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Comparative equilibrium study of Fresh lemon juice and Amla juice by analytical evaluation

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Abstract
The objective of this study is to examine the conclusions of iodometry and colorimetry in order to ascertain the stability of the vitamin C present in fresh lemon juice and amla juice. The results of this study, ascorbic acid degrades more quickly at low temperatures than it does at normal temperature. Iodometry and colorimetry results are in close agreement. As a result, we could say that the iodometry method is a cheap and simple way to estimate the amount of ascorbic acid comprised in both liquids. Results of qualitative tests are also reported. The results of TLC, Paper Chromatography, and RF analysis are all almost identical. Results from UV spectroscopy are similarly within range. Thus, stability testing for vitamin C has been validated by all of these tests.

Keywords: Colorimetry, Iodometry, Qualitative test, UV Spectroscopy, TLC, Paper Chromatography

Introduction
Water-soluble vitamin C, often referred to as L-ascorbic acid ((5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4- dihydroxy-2,5-dihydrofuran-2-one), is found in certain foods naturally, is added to others, and may also be purchased as a dietary supplement. Because vitamin C cannot be synthesised by humans, unlike other animals, it is a vital nutritional component. The manufacture of collagen, L-carnitine, and a few neurotransmitters depends on vitamin C, which also plays a role in protein metabolism. Collagen is a crucial component of connective tissue, which is crucial for the healing of wounds. Alpha-tocopherol (vitamin E) and vitamin C have both been found to replenish other antioxidants in the body, making them both significant physiological antioxidants. The finest sources of vitamin C are fresh fruits and vegetables. The main sources of vitamin C in the American diet are potatoes, tomatoes, and tomato juice. Red and green peppers, kiwifruit, broccoli, strawberries, Brussels sprouts, and cantaloupe are other healthy dietary options. Although grains don't naturally contain vitamin C, certain breakfast cereals with added vitamin C do. Because ascorbic acid is water soluble and is degraded by heat, extended storage and cooking may lower the vitamin C content of food. Ascorbic acid's stability is also affected by pH, light, humidity, other elements, storage conditions, and container. According to the literature review, iodometry, colorimetry, UV-Visible spectroscopy, and chromatography were used to test the stability of the ascorbic acid contained in juices.

Chemical constituents of Amla

Fig 1: Amla juice and Powder

Types	Chemical constituents
Hydrolysable Tannins	Emblicanin A and B, Punigluconin, Pedunculagin, Chebulinic acid (Ellagitannin), Chebulagic acid (Benzopyran tannin), Corilagin (Ellagitannin), Geraniin (Dehydroellagitannin), Ellagotannin
Alkaloids	Phyllantine, Phyllembein, Phyllantidine
Phenolic compounds	Gallic acid, Methyl gallate, Ellagic acid, Trigallayl Glucose
Amino acids	Glutamic acid, Proline, Aspartic acid, Alanine, Cystine, Lysine
Carbohydrates	Pectin
Vitamins	Ascorbic acid
Organic acids	Citric acid

Chemical constituents: Lemon

The constituents of the lemon peel include volatile oil, hesperidin and vitamin B. The chief constituent of the juice is 6 to 9 percent citric acid and also Vitamin C.

Fig 2: Lemon Juice

Extraction of Lemon juice

Squeeze the lemon hard while holding it over a large bowl. Take your lemon and hold it over a large bowl, just beneath the rim. Wrap your palm around the peeled lemon and point the exposed membrane down towards the bowl. Squeeze hard to remove the majority of the juice. With slices, hold the opposite ends between your index finger and thumb. Squeeze your fingers together to remove most of the juice.

Fig 3: Amla Juice

Extraction of Amla juice

Cut the amla in half width-wise if we are going to use a juicer. After cutting your amla, place one half on top of your juicer with the skin facing up. Press down while twisting the amla into the blades. Do this for 45-60 seconds to extract the juice.

Table 2: Preliminary Test: Lemon Juice

Test	Observation	Inference
Nature		
Lemon juice	Acidic	Acidic in nature
PH		
Lemon juice	Red colour	Acidic
Solubility		
Lemon juice	Less soluble	Less soluble
Microscopic test		
Taste	Sour	Sour taste
Odour	Citrus	Citrus smell

Table 3: Preliminary Test: Amla Juice

Test	Observation	Inference
Nature	Acidic	Acidic
PH	Red colour	Acidic
Solubility	Not soluble	Insoluble
Taste	Sour	Sour taste
Odour	Musky	Musky

Table 4: Qualitative Test: Lemon juice

Test	Observation	Inference
Solution + add NH4OH till neutralized the sample		
1. Above sample + CaCl3 solution	No immediate ppt	Oxalic acid absent
2. Boil the above mixture and cool	Ppt on heating	Citric acid present
Solubility test 1. Lemon juice + water	Less soluble	Less soluble
2. Ph test	Red colour	Acidic in nature
Sodium bicarbonate test 1. NaHCO3 + Sample	Effervescence	Citric acid present
Action of KMnO4 1. NaCo3 + KMnO4 + sample solution	Violet to yellow to colourless	
Test of functional group a) Carboxylic acid	Effervescence	Citric acid confirmed
b) NaCo3 + sample	Effervescence	Citric acid confirmed
C.T. for citric acid NaHCO3 + sample solution	Effervescence	Citric acid confirmed
Add few drops of Denige's reagent to the sample solution then add dilute KMnO4 solution heat the mixture	Permanganate colour is discharged with turbidity	Presence of citric acid

Table 5: Preliminary Test: Amla

Test	Observation	Inference
Ferric chloride test 1. Aq. Sample solution + FeCl3	Blue colour observed	Compound present
1. Gelatin + NaCl + Aq. Sample solution	Milky white colour observed	Compound present
2. Aq. Sample + Lead acetate then filtrate it then add 2,6-dichlorophenol- indophenol	Colour disappear to Aq. White	Compound present
C.T. for citric acid NaHCO3 + sample solution	Effervescence	Citric acid confirmed

Qualitative Tests of Lemon & Amla juices

Iodometry: Procedure

Put 10 ml of juice and 10 ml of water in a flask. Add 1 to 2 cc of starch indicator to the flask. Use iodine as a burette titrant. Drop by drop, shake the flask as you add the iodine solution. Note how the orange colour transforms into black at the conclusion point. Track volumetric concentration.

Table 6: Observation: Lemon juice

Sr. No.	Concentration / volume
1.	4.5
2.	3.5
3.	4

Table 7: Amla juice

Sr. No.	Concentration/ volume
1.	16
2.	17
3.	16.5

Colorimetry

- **Preparation of 0.1N Oxalic acid**

Weigh 1.26 g of oxalic acid and dilute with 200 ml distilled water.

Preparation of acetate buffer at pH 4.2

Weigh 0.068 g of sodium acetate and add 3.85 g of ammonium acetate in 250 ml of distilled water and add 12.5 ml of glacial acetic acid solution.

- **Preparation of 0.01% methylene blue**

Weigh 1g of methylene blue and dilute with 100ml distilled water.

- **Preparation of sample**

Pipette out 1 ml of juice samples in 10 ml volumetric flask and makeup to 10 ml with distilled water.

- **Procedure**

Measure 20 ml of oxalic acid and add 0.2ml of 0.01% of methylene blue and add 1ml of acetate buffer of pH 4.2 and add 1 ml of sample solution and measure the absorbance at 540 nm

Table 8: Observation

Sample	Absorbance
a) Amla Juice	1.18
b) Lemon Juice	1.02

UV Spectroscopy

Instrument

a) UV- Visible spectrophotometer (Double beam) having matched quartz cells of light path
b) 10 cm
c) Software: UV probe Version of software: 2.42
d) Electronic analytical weighing balance (REPTECH)
e) Volumetric flask (Borosilicate),
f) Pipettes
g) Conical flask

- **Sample**

a) Lemon Juice
b) Amla Juice

- **Standard ascorbic acid solution**

Standard ascorbic acid solution was prepared by dissolving 50 mg of AA in 100ml of distilled water (500 µg/ml).

- **Preparation of calibration curve**

Calibration curve of different concentration i.e. 5, 10, 15, 20, 25 µg/ml was prepared by proper dilution.

- **Prepare sample extracts**

By mixing 10 g of the sample in the blender, the sample extract is created. A 250 ml conical flask was then filled with the sample and 50 ml of a 5% metaphosphoric acid acetic acid solution.
The flask was filled with the remaining 50 ml of phosphoric acid solution.

After using Whatman filter paper to filter the solution, the filtrate was collected to be tested for vitamin C.

- **Procedure for estimation of vitamin C**

A little amount of bromine solution was added and blended with the filtered sample solution. To eliminate the bromine solution, a few drops of thiourea solution were then added to the sample solution. The sample solution and the whole of the standard calibration curve (5,10,15,20,25 g/ml) were then mixed with 1 ml of a 2,4 DNPH solution. 2,4 DNPH solution causes the coupling process. All of the standards and sample solution were held at 37°C for 3 hours to allow the reaction to finish. 5ml of H2SO4 was added after the solutions had cooled on an ice bath for three hours. As a consequence, coloured solutions were produced, and their absorbance at particular wavelengths was measured.

- **Reaction**

a) Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.
b) L-dehydroascorbic acid reacts with 2,4dinitrophenylhydrazine and produces an osazone which on treatment with H2SO4 forms red coloured solution.

- **Calibration curve**

The absorbance of all the standards was used to generate the calibration curve once the maximum concentration of the coloured solution was determined. Plotting absorbance vs concentration allowed for the construction of the calibration curve.

Table 9: Observation

Sample	Biological Name	Amount of Vitamin C (mg / 100 gm)
Lemon Juice	Citrus limon	56.4
Amla Juice	Emblica officinalis	60.5

Thin layer chromatography

Procedure

Prepare the mobile phase (6:2:2) parts n-butanol, acetic ac and water. 30ml of the mobile phase should be placed in a beaker, and the beaker should be covered with aluminium foil to enable the mobile phase to saturate for a while. next take a TLC Plate that is 7 cm wide and 20 cm long. Next, using water and silica-G powder, create a paste. Pour it onto a TLC Glass plate and adjust the thickness so that it may be distributed evenly on all four sides. The TLC plate was dried in the oven at 150°C for 10-15 minutes. Once the TLC plate has dried, remove it. Make a fusion tube now, then prepare two samples and one standard solution. Draw a line 2 cm up from the TLC plate's bottom. Add three points to the drawing.

Report

The RF values for ascorbic acid 0.1N sodium hydroxide solution and pure ascorbic acid with water were determined to be 0.64 and 0.67, respectively. Compared to pure ascorbic acid, the RF value of degraded ascorbic acid was somewhat higher.

Observation

1. Distance of solvent travelled = 15 cm
2. Distance of Std. travelled = 12.5 cm

Calculations

Std. solution (Ascorbic acid) = Distance travelled by Solute / Distance travelled by Solvent
= 12.5 / 15
= 0.83
Amla = Distance travelled by Solute / Dist. travelled by Solvent
= 12/15
= 0.8
Lemon = Distance travelled by Solute / Dist. travelled by Solvent
= 10.5/15
= 0.7

Result

1. The RF value of standard solution (ascorbic acid) = 0.83
2. The RF value of Amla juice = 0.8
3. The RF value of Lemon juice = 0.7

Paper chromatography

Procedure

Prepare the mobile phase (6:2:2) of n-butanol, acetic acid, and water. The dynamic phase of
Take 20ml in a beaker and cover it with aluminium foil to let it sit and soak for a while. Then, pick a piece of filter paper that is 3 cm wide and 10 cm long. Make a fusion tube now, then prepare two samples and one standard solution.

Draw a line 1 cm up from the filter paper's bottom. Place three spots along the paper's drawn line and label them standard 1, sample 1, and sample 2, correspondingly. Put the paper in the chamber and allow it to run till 80%. The paper should then be removed and placed somewhere to dry. Spray ninhydrin solution, then let it air dry. Watch the spots and determine the RF value.

Observation

1. Distance of solvent travelled = 7.6 cm
2. Distance of Std. travelled = 4.9 cm

Calculation

Standard. solution (ascorbic acid) = Dist. travelled by Solute/ Dist. travelled by Solvent
= 4.9/7.6
= 0.6
Amla = Distance travelled by Solute/ Dist. travelled by Solvent
= 3.8/7.6
= 0.5

Lemon = Dist. travelled by Solute/ Dist. travelled by Solvent
= 3.4/7.6
= 0.4

Result

1. The RF value of standard solution (ascorbic acid) = 0.6
2. The RF value of Amla juice = 0.5
3. The RF value of Lemon juice = 0.4

Conclusion

Degradation was more pronounced in commercial lemon juice and fresh lemon juice with salt and sugar, respectively, due to greater ascorbic acid concentrations and the presence of sugars. While ascorbic acid degraded because of humidity exposure in refrigerator settings, it did so slowly because of the low temperature. Water fosters the growth of microorganisms and catalyses chemical processes such as oxidation, hydrolysis, and reduction reactions. Due to lower concentration and the absence of sugar, deterioration in lemon juice with salt was slower. However, temperature and humidity also contribute to the breakdown of ascorbic acid.
Due to the shift in ambient temperature, drug degradation was more pronounced in all samples in reproducibility testing than in earlier investigations. Because oxidation, reduction, and hydrolysis reactions that result in drug degradation are sped up by high temperatures and light. But Results obtained in a refrigerator were comparable to those obtained before. This made the procedure repeatable.
Thus, we may infer that lemon juice made with salt can be kept in glass containers at room temperature for 8 days under normal weather and for 5 days during mild temperature circumstances. When comparing the findings of colorimetry and iodometry, colorimetry's sensitivity was higher, although both sets of data were close to one another. As a result, we may conclude that the iodometry method is a cheap and simple way to estimate the amount of ascorbic acid in lemon juice.
In light of the comparison investigations, we deduced from the aforementioned tests that citric acid and vitamin C were present in amla juice and lime juice.

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