CHAPTER (1)

Introduction

Introduction

(1-1) History of Vitamin C :

A published work in 1753 suggested that citrus fruits (limes) contained certain compounds that could treat scurvy. Scurvy was endemic between the 17th and 19th centuries because of insufficient intake of fruits and vegetables. Today, we know that Vit C also known as ascorbic acid, has the ability to cure and prevent scurvy.

The Vit C discovery began in the late 16th century when French explorers were saved from efforts of scurvy by drinking a tea made from the arbor tree during long sea voyages. Later, it was noted that lemon juice can prevent people from getting scurvy and by 1734, it was concluded that people who did not eat fresh vegetables and greens would get the disease, and all seamen were thus provided with citrus fruits. British explorer Cook also supplied his men with limes during their long voyages in the late 18th century. These observations led to important breakthroughs in the understanding of scurvy by conducting experiments on guinea pigs and became one of the first examples of the use of animal models to study nutritional diseases. The first isolation of “ascorbic acid” was achieved in 1937 by Svirbely and Szent-Gyorgyi, who went on to win the Nobel Prize in Medicine. The first synthesis of Vit C was achieved by Haworth and Hirst, also resulting in a Nobel Prize in Chemistry in 1937. Mass production of Vit C by Hoffmann-La Rochecame 20 years later.

(figure 1-1) Vitamin **C**

[1]

(1-2) Definition of Ascorbic Acid :

Ascorbic Acid is ,naturally, occurring organic compound with antioxidant properties. It is a white solid impure samples can appear yellowish. It dissolves well in water to give mildly acidic solutions. Ascorbic is one form “vitamer” of vitamin C. It was originally called L-hexuronic acid ,but when it was found to have vitamin C activity in animals “vitamin C” being defined as an activity vitamin ,the suggestion was made to rename L-hexuronic acid .The new name is derived from a- (meaning no) and scorbutus (scurvy), the disease caused by a deficiency of vitamin C.

Because it is derived from glucose many animals are able to produce it, but humans require it as part of their nutrition. Other vertebrates lacking the ability to produce A A include other primates, Guinea pigs, tallest fishes, bats and some micro nutrient. There exists an A A which doesn’t occur in nature. It maybe synthesized artificially. I t has identical antioxidant properties to L-ascorbic acid yet has far less vitamin C activity (although not quite zero). This fact is taken as evidence that the antioxidant properties of A A are only a small part of its effective vitamin activity.

To be specific L-ascorbate is known to participate in many specific enzyme reactions that require the correct epimer (L-ascorbate and not D-ascorbate).

[2]

(1-3) Sources of Vitamin C :

Vegetables and fruits are the best sources of vitamin C. This table will help you choose foods that are high in vitamin C.

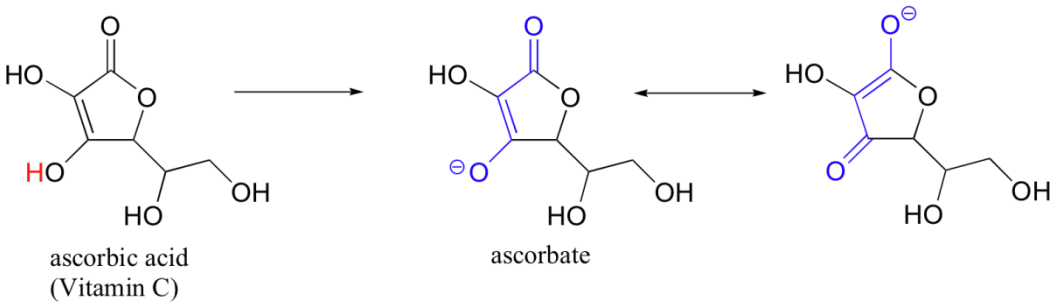
- Table shows sources of vitamin C :

Food	**Serving size**	**Vitamin C (mg)**
Vegetables and Fruit		
Vegetables		
Peppers (red, yellow) raw	125 mL (½ cup)	101-144
Peppers (red, green), cooked	125 mL (½ cup)	121-132
Peppers, green, raw	125 mL (½ cup)	63
Broccoli, cooked	125 mL (½ cup)	54
Cabbage, red, raw	250 mL (1 cup)	54
Tomato sauce, canned	125 mL (½ cup)	15
Fruit		
Guava	1 fruit	206
Papaya	½ fruit	94
Kiwifruit	1 large	84
Orange	1 medium	59-83
Lychee	10 fruits	69
Strawberries	125 mL (½ cup)	52

Pineapple	125 mL (½ cup)	39-49
Grapefruit, pink or red	½ fruit	38-47
Clementine	1 fruit	36
Cantaloupe	125 mL (½ cup)	31
Mango	½ fruit	29
Avocado, Florida	½ fruit	26
Soursop	125 mL (½ cup)	25
Tangerine or mandarin	1 medium	22
Persimmon	125 mL (½ cup)	17
Berries (raspberries, blueberries, blackberries)	125 mL (½ cup)	14-17
Juice		
Juice (orange, grapefruit, apple, pineapple, grape) , Vitamin C added	125 mL (½ cup)	23-66
Fruit and vegetable cocktail	125 mL (½ cup)	35-40
Guava nectar	125 mL (½ cup)	26
Grain Products ,Milk and Alternatives and Meats and Alternatives	These food groups contain very little of this nutrient.	

[3]

(1-4) Acidity :



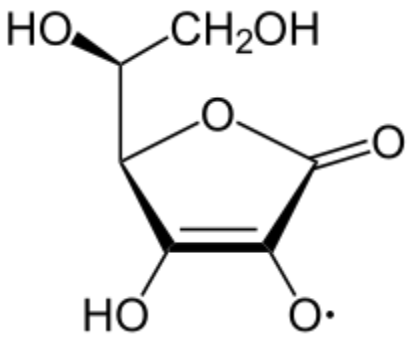
(figure 1-2) Structures for The Ascorbate Anion

The ascorbate anion is stabilized by electron delocalization, as shown above in terms of resonance between the two structures. For this reason A A is much more acidic than would be expected if the compound contained only isolated hydroxyl groups.

[4]

(1-5) Antioxidant Mechanism :

The ascorbate ion is the predominant species at typical biological pH values. It is a mild reducing agent and antioxidant. It is oxidized with loss of one electron to form a radical cation and then with loss of a second electron to form Dehydroascorbic acid. It typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical. Such radicals are damaging to animals and plants at the molecular level due to their possible interaction with nucleic acids, proteins and lipids. Sometimes these radical initiate chain reactions. Ascorbate can terminate these chain radical reactions by electron owing to the resonance stabilized nature of its own radical ion called “Semidehydroascorbate”.



(figure 1-3) Semidehydroascorbate radical

The oxidized forms of ascorbate are relatively unreactive and don`t cause cellular damage. However, being a good electron donor, excess ascorbate in the presence of free metal ions can not only promote but also initiate free radical reactions, thus making it a potentially dangerous pro-oxidative compound in certain metabolic contexts. In exposure to oxygen ascorbic acid undergo further oxidative decomposition to various products including diketogulonic acid, xylonic acid, threonic acid and oxalic acid.

[5]

(1-6) Food Chemistry :

Ascorbic acid and its Sodium Potassium and Calcium salts are commonly used as antioxidant additives. These compound are water soluble and thus can`t protect fats from oxidation. For this purpose the fat soluble esters of ascorbic acid with long chain fatty acids (ascorbyl palmitate or ascorbyl stearate) can be used as food antioxidants.
Eighty percent of world`s supply of ascorbic acid is produced in China.

The relevant European food additive E numbers are :

- E300 Ascorbic Acid (approved for use as a food additive in the EU, USA, Australia and New Zealand).
- E301 Sodium Ascorbate (approved for use as a food additive in the EU, USA, Australia and New Zealand)

- E302 Calcium Ascorbate (approved for use in the the EU, USA, Ausralia and New Zealand).
- E303 Potassium Ascorbate.
- E304 fatty acid esters of Ascorbic Acid :
    i. Asorbyl Palmitate.
    ii. Ascorbyl Stearate.

It creates volatile compounds when mixed with glucos e and amino acids in 90 C.

[6]

(1-7) Biosynthesis :

Ascorbic Acid is found in plants and animals where it is produced from gucose. Animals must either produce it or digest it, otherwise a lack of vitamin C may cause scurvy, which may eventually lead to death. Reptiles and older orders of make Ascorbic acid in their kidneys. Recent orders of birds and most mammals make Ascorbic acid in their liver where the enzyme L-gulonolactone oxidize is required to convert Glucose to Ascorbic acid. Humans, some other primates and Guinea pigs are not able to make L-gulonolactone oxidize because of genetic mutation. Synthesis and signaling properties are still under investigation.

(1-7-1) Animal Ascorbic Acid Biosynthesis Pathway :

The biosynthesis of Ascorbic acid starts with the formation of UDP-Glucuronic acid. UDP-glucuronic acid is formed when UDP-glucose undergoes two oxidations catalyzed by the enzyme UDP-glucose-6-dehydrogenase. UDP-glucose-6-dehydrogenase uses the co-factor NAD+ as the electron acceptor. Pyrophosphorylase removes a UMP and Glucuronokinase, with the co-factor ADP, removes the final Phosphate leading to D-glucuronic acid. The aldehyde group of this is reduced to a primary alcohol using the enzyme Glucuronate reductase and the co-factor NADPH, yielding L-gulonic acid. This is followed by lactone formation with the hydrolase gluconolactonase between the carbonyl on C1 and hydroxyl group on the C4. L-gulonolactone then reacts with

Oxygen, catalyzed by the enzyme L-gulonolactone oxidase (which is nonfunictional in humans and other primates) and the co-factor FAD+. This reaction produces 2-oxogulonolactone, which spontaneously undergoes enolization to form Ascorbic acid.

(1-7-2) Plant Ascorbic Acid Biosynthesis Pathway :

There are many different biosynthesis pathways for Ascorbic acid in plants. Most of these pathways are derived from products found in glycolysis and other pathways. For example, one pathway goes through the plant cell wall polymers. The plant Ascorbic acid biosynthesis pathway most principal seems to be L-galactose. L-galactose reacts with the enzyme L-galactose dehydrogenase, where by the lactone ring opens and forms again but with between the carbonyl on C1 and hydroxyl group on the C4, resulting in L-galactonolactone. L-galactonolactone then reacts with the mitochondrial flavoenzyme L-galactonolactone dehydrogenase to produce Ascorbic acid.
An interesting fact about L-Ascorbic acid is that it has shown to have a negative feedback on L-galactose dehydrogenase in spinach. Ascorbic acid efflux by embryo o dicots plants is a well-established mechanism of iron reduction and a step obligatory for iron uptake.
[7]

(1-8) Industrial Preparation :

Ascorbic acid is prepared in industry from glucose in a method based on the historical Reichstein process. In the first of a five-step process, glucose is catalytically hydrogenated to sorbitol, which is then oxidized by the microorganism Acetobacter suboxydans to sorbose. Only one of the six hydroxyl groups is oxidized by this enzymatic reaction. From this point, two routes are available. Treatment of the product with acetone in the presence of an acid catalyst converts four of the remaining hydroxyl groups to acetals. The unprotected hydroxyl group is oxidized to the carboxylic acid by reaction with the catalytic oxidant TEMPO (regenerated by Sodium Hypochlorite — bleaching solution). Historically, industrial preparation via the Reichstein process used Potassium Permanganate as the bleaching solution. Acid-catalyzed hydrolysis of this product performs the dual function of removing the two acetal

groups and ring-closing lactonization. This step yields ascorbic acid. Each of the five steps has a yield larger than 90%.

More biotechnological process, first developed in China in the 1960s, but further developed in the 1990s, by passes the use of acetone-protecting groups. A second genetically modified microbe species, such as mutant Erwinia, among others, oxidises sorbose into 2-ketogluconic acid (2-KGA), which can then undergo ring-closing lactonization via dehydration. This method is used in the predominant process used by the Ascorbic acid industry in China, which supplies 80% of world's Ascorbic acid. American and Chinese researchers are competing to engineer a mutant that can carry out a one-pot fermentation directly from Glucose to 2-KGA, by passing both the need for a second fermentation and the need to reduce Glucose to Sorbitol.

[8]

(1-9) Properties :

- Table shows properties of vitamin C :

Properties	
Chemical formula	$C_6H_8O_6$
Molar mass	176.12 g·mol^{-1}
Appearance	White or light yellow solid
Density	1.65 g/cm^3
Melting point	190 to 192 °C (374 to 378 °F; 463 to 465 K) decomposes
Solubility in water	330 g/L
Solubility in ethanol	20 g/L

Solubility in glycerol	10 g/L
Solubility in propylene glycol	50 g/L
Solubility in other solvents	insoluble in diethyl ether, chloroform, benzene, petroleum ether, oils, fats

[9]

(1-10) Ascorbic Acid Oral Use :

Ascorbic acid is used to prevent or treat low levels of vitamin C in people who do not get enough of the vitamin from their diets. Most people who eat a normal diet do not need extra Ascorbic acid. Low levels of vitamin C can result in a condition called scurvy. Scurvy may cause symptoms such as rash, muscle weakness, tiredness and tooth loss.
Vitamin C plays an important role in the body. It is needed to maintain the health of skin, teeth, bone and blood. It is also used to protect your body`s cells from damage. It is known as an antioxidant.
Other Uses :
This vitamin may also be used with other vitamins for a certain eye condition (macular degeneration). Free radicals molecules produced by the body that can damage cells and DNA, may also be involved in the destruction of cartilage. Antioxidants such as this vitamin appear to limit the damage caused by free radicals. However, that said no evidence suggests that taking vitamin osteoarthritis (OA), putting pressure on bones and joints. In addition, some researches think C supplements well help treat or prevent OA. What the evidence does show as that people who eat diets rich in vitamin C are less likely to be diagnosed with arthritis.
Taking nonsteroidal anti-inflammatory drugs can lower your levels of vitamin C. If you take these drugs regularly for OA, you might want to take a vitamin C supplement.

(1-10-1) Age-related Macular Degeneration

Vitamin C (500 mg) appears to work with other antioxidants, including zinc (80 mg), beta-carotene (15 mg), and vitamin E (400 IU) to protect the eyes against developing macular degeneration (AMD), the leading cause of legal blindness in people over 55 in the United States. The people who seem to benefit are those with advanced AMD. It isn't known whether this combination of nutrients helps prevent AMD or is beneficial for people with less advanced AMD. This combination includes a high dose of zinc, which you should only take under a doctor's supervision.

(1-10-2) Pre-eclampsia

Some studies suggest that taking vitamin C along with vitamin E may help prevent pre-eclampsia in women who are at high risk. Pre-eclampsia, characterized by high blood pressure and too much protein in the urine, is a common cause of premature births. Not all studies agree, however.

(1-10-2) Asthma

Studies are mixed when it comes to the effect of vitamin C on asthma. Some show that low levels of vitamin C are more common in people with asthma, leading some researchers to think that low levels of vitamin C might increase the risk for this condition. Other studies seem to show that vitamin C may help reduce symptoms of exercise-induced asthma.

[10]

(1-11) Ascorbic Acid in Psidium Guajava Leaves :

Psidium Guajava Leaf is an important part of Guava tree which is useful in curing many health problems. Guava leaves having properties like antibactrial, anti oxidant, anti cancer and it is used in many diseases. In this research different extraction methods are discussed. In this study, aqueous extract shows it is widely use in medical uses. Aqueous extract of Guajava leaves prevent neurodegenerative and cardiovascular

disease also it shows anti prostate cancer activity, anti hyperglycaemic and anti cancer activities.

(1-11-1) Chemical Composition :

The leaves contain essential with the main components being α-Pinene, β-Pinene, Limonene, Menthol, Terpenyl Acetate, Isopropyl alcohol, Longicyclene, Caryophyllene, β-Bisabolene, Caryophyllene Oxide, β-Copanene, Farnesene, Humulene, Selinene, Cardinene and Curcumene. The essential oil from the leaves has been shown to contain Nerolidiol, β-Sitosterol, Ursolic, Crategolic and Guayavolic acids have also been identified. The leaves contain Fixed oil 6%, Volatile oil 0.365%, 3.15% Resin, 8.5% Tannin and a number of other fixed substances.

The essential oil contains Eugenol, Mallic acid and Tannin from 8-15%.

The leaves contain an essential oill rich in Cineol and four triterpenic acids as weel as three flavonoids leaves contain Resin, Fat, Cellulose, Tannin, Volatile oil, Chlorophyll and Mineral salts.

(1-11-2) Medicinal Properties of Psidium Guajava Leaves :

Guava leaves: guava leaves have some wonderful medicinal properties which are safe and also effective. They are discussed below:

It’s an excellent mouth freshener and can readily replace any toothpaste or other cosmetic tooth remedy.

How to : It’s just simple to use, a fresh tender guava leave can be chewed to paste and then rubbed on the gums with fingers, it’s a very common morning mouth freshener in India and it’s so effective that any one practicing it once is bound to love it, it’s a potent germs killer and people practicing it regularly do not need to see their dentist, so it can be said “a guava leave a day, keeps the dentist away”.

The guava leaves concoction is an effective remedy for diarrhea, it can be made by boiling fresh leaves in water, and the concoction can be

stored as refrigerated for future use. It`s a poor man's remedy for diarrhea and have indeed worked as a life saver in many occasions.

It's a proven remedy for type two diabetes and in Japan its extracts are used commonly to reduce blood sugar levels and indeed its extracts is successfully marketed by herbal medicine vendors as a natural remedy for diabetes; any form of intake of the guava leaves is bound to benefit the diabetics.

Obesity is another disease which can be managed successfully by the guava leaves and its extracts. The guava leaves tea is an excellent dieting supplement and effective in reducing body weight. Thus it has a double impact on diabetes.

The guava leaves extracts have shown potential effect against the prostate cancer and the subject is a new buzzword in the cancer management circles around the world in the recent times.

The guava leaves have a wide spread use as a liver and jaundice remedy in India, research has proved the liver protective properties of the guava leave extract ,thus it can be consumed as a liver tonic.

It`s a proven remedy for a wide spectrum of skin ailments, Eczema, to be more precise is well treated with guava leave extracts, it`s very effective as a local application treatment for psoriasis also.

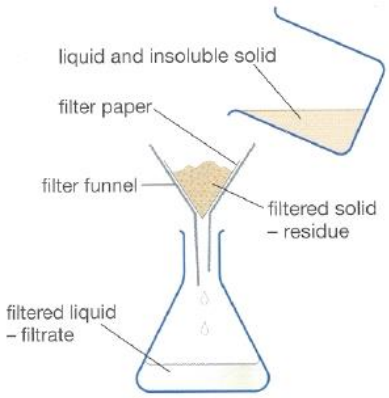
It's an excellent antioxidant and thus has sure shot benefits against many body ailments and has a protective property against some serious ailments. The guava leave extract are one of the most easily available nature's health tonic.

[11]

(1-12) Methods of Ascorbic Acid Extraction from Psidium Guajava Leaves :

i. Solvent Extraction of Ascorbic Acid at Ambient Temperature :

In this technique we will place our sample in the solvent in room temperature and pressure for few days then the precipitate will collect by filtration.



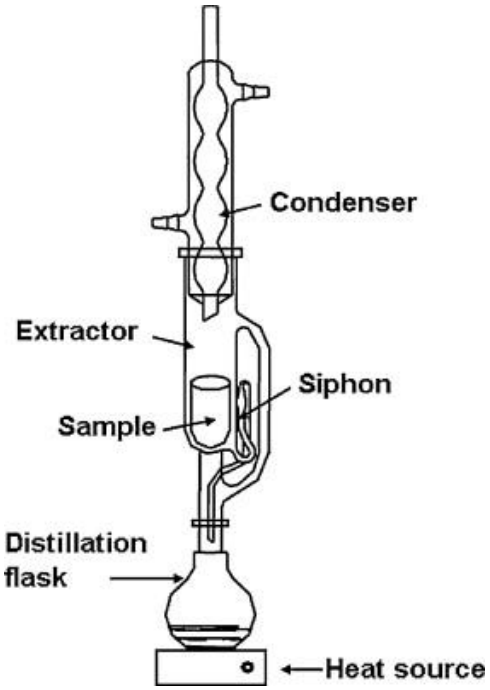
(figure 1-4) Filtration

ii. Soxhlet Extraction :

Soxhlet have many components like condenser, thimble and flask.

To do the extraction using soxhlet we well accurately weight 10g of leaves and place it in the thimble which loaded into soxhlet and the solvent well be in the flask. Solvent vapor moves up to the column and floods into the chamber. Some parts of non volatile compounds dissolve in solvent. Process repeats many times.

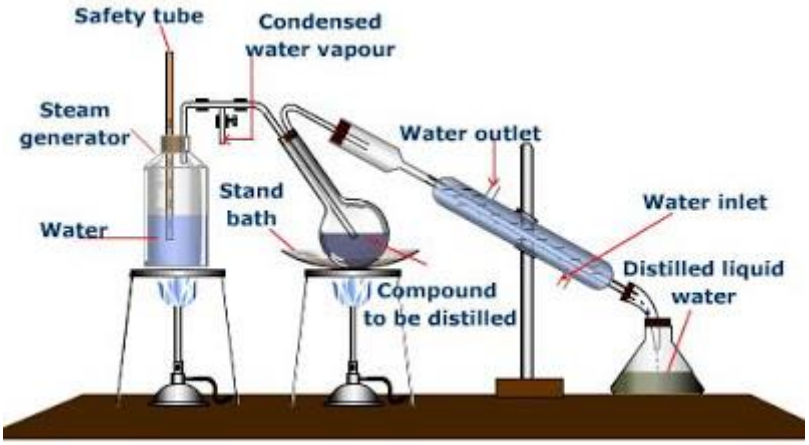
The extraction has been done at boiling temperature of the solvent in 100ml of Ethanol for 3 hours.



(figure 1-5) Soxhlet

iii. Steam Distillation :

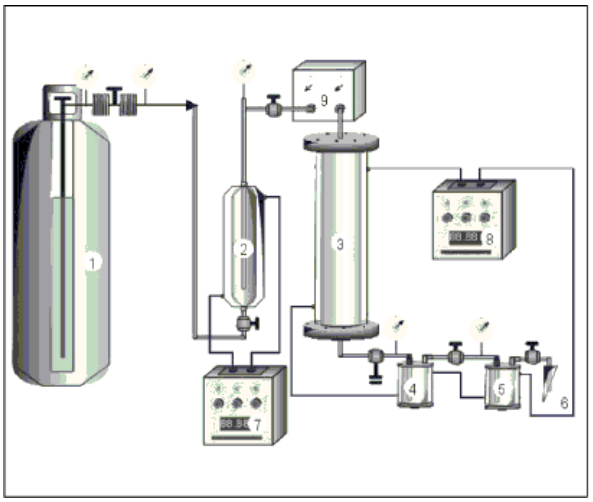
It is used to separate two miscible liquids which are highly heat sensitive and may possibly decompose below their boiling point. It is commonly used in the extraction of essential oils.



(figure 1-6) Steam Distillation

iv. Supercritical Fluid Extraction (SFE) :

This technique resembles soxhlet extraction except that the solvent used is a supercritical fluid, substance above its critical temperature and pressure. This fluid provides a broad range of useful properties. One main advantage of using SFE is the elimination of organic solvents, thus reducing the problems of their storage and disposal in the lipidologist laboratory. Furthermore, several legislative protocols (such as the EPA Pollution Prevention Act in the USA) have focused on advocating a reduction in the use of organic solvents which could be harmful to the environment.

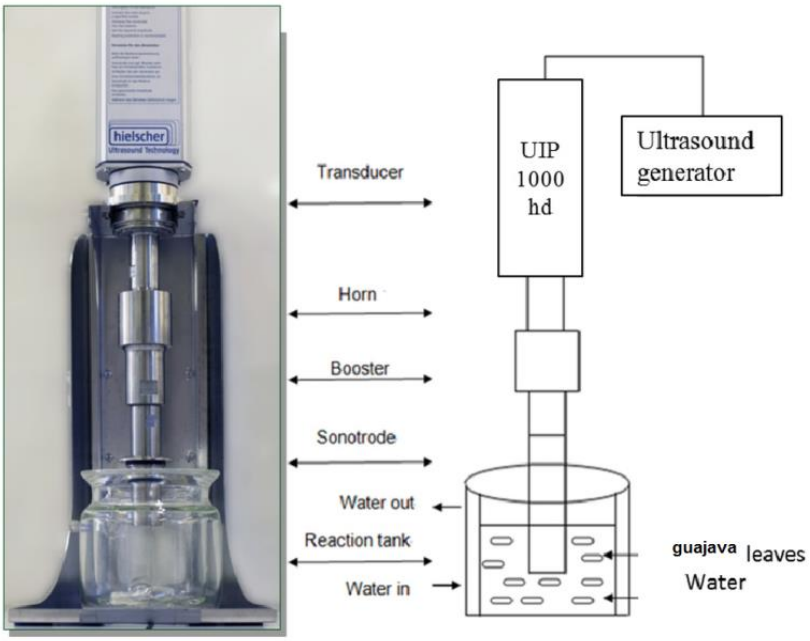


(figure 1-7) SFE

v. Ultrasound Extraction :

Ultrasound apparatus consist of horn, booster, sonotrode, water tank and ultrasound generator.

If we use this tichnique, we well take 1.5mg of solid sample in the apparatus.



(figure 1-8) Ultrasound Apparatus

[12]

CHAPTER (2)

Materials & Method

(2-1) Materials :

Psidium Guajava leaves was taken from Abo Adam region and dried under sun then prepared in small shape using mortar and pestle.

(2-2) Chemicals :

- Ethanol
- Iodine (0.005M)
- Starch (1g/50ml)
- Diethylether (Assay 98%, M.wt 74.12, Alpha Chemica)
- Distilled Water

(2-3) Method :

i- Extraction of Ascorbic Acid Using Solvent in Ambient Condition :

10g of dried Guajava Psidium Leaves was weighted by using sensitive balance and putted in a beaker. 125ml of Ethanol was added and the beaker was covered and putted away for four days then it was filtered by using filter paper. After that, the solvent was evaporated by using rotary evaporating and the percentage of yield was calculated.

ii- Soxhlet Extraction :

10g of the dried leaves was weighted accurately and placed in the thimble which was loaded into soxhlet. Vessel having flask containing extractor solvent. The sample was extracted with 250ml of Ethanol. The extraction has been done for 3 hours.

The abstract was collected and transferred into beaker (250ml), the solvent was separated from extraction used rotary vacuum distillation. Then the abstract was put for two days without covering, after that, the

precipitate was washed with five portions (20ml) of Diethylether with filtration.

Then the precipitate was dried, weighted and the yield percentage was calculated.

- **Preparation of sample solution :**

. 0.1g of sample precipitate was dissolved in a few quantity of distilled water and transferred to (100ml) volumetric flask, the solution was completed to the mark by using distilled water.

iii- Iodine Titration Determination :

25ml of sample solution which was prepared then transferred to a conical flask after that titrated against standard Iodine solution (0.005M) using 1ml of starch solution as indicator.

iv- IR :

A little of Potassium Bromide was added to the extract after the sample was powdered by pestle and mortar then it was placed between discs by pressing such as a thin layer ,carefully, without making any space or agglomerates. After that, the prepared sample was placed through the path of Infra Red radiation and the result was printed.

- **Calculation :**

Yield percentage : (Practical wt. \ Theoretical wt.) x 100%

Molar concentration : (MxV) \ n = (MxV) \ n

M = molar concentration

V = volume

n = number of moles

CHAPTER (3)

Results & Discussion

(3-1) Results

- Table shows results of extraction in ambient conditions :

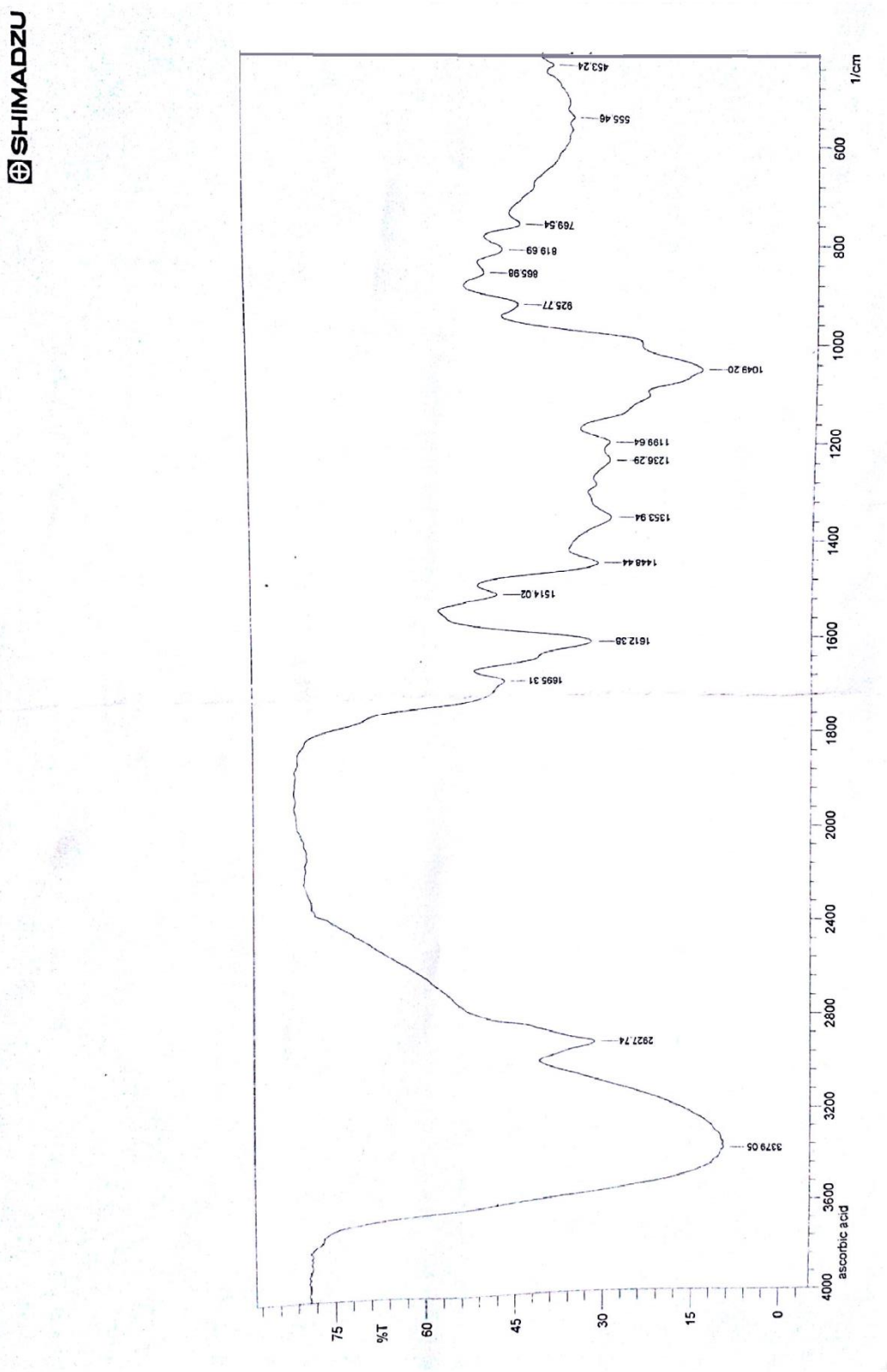
Weight of beaker/g	Weight of beaker+extract/g	Weight of extract/g	Yield percentage
98.20g	99.71g	1.51g	15.1%

- Table results of extraction using soxhlet :

Weight of filter paper/g	Weight of filter paper+extract/g	Weight of extract/g	Yield percentage
1.06g	3.13g	2.07g	20.7%

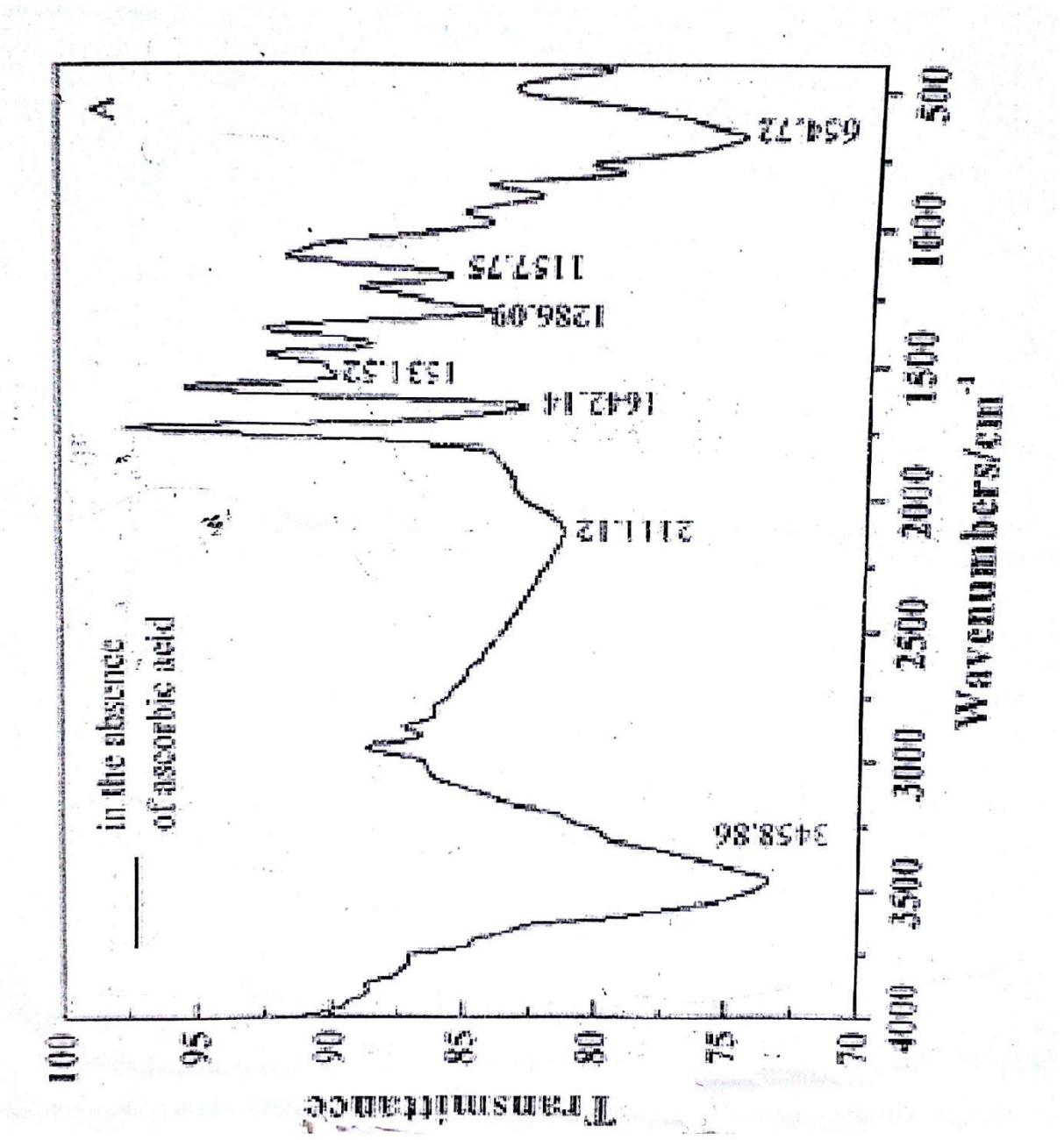
- Table shows the Iodine titration results :

Initial volume\ml	Final volume\ml	Used volume\ml	Molar concentration
0.00	7.00	7.00	0.00141 mol\l 0.000141 mol\100 ml
7.00	14.10	7.10	



(figure 3-1) IR sample identification

(figure 3-2) IR standard sample identification



(3-2) Calculations

- Yield percentage (ambient conditions) =
  (extract wt.\ sample wt.) x 100
  = 15.1%

- Yield percentage (soxhlet conditions) =
  (extract wt. \ sample wt.) x 100
  = 20.7%

- Iodine titration :
  (MxV) \ n = (MxV) \ n
  M = 0.00141 mol \ l
  = 0.000141 mol \ 100 ml

(3-3) Discussion

In this research extraction of Ascorbic acid A A (Vitamin C) from Psidium Guajava Leaves and it`s properties were under studying.

The extraction done using two methods. Firstly,solvent extraction in ambient conditions using Ethanol and in other method soxhlet extraction was used.

Yield percentage in ambient condition extraction 15.1% but in soxhlet extraction 20.7%. The difference may due to difference in temperature, methods used and conditions.

Maybe the drying under sun the reason of low percentages, also, a mistake in preparation method may caused the low percentages and the difference..

The identification by using IR technique was done and comparing between sample's peaks values with standard`s peaks values was done to be sure that our sample is Ascorbic acid.

- (3379 and 3458 this range represent hydroxyl in carboxylic functional group)
- (2927.74 and 2111.12 this range represent C-H stretching vibration)
- (1531.52 and 1514.02 this range represent carbonyl functional group)

There was some other peaks in different ranges and they may caused by impurities or due to non selective solvent that used to extract our substance.

Ascorbic acid quantity was measured using redox titrations.

In Iodine titration, the molar concentration calculated 0.000141 mol\100ml.

(3-4) Suggestions

- Maybe the drying under sun the reason of low percentages, also, a mistake in preparation method may caused the low percentages and the difference.
- Using other material like lemon or orange to get high extraction percentages.
- The solvent used must be selective and high purity.
- Using high accuracy technique like HPLC.
- Accuracy in working.

(3-5) References

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