WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 22, 1163-1173. Research Article ISSN 2277–7105

COMPARATIVE STABILITY STUDY OF FRESH LEMON JUICE AND AMLA JUICE BY ANALYTICAL EVALUATION

S. M. Wakchoure*, Shubham Lavekar[1], Zuhair Baig[2], Omkar Magar[3], Shitija Kurane[4], Omkar Magar[5]

*Assistant Professor Department of Pharmacognosy, Shivajirao S.Jondhle College of Pharmacy, Asangaon, Thane.

[1,2,3,4,5]Students Shivajirao S.Jondhle College of Pharmacy, Asangaon, Thane.

Article Received on
31 October 2023,

Revised on 21 Nov. 2023,
Accepted on 11 Dec. 2023

DOI: 10.20959/wjpr202322-30630

***Corresponding Author**
S. M. Wakchoure
Assistant Professor
Department of Pharmacognosy, Shivajirao S.Jondhle College of Pharmacy, Asangaon, Thane.

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.

Figure:1 Amla juice and Powder.

Chemical constituents of Amla

Table No. 1.

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 ofthe juice is 6 to 9 percent citric acid and also Vitamin C.

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.

Figure no 2: Lemon Juice.

Figure no 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 ontop of your juicer with the skin facing up. Press down while twisting the amla into the blades. Do this for45-60 seconds to extract the juice.

Table no 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 no 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 no 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)** Carboxylicacid	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 no 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 itthen 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 no 6: Observation:Lemon juice.

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

Table no 7: Amla juice.

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

Colorimetry

- **Preparation of 0.1N Oxalic acid**

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

- **Preparation of acetate buffer at pH 4.2**

Weigh 0.068g of sodium acetate and add 3.85g of ammonium acetate in 250ml of distilled water andadd 12.5ml 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 1ml of juice samples in 10ml volumetric flask and makeup to 10ml with distilled water.

- **Procedure**

Measure 20ml 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 1ml of sample solution and measure the absorbance at 540nm.

Table no. 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 50mg of AA in 100ml of distilled water(500µg/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 10g of the sample in the blender, the sample extract is created. A 250ml conical flask was then filled with the sample and 50ml of a 5% metaphosphoric acid acetic acid solution.

The flask was filled with the remaining 50ml 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**

i. Ascorbic acid is oxidized to dehydroascorbic acid by the action of bromine solution.

ii. L-dehydroascorbic acid reacts with 2,4dinitrophenylhydrazine and produces an osazone whichon 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 no 9: Observation.

Sample	Biological Name	Amount Of Vitamin C (mg/100gm)
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.

REFERENCE

1. Text Book of Pharmacognosy by C. K. Kokate, Purohit , Gokhlae (2007), 37th Edition, NiraliPrakashan, New Delhi.
2. Text Book Of Pharmacognosy by T. E. Wallis.
3. W.C.Evans, Trease and Evans Pharmacognosy, 16th edition, W.B. Sounders & Co., London, 2009.
4. Mohammad Ali. Pharmacognosy and Phytochemistry, CBS Publishers & Distribution, New Delhi.
5. A.N. Kalia, Textbook of Industrial Pharmacognosy, CBS Publishers, New Delhi,2005.
6. Essentials of Pharmacognosy, Dr. S. H. Ansari, IInd edition, Birla publications, New Delhi, 2007.
7. https://scholar.google.co.in/scholar?q=synthesis,+characterization+and+antimicrobial +evaluation+of+some+benzopyran+derivatives&hl=en&as_sdt=0&as_vis=1&oi=sch olart
8. https://rjptonline.org/HTMLPaper.aspx?Journal=Research+Journal+of+Pharmacy+an d+Technology%3bPID%3d2019-12-10-24
9. https://www.researchgate.net/publication/317237453_Stability_Studies_on_Ascorbic _Acid_content_in_various_fruits_Vegetables_and_their_Cocktail_Juices
10. https://youtu.be/_HbpNPeyWNU
11. https://lupinepublishers.com/surgery-case-studies-journal/fulltext/process-for-preparation-of-vitamin-c-and-method-for-determination-of-vitamin-c-in-tablets.ID.000114.php#:~:text=Ultraviolet%20(UV)%20Spectrophotometry,-Go%20to&text=Direct%20ultraviolet%20spectrophotometry%20is%20a,pH2%20(Ya nshan%2C%201997)
12. https://youtu.be/buAYVpzb1BA

13. https://rjptonline.org/HTMLPaper.aspx?Journal=Research+Journal+of+Pharmacy+and+Technology%3bPID%3d2019-12-10-24
14. https://youtu.be/Br4gVXY_SXg
15. https://lupinepublishers.com/surgery-case-studies-journal/fulltext/process-for-preparation-of-vitamin-c-and-method-for-determination-of-vitamin-c-in-tablets.ID.000114.php#:~:text=For%20standard%20solution%20Mass%20Ascorbic,2.5%20(250%2F100)
16. https://www.researchgate.net/publication/7560404_Fluorimetric_Determination_of_Ascorbic_Acid_in_Vitamin_C_Tablets_Using_Methylene_Blue https://rjptonline.org/HTMLPaper.aspx?Journal=Research+Journal+of+Pharmacy+and+Technology%3bPID%3d2019-12-10-24
17. https://www.slideshare.net/ceutics1315/testing-the-degradation-of-ascorbic-acid-by- tlc
18. https://foodandnutrition.org/wp-content/uploads/lemons-and-juice-510180397- 780x520.jpg
19. https://image.made-in-china.com/202f0j00vheYpHTMHjbP/Instant-Fruit-Juice- Dried-Lemon-Powder.jpg
20. https://tamilcrew.com/wp-content/uploads/2021/04/Frozen-Indian-Gooseberry- Juice.jpg
21. https://5.imimg.com/data5/SP/DA/MY-5751538/amla-juice-powder-500x500.jpg
22. https://cdn.shopify.com/s/files/1/0066/7569/3639/articles/vitamin-c-benefits.jpg?v=1623791337
23. https://www.glentham.com/static/images/structures/large/50-81-7.png
24. https://homework.study.com/cimages/multimages/16/7140542734272140426453317 4.png
25. https://pubchem.ncbi.nlm.nih.gov/image/imgsrv.fcgi?cid=54670067&t=l
26. https://upload.wikimedia.org/wikipedia/commons/thumb/a/ae/Palmitins%C3%A4urea scorbylester_Strukturformel.svg/1200px-Palmitins%C3%A4ureascorbylester_Strukturformel.svg.png
27. https://www.nutraskinusa.com/wp-content/uploads/2019/11/113170-55-1.png
28. https://s3.amazonaws.com/images.icliniq.com/infographics/Vitamin+C+Toxicity+%282%29.jpg