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Isolation Evaluation and Estimation of Calcium Citrate from Herbal Source

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Research Article

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Abstract

In the modern organic chemistry, various methods are now a day widely utilized for isolation of different organic compounds. From the ancient era to the present the various methods of extraction like Maceration, Percolation, Decoction and Soxhlet extraction are widely utilized. Calcium citrate also known as calcium salt of citric acid which is a member of tricarboxylic acid derivatives used occasionally as food ingredients and preservatives but it is well known source of calcium and utilized as calcium supplement.

Thus the present study was initiated with aim to isolate, evaluate and estimate the calcium citrate from lemon juice by using decoction method. Calcium chloride was used for the crystallization of calcium isolated from lemon juice. Theoretical and practical yield of calcium citrate after isolation were determined. For further evaluation of isolated calcium citrate basic identification tests like solubility, melting point (MP), loss on drying (LOD) and assay of calcium citrate were done.

The present study further proceeded to confirmatory evaluation by UV-Vis spectroscopy and thin layer chromatography (TLC). The basic identification tests found the presence of calcium citrate with M.P in the range of 119°C-121°C with percentage practical yield of 24.02%w/w and LOD was 9.40%. The UV-Vis absorbance shown at λ-Max 0.036 and TLC reported Rf value 0.68 and 0.53 using various polar, semipolar and non polar solvents. As ample amount of calcium citrate was isolated from herbal source i.e. lemon juice, the study reached to the conclusion that instead of synthetic sources, the herbal source can be very useful and economical approach for the isolation of calcium citrate from lemon juice.

Keywords: Calcium Citrate; Lemon Juice; Decoction; TLC; UV Spectroscopy

Abbreviations: MP: Melting Point; LOD: Loss on Drying; TLC: Thin Layer Chromatography; SFC: Super Critical Fluid Extraction; PLE: Pressurised Liquid Extraction; MAE: Microwave Assisted Extraction; PTH: Parathyroid Hormone; TLC: Thin Layer Chromatography.

Introduction

In organic chemistry, isolation is utilized to remove individual compound from a more complicated matrix. The isolation of organic compounds can be done using a variety of methods. One of these, called extraction, entails removing an organic substance from a matrix. Crystallization, sublimation, and distillation are other isolation methods. These methods are employed frequently; for instance, distillation columns are used in the oil business to separate organic molecules based on their boiling points. The procedures of liquid-liquid and solid-liquid extractions are also extensively employed; however they rely on selecting the right solvent based on the characteristics of the substance to be separated [1].

Crystallization

When a solid material is created from a liquid solution or melt, the process is known as crystallization, and the resulting solid material has a crystalline structure. The following qualities are typically present throughout a crystallization process. The input material is a liquid that is either above the melting temperature of the solid phase or in solution. If in solution, more than one solvent might be present. Impurities could be solid or dissolved in the mixture. Some impurities, particularly byproducts of organic reactions, may have a lot of characteristics with the solute. Impurities may crystallize separately during the crystallization process, stay in solution, or somehow merge into the finished crystals. The product's substance is solid and comes in a variety of sized particles.

The Advantages of Crystallization as a Process are:

- In a single process, high purification can be attained.
- Uses less energy and operates at a lower temperature than similar distillation separations.
- Plants can be basic, straightforward, and low-maintenance.
- Possibly more cost-effective than alternative separation methods.
- Phase equilibrium controls yield [2] (Figure 1).

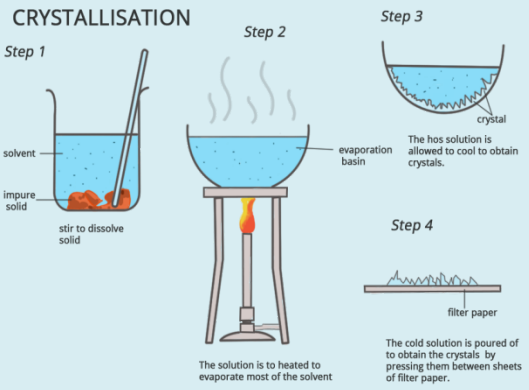


Figure 1: Method of Crystallization.

Extraction

The initial stage in separating the desired natural products from the raw ingredients is extraction. According to the extraction principle, there are several extraction procedures, including solvent extraction, distillation, pressing, and sublimation. The technique with the highest usage is solvent extraction.

The Process of Extracting Natural Products Involves the Following Steps:

- Allowing the solvent to permeate the solid matrix.
- Allowing the solute to dissolve in the solvents.
- Allowing the solute to diffuse out of the solid matrix; and
- Collecting the extracted solutes.

The extraction will be made easier by any element that increases the above steps' diffusivity and solubility. The qualities of the extraction solvent, raw material particle size, solvent to solid ratio, extraction temperature, and extraction time all have an impact on how well materials are extracted. For solvent extraction, the choice of the solvent is essential. The selection of solvents should take into account selectivity, solubility, cost, and safety. Solvents with polarity values close to the polarity of the solute are likely to perform better and vice versa, according to the law of resemblance and inter miscibility (like dissolves like). Alcohols (EtOH and MeOH) are all-purpose solvents used in solvent extraction for research on Phytochemical.

Solubility and diffusion are both increased at high temperatures. However, excessively high temperatures can result in the loss of solvents, which can extract unwanted impurities and induce the destruction of thermo labile components. With longer extraction times within a specific time window, extraction efficiency rises. Once the solute has reached equilibrium both within and outside of the solid substance, adding more time won't have any effect on extraction. The extraction yield increases with the solvent to solid ratio; however, a solvent to solid ratio that is excessively high can result in excessive extraction solvent and take a long time to concentrate [1].

The common organic solvents used in the conventional extraction techniques, such as maceration, percolation, and reflux extraction, require a significant amount of solvent and a lengthy extraction period. The extraction of natural products has also been carried out using some more recent or environmentally friendly extraction techniques, such as super critical fluid extraction (SFC), pressurised liquid extraction (PLE), and microwave assisted extraction (MAE), which have the advantages of using less organic solvent, taking less time, and having higher selectivity. However, several extraction techniques, such sublimation, expeller pressing, and effleurage, are hardly utilised in modern phytochemical research.

Maceration

This is a very simple extraction technique; however it has the drawbacks of a lengthy extraction time and poor extraction efficiency. It might be used to extract components

that are thermo labile (Figure 2).

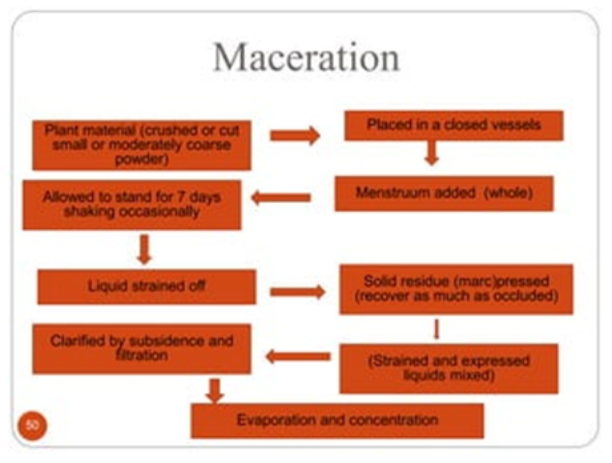


Figure 2: Maceration method.

Percolation

Because percolation is a continuous process in which the saturated solvent is continuously replenished by new solvent, it is more effective than maceration [2] (Figure 3).

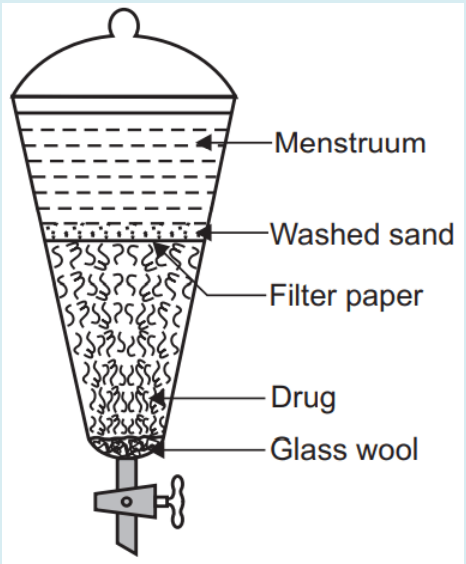


Figure 3: Percolation method.

Decoction

There are numerous water-soluble contaminants in the decoction extract. The extraction of volatile or thermo labile components cannot be accomplished by decoction [3] (Figure 4).

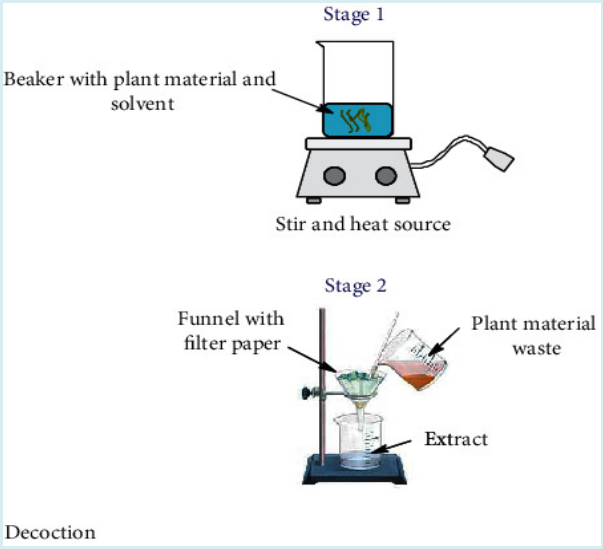


Figure 4: Decoction method.

Soxhlet Extraction

The Soxhlet extraction method, which employs the principles of reflux and siphoning to constantly extract the herb with new solvent, combines the benefits of the reflux extraction and percolation [4]. In comparison to maceration or percolation, the Soxhlet extraction is an automatic continuous extraction technique with great extraction efficiency. Thermal deterioration is more likely given the Soxhlet extraction's high temperature and lengthy extraction period [3] (Figure 5).

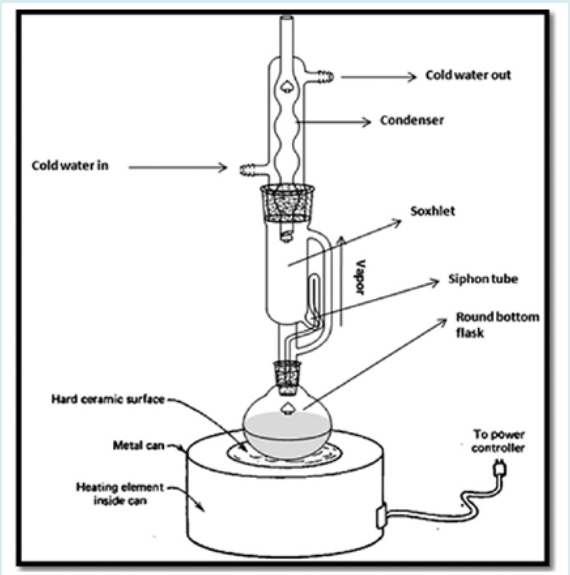


Figure 5: Soxhlet method.

Calcium Citrate

Calcium citrate is the name given to the calcium salt of citric acid. Citrate calcium is occasionally used as a food ingredient and preservative [5-8]. Tricalcium citrate and cal citrate are other names for calcium citrate [9]. It is an organic substance that is a member of the tricarboxylic acids derivatives class. Three carboxyl groups are typically present in these acids [9,10]. It can also be present in some dietary calcium supplements like Castrate or Citracal, in addition to natural food products. In terms of mass, calcium makes up 24.1% of calcium citrate (anhydrous) and 21.1% of calcium citrate (tetrahydrate) (Figure 6). The mineral known as Earlandite is a tetrahydrate form of calcium citrate [9,10] (Table 1).

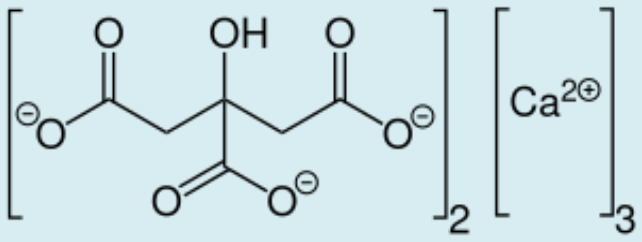


Figure 6: Calcium Citrate.

Product Name	Calcium Citrate
Synonym	Tricalcium dicitrate, Tricalcium citrate, Acicontral
IUPAC	2-hydroxy-1,2,3-propane-tricarboxylic acid calcium salt (2:3)
Molecular Formula	$C_{12}H_{10}Ca_3O_{14}$
Molecular Weight	498.46 g/mol
Melting point	120°C
Solubility	Slightly soluble in water, freely soluble in dil. HCL & insoluble in alcohol.

Table 1: Characteristics of Calcium citrate.

One of the widely used plant acids is citric acid. About 30% of the lemon fruit's juice which is used for its vitamin C content. Hesperidin, other flavanol glycosides, and volatile oil make up roughly 2.5% of dried lemon peel. Calcium chloride is added to lemon juice and citric acid is obtained in form of calcium citrate, which is carried out in basic conditions (Figure 7).

Figure 7: Calcium Citrate.

Mechanism of Action

Plasma calcium levels are raised with calcium citrate. As a result, less parathyroid hormone (PTH) is secreted, lowering the calcium flux from osteocyte activity. This is accomplished by calcium activating a calcium receptor that is G-protein coupled on the surface of parathyroid cells. The amount of calcium that is deposited in bone rises due to the reduction in calcium flux, increasing bone mineral density. PTH secretion is decreased, and less vitamin D is converted into calcidiol, which is its active form.

A decrease in calcidiol causes reduced calcium absorption because it raises the expression of calcium dependent ATPase and transient receptor potential cation channel subfamily V member 6 (TRPV6), both of which are important in calcium uptake from the gut. Additionally, when PTH secretion is decreased, TRPV5, the channel responsible for calcium reabsorption in the kidney, is down regulated, increasing calcium excretion through the kidneys. During times of high plasma calcium, the hormone calitonin is probably involved in reducing bone resorption [10].

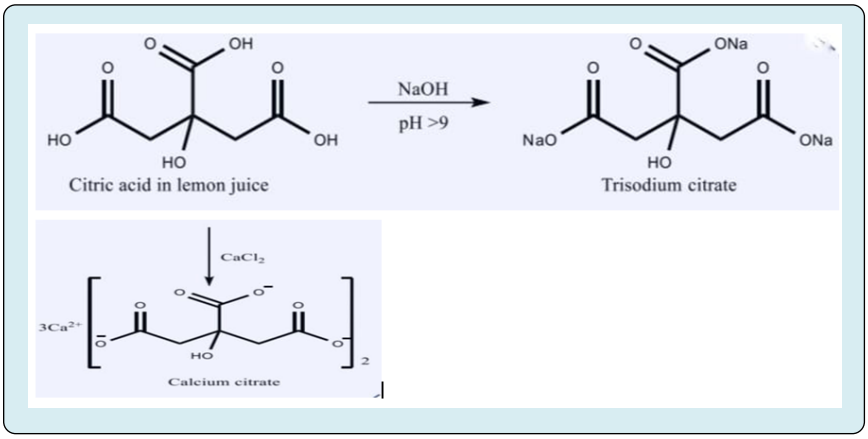
Uses of Calcium Citrate

- Low calcium problems are treated, including osteoporosis, rickets, and weak bones (osteomalacia).
- It lessens latent tetany and some muscle illnesses like hyperparathyroidism in the parathyroid gland.
- It is used in some individuals to make sure they get enough calcium, particularly for women who are nursing, pregnant, or dealing with postmenopausal problems.
- It can be found in drugs like phenobarbital, phenytoin, or prednisone.
- This medicine aids in lowering the risk of cancer since calcium has a protective effect on cells.
- It lowers the risk of developing cardiovascular disease. However, some studies disputed this result [10].

Result & Discussion/ Material & Methods

Isolation of Calcium Citrate

Reaction of Calcium Citrate



Procedure

- Take about 50.0 ml of lemon juice and add 100% sodium hydroxide with constant stirring until mixture is slightly alkaline as indicated by the color change from clear yellow to dark yellow.
- Strain the reaction mixture through a muslin cloth to remove the pulp.
- Again filter the filtrate through the Buchner funnel under vacuum and if the filter paper gets clogged, replace it as required for complete filtration.
- To the filtrate obtained add 5.0 ml of 10.0% calcium chloride for each of 10.0 ml of filtrate and heat the mixture to boiling.
- Filter the copious precipitate of calcium citrate while hot through a Buchner funnel.
- Wash the precipitate with small volumes of boiling water and dry [5] (Figure 8).

Figure 8: Isolation of Calcium Citrate.

Properties of Calcium Citrate

- It is the crystalline powder that is white, odorless, and freely soluble in Dil. HCL but insoluble in alcohol [11].
- Tricalcium dicitrate tetrahydrate $[ca_3(C_6H5O_7)_2(H_2O)_2]$ needle-shaped crystals by use of hydrothermal synthesis, $2H_2O$ was produced.
- In addition to being a popular food additive, calcium citrate is also present in several dietary calcium supplements, such as Citracal and Caltrate.
- Citric acid and other citrates are produced using it.
- It is employed to enhance the capabilities of flour in

baking [10]

Theoretical Yield Practical Yield and % Practical Yield

- **Theoretical Yield:**

Citric acid ($C_6H_8O_7$) Calcium citrate [Ca_3 ($C_6H5O_7)_2$]
192 498
192 gm of citric acid gives 498 gm of calcium citrate.
26 gm of citric acid gives x gm of calcium citrate.
192 = 498
26 = x
26 x 498/ 192
X = 67.43 gm

Practical yield = 16.20 gm
% practical yield = practical yield / Theoretical yield x 100
= 16.20 / 67.43 x 100
= 24.02% w/w

Identification Test

- **Test:** Dissolve 0.5 g in a mixture of 10 ml of water and 2.5 ml of 2N nitric acid. Add 1 ml mercuric sulfate TS, heat to boiling and add 1.0 ml of potassium permanganate [12,13].
- **Observation:** White precipitate is formed (Figure 9).

Figure 9: White ppt is formed.

- **Test:** Ignite completely the sample at low a temperature as possible, cool and dissolve the residue in dilute glacial acetic acid (1:10). Filter and add 10 ml of ammonium oxalate TS to the filtrate (Figure 10). Acceptance criteria: a voluminous white precipitate that is soluble in hydrochloric acid is formed (Table 2).
- **Observation:** White precipitate is formed [14] (Figure 11).

Figure 10: White ppt is formed.

Sr.No	Solvent	Solubility
1	Water	Slightly soluble
2	Methanol	Insoluble
3	Ethanol	Insoluble
4	Dil. Hcl	Freely soluble
5	Petroleum ether	Insoluble
6	Benzene	Insoluble
7	Chloroform	Insoluble

Table 2: Solubility test for Isolated Calcium citrate.

Figure 11: Solubility Test.

Melting Point

Take Thiele's tube and fill it with liquid paraffin. Fill the Capillary tube with powdered Aspirin with one-fourth part, and tie tightly with a thermometer to the tip (Figure 12). Heat the Thiele's tube on the burner till the powder melt and note down the temperature (Table 3).

Figure 12: Melting Point.

Sr. No.	Melting point	**Melting range**
1.	119°C	119°C-121°C
2.	121°C	
3.	121°C	

Table 3: Melting point and melting range.

Loss on Drying

- **Limit:** NLT 10.0% and NMT 13.3%.
- **Procedure:** Weigh 1.000 g of substance in a clean and dried pre weighed LOD Bottle. Cover the stopper and gently shake to distribute material to not more than 10 MM height. Place the LOD Bottle in the oven and remove the cover and leave it also inside the oven. Dry the sample at 150°C for 4 hr. On opening the chamber, immediately close the LOD Bottle, transfer it to the desiccators' and bring it to room temperature. Weigh up to constant weight [15].

Calculation:
Where:
W1 = Weight of empty clean and dried LOD Bottle.
W2 = Weight of LOD Bottle + sample.
W3 = Weight of LOD Bottle + sample. (After drying)

W1= Weight of LOD bottle (g) = 10.730g
W2= Weight of LOD bottle + Weight of sample = 10.730 + 1.0= 11.730g
W3= Weight of sample taken = 1.0g
After Drying,
Weight of LOD bottle + Weight of sample = 11.7g
Weight of sample after drying =11.7 - 10.730 = 0.97g
% Loss on drying = W2-W1/W2-W3 x 100
=11.740 - 10.730/11.740 – 1.0 x 100
= 1.01/10.74 x 100
= 9.40%

Assay

- **Limit:** NLT97.5% and NMT 100.5% calculated on dried substance.
- **Reagent Required:** 3N Hydrochloric acid, 0.05M Disodium Edetate, Sodium hydroxide solution, Hydroxy Naphthol blue.

Procedure

Dissolve about 350mg of Calcium Citrate, previously dried at 150° to constant weight and accurately weighed, in a mixture of 10ml of water and 2ml of 3N hydrochloric acid, and dilute with water to about 100ml. While stirring, add about 30ml of 0.05 M Edetate Disodium VS from a 50ml burette. Add 15ml of 1N sodium hydroxide and 300mg of Hydroxy Naphthol blue, and continue the titration to a blue endpoint. Each ml of 0.05 M Edetate Disodium is equivalent to 8.307 mg of $Ca_3 (C_6H_5O_7)_2$ [15] (Figure 13).

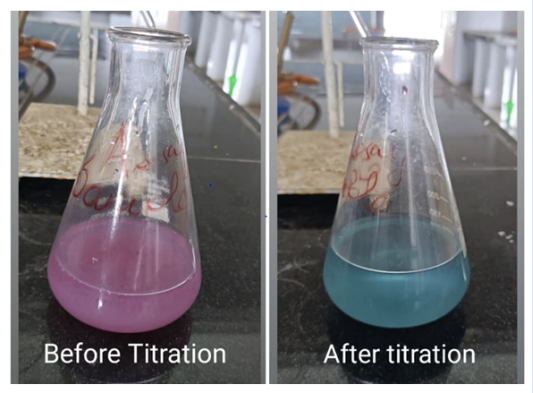


Figure 13: Assay of Calcium Citrate.

Calculation: Percentage Purity
- Standard 100 mg in 100 ml 3 N HCL
- 1 ml standard solution in 100 ml water
- Sample 100 mg in 100 ml 3 N HCL
- 1 ml sample solution in 100 ml water

Standard Dilution absorbance = 0.538
Sample dilution absorbance = 0.565
= Absorbance sample/Absorbance standard x wt. of standard/ wt. of sample x 99.68
= 0.565/0.538 x 0.101/0.106 x 99.68
= 99.74%

UV Absorbance

Shimadzu UV-1800 spectrophotometer measurements were made in quartz cells with a standard optical path length of l = 1 cm. At a temperature of 25°C, the solution's absorbance (A) was measured in the middle-UV region (190-400 nm). Except for the least concentrated solutions, the solute concentrations in the standard solution and sample solution were suitable for maintaining peak absorbance in the range of 0.1–1.0, with the lowest relative error of the measured concentrations due to apparatus noise.

To confirm the calibration's linearity, absorbance measurements were made for standard solutions. There were three measurements made for each concentration. Both of the analysed series revealed the rectilinear dependency of absorbance on concentration, which was supported by an excellent linear fit (Table 4). The aforementioned procedure therefore complies with the Beer-Lambert law and is effective for the quantitative measurement of citrates in the investigated concentration range of 0.5-5.0 mmol/L (real concentrations obtained after mixing analysed materials with the Hcl solution) [16] (Figure 14).

Wavelength (nm)	Absorbance λ
200	0.061
225	0.842
250	0.903
275	0.909
300	0.909

Table 4: Absorbance.

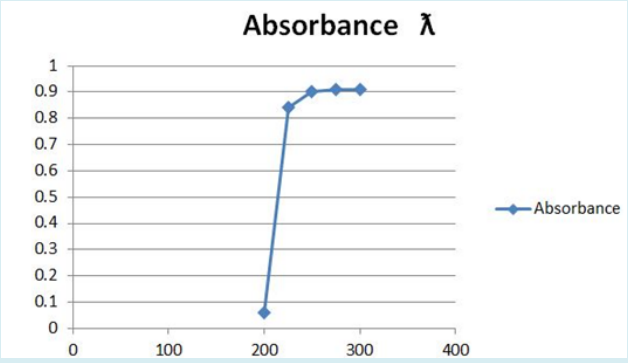


Figure 14: calibration curve of isolated calcium citrate.

Thin Layer Chromatography (TLC)

The TLC plate was created using silica gel on a clean glass plate (8 x 12 cm), dried, and then activated in the oven for roughly an hour at 110 °C. Following activation, 20 L of tri-sodium citrate (a positive control) and every plant extract in the usable range of 20 mg to 100 mg were placed in spot type onto the TLC plates. The TLC plates should be left at room temperature for 30 minutes to allow the solvent to drain before using the TLC bio autographic procedure [16] (Figures 15-20).

Development of TLC

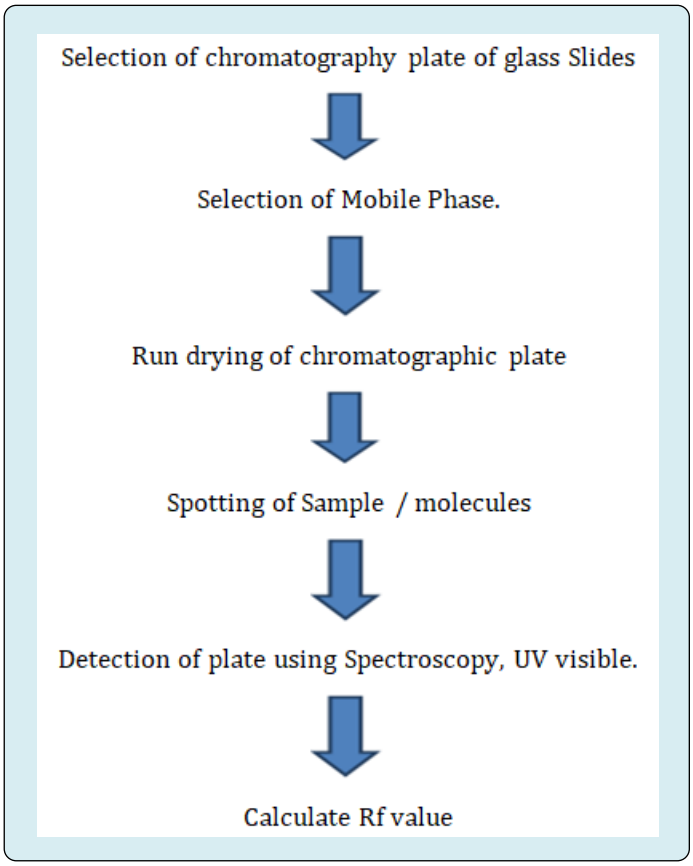


Figure 15: TLC of Calcium Citrate.

Solvent System Development (Table 5)

Sr. no.	Solvent system (Mobile phase)	Ratio
1	Chloroform	10
2	Methanol	10
3	Toluene	10
4	Ethyl alcohol	10
5	N-Hexane	10
6	Ethyl acetate	10
7	Ethyl alcohol+ammoniumhydroxide+water	8:0.4:1.6
8	Ethyl alcohol+chloroform	9:01
9	Ethyl alcohol+chloroform	8:02
10	Ethyl alcohol+chloroform	7:03
11	Ethyl alcohol+chloroform	6:04
12	Ethyl alcohol+chloroform	5:05
13	Chloroform+ethyl alcohol	6:04
14	Chloroform+ethyl alcohol	7:03

Table 5: Chloroform.

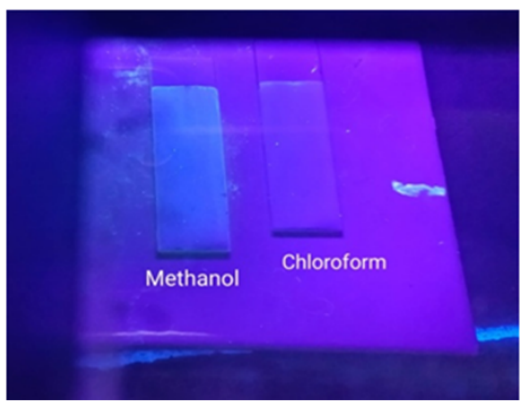


Figure 16: Using Individual solvent system for TLC.

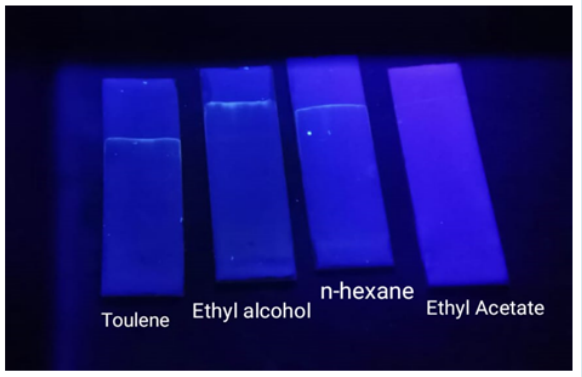


Figure 17: Solvent system of toluene, ethyl alcohol, n-hexane and ethyl acetate.

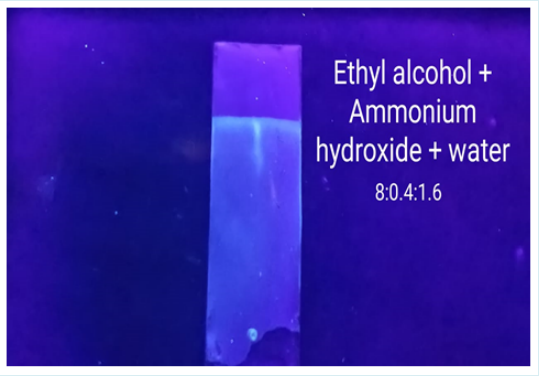


Figure 18: Ethyl alcohol+ammoniumhydroxide+water (8:0.4:1.4).

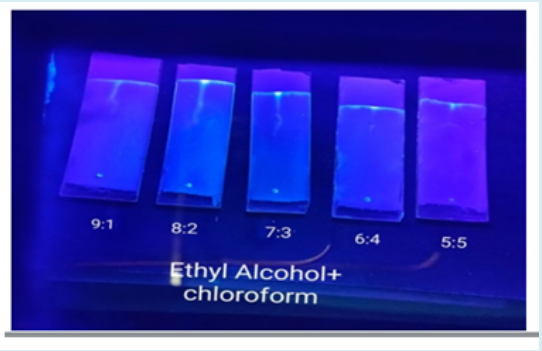


Figure 19: Ethyl alcohol + chloroform (9:1/8:2/7:3/6:4/5:5).

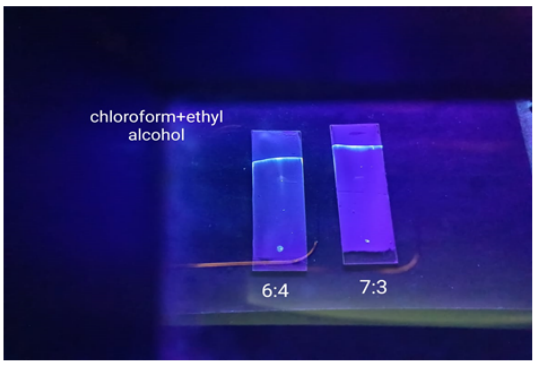


Figure 20: Chloroform + Ethyl alcohol (6:4 and 7:4).

Rf value = distance travelled by the solute/ distance travelled by the solvent
Chloroform : Ethyl alcohol (6:4)
Rf = 3.4/5
Both solvent system which is best for the Calcium citrate = 0.68
Chloroform : Ethyl alcohol (7:3)
Rf = 3.2/6 = 0.53

Conclusion

In the present study it was concluded that: the Preliminary identification test found the presence of calcium citrate. The melting point of isolated calcium citrate is estimated at 119°C-121°C. Among the isolated calcium citrate percentage yield calculated on 24.02% w/w. It was performed thin layer Chromatography, calculated Rf value is 0.68 and 0.53 using polar, semipolar and non polar solvents. It was performed UV Spectrophotometry the calculated λ max is 0.036. As ample amount of calcium citrate was isolated from herbal source i.e. lemon juice, the study reached to the conclusion that instead of synthetic sources, the herbal source can be very useful and economical approach for the isolation of calcium citrate from lemon juice.

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