our experiment we found that 1 gm frozen amla contains around 333.33mg of Vitamin-C which is the highest between lemon and amla products. So, even if we take around 0.5 gm of frozen amla every day, it can satisfy our daily need of ascorbic acid.

For people with diabetes, we recommend amla/lemon products less in sugar such as pickle, raw, and even sharbat which contain enough Vitamin-C needed for our body.

For people with high blood pressure, we suggest amla/lemon products less in salt such as raw, sharbat, candies, juice which contain daily required Vitamin-C.

Overall, we suggest people not to take synthetic Vitamin-C supplements but rather take natural products in the form of amla/lemon which provide higher amount of Vitamin-C without any side effects and are cheaper.

## 6. Recommendation

Amla and Lemon are the natural sources of Vitamin C, which are easily available throughout the year and affordable for everyone. They can be preserved in various forms without the loss of Vitamin-C from them and consumed in various ways.

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