Chapter one

Introduction

1-1 Ascorbic acid :-

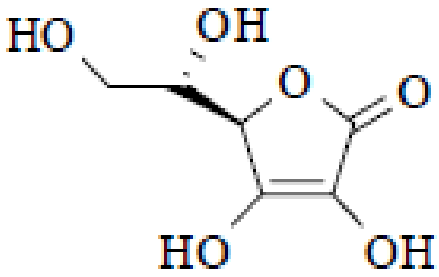
1-1-1 Definition:

Vitamin C (Ascorbic acid) is a powerful antioxidant naturally present in many foods, especially fruits and vegetables, which play an important role in the prevention of infectious diseases. It is important in processes of oxidation and reduction in human organism, participating in several metabolic reactions.

Vitamin C, the L-enantiomer of ascorbic acid, is a water-soluble vitamin used by the body for several purposes. Most animals can synthesize their own vitamin C, but some animals, including primates, guinea pigs, and humans, cannot. Vitamin C was first isolated in 1928, and in 1932 it was proved to be the agent, which prevents scurvy. Citrus fruits (lime, lemon, orange, grapefruit) and tomatoes are good common sources of vitamin C. Other foods that are good sources of vitamin C are papaya, broccoli, brussels sprouts, blackberries, strawberries, cauliflower, spinach, cantaloupe, and blueberries.

The amount of Vitamin C in fruit depends on the precise variety of the fruit, the soil and climate in which it grew, and the length of time since it was picked[1].

1-1-2 Structural formula:



L-Ascorbic acid

1-1-3 Names:

- Ascorbic acid.
- VitaminC.
- L-3-Ketothreohexuronic acid.
- Ascorbate .
- VicominC.
- Ascobicap.
- Acid Ascorbic.
- Antiscorbic vitamin .
- Keto-L-gulofuranolactone-3.
- Lactone.
- L-lyxoascorjbic acid .
- Vitacimin .
- Vitascorbol.[3]

1-1-4 properties:

1-1-4-1Computed Properties:

Molecular Weight	176.12412 g/mol
Molecular Formula	$C_6H_8O_6$
XLogP3	-1.6
Hydrogen Bond Donor Count	4
Hydrogen Bond Acceptor Count	6
Rotatable Bond Count	2
Exact Mass	176.032088 g/mol
Monoisotopic Mass	176.032088 g/mol
Topological Polar Surface Area	107 A^2
Heavy Atom Count	12
Formal Charge	0
Complexity	232
Isotope Atom Count	0
Defined Atom Stereocenter Count	2
Undefined Atom Stereocenter Count	0
Defined Bond Stereocenter Count	0
Undefined Bond Stereocenter Count	0
Covalently-Bonded Unit Count	1

1-1-4-2physical properties:

- *description*

White to very pale yellow crystalline powder with a pleasant sharp acidic taste. Almost odorless.

- *Color*

White to slightly yellow crystals or powder, gradually darkens on exposure to light.

- *Odor*

Odorless.

- *Test*

Pleasant, sharp, acidic taste.

- *Melting point*

190-192 deg C (some decomposition).

- *Solubility*

Insoluble in ether, chloroform, benzene, petroleum ether, oils, fats, fat solvents. The solubility in g/mL is 0.33 in water, 0.033 in 95 wt% ethanol, 0.02 in absolute ethanol, 0.01 in glycerol USP, 0.05 in propylene glycol.

- *Density*

1.65 g/cm3.

- *Vapor pressure*

$9.28X10^{-11}$ mm Hg at 25 deg C.

- *LogP*

log Kow = -1.85

- *Stability*

Stable to air when dry; impure preparation and in many natural products vitamin oxidizes on exposure to air and light. Aqueous solutions are rapidly oxidized by air, accelerated by alkalies, iron, copper.

- *Decomposition*

When heated to decomposition it emits acrid smoke and irritating fumes.

- *pH*

pH = 3 (5 mg/mL); pH = 2 (50 mg/mL).

- *pKa*

4.7 (at 10 °C). [4]

1-1-4-3 Biological function :

Vitamin C is a water soluble organic compound involved in many biological processes (Figure 1-1).

Ascorbic Acid(AA) plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism . Although all the functions of Ascorbic Acid are not fully explained, it is likely that it is also involved in maintaining the reduced state of metal cofactors, for example at monooxygenase (Cu^{+}) and dioxygenase (Fe^{2+}) . In cells the other role of Ascorbic Acid is to reduce hydrogen peroxide (H_2O_2), which preserves cells against reactive oxygen species. Besides this, primates and several other mammals are not able to synthesis ascorbic acid . The animal species, which are able to produce this molecule, biosynthesis AA from glucose catalyzed Lgulonolactonoxidase.

In spite of the ability to synthesize this molecule both groups of animal species suffer from deficiency of Ascorbic Acid. [2]

vitamin C is needed for the production of collagen in the connective tissue. Some tissues have a greater percentage of collagen, including: skin, mucous membranes, teeth, bones .

Vitamin C is also required for synthesis of dopamine, noradrenaline and adrenaline in the nervous system or in the adrenal glands. It is a strong antioxidant. [1]

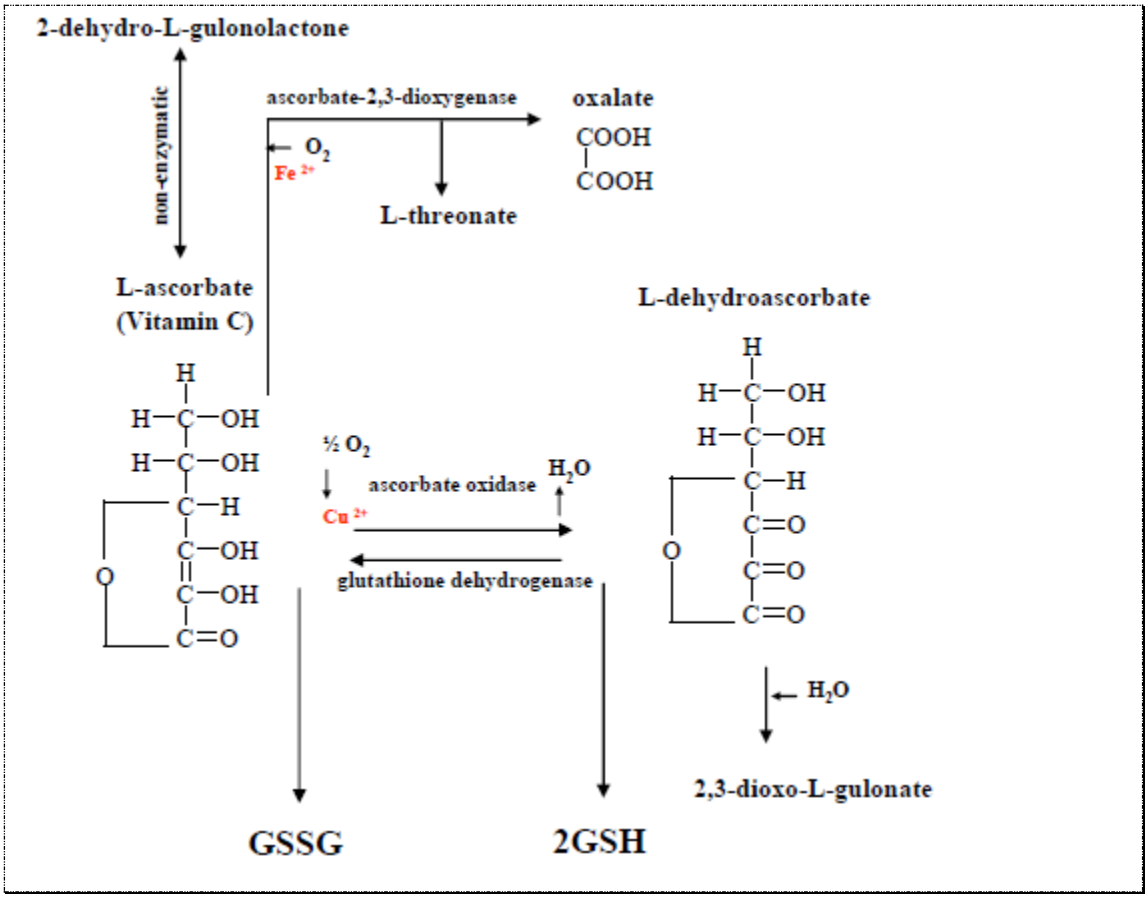


Figure1-1. Scheme of a biological function of ascorbic acid (GSH – reduced glutathione, GSSG – oxidized glutathione)

1-1-5 general uses:

• *Cancer prevention:*

Vitamin C is a known antioxidant and has been associated with reduced risk of stomach, lung, colon, oral, and prostate cancer.

• *Cataract prevention:*

Long-term studies on vitamin C supplementation and cataract development have shown that supplementation significantly reduces the risk of cataracts, particularly among women. One study published in 2002 found that adequate vitamin C intake in women under 60 years of age reduced their risk of developing cataracts by 57%.

• *Collagen production:*

Vitamin C assists the body in the manufacture of collagen, a protein that binds cells together and is the building block of connective tissues throughout the body. Collagen is critical to the formation and ongoing health of the skin, cartilage, ligaments, corneas, and other bodily tissues and structures. Vitamin C is also thought to promote faster healing of wounds and injuries because of its role in collagen production.

• *Diabetes control:*

Vitamin C supplementation may assist diabetics in controlling blood sugar levels and improving metabolism.

• *Gallbladder disease prevention:*

A study of over 13,000 subjects published in the Archives in Internal Medicine found that women who took daily vitamin C supplements were 34% less likely to contract gallbladder disease and gallstones, and that women deficient in ascorbic acid had an increased prevalence of gallbladder disease.

• *Immune system booster:*

Vitamin C increases white blood cell production and is important to immune system balance. Studies have related low vitamin C levels to increased risk for infection. Vitamin C is frequently prescribed for HIV-positive individuals to protect their immune system.

• *Neurotransmitter and hormone building:*

Vitamin C is critical to the conversion of certain substances into neurotransmitters, brain chemicals that facilitate the transmission of nerve impulses across a synapse (the space between neurons, or nerve cells). Such neurotransmitters as serotonin, dopamine, and nor epinephrine are responsible for the proper functioning of the central nervous system, and a deficiency of neurotransmitters can result in psychiatric illness. Vitamin C also helps the body manufacture adrenal hormones. Dining Services.[5]

1-1-6 Daily needs:

The only way humans uptake ascorbic acid is via food , but the daily needs of vitamin C for a human are not clear yet. Linus Pauling postulated that people's needs for vitamins and other nutrients vary markedly and that to maintain good health, many people need amounts of nutrients much greater than the recommended doses.

According to his suggestions, daily uptake of vitamin C has to be within units of grams of AA to reduce the incidence of colds and other diseases. These “huge” amounts of AA have not been ever proved as the reason for large reducing of the incidence of illnesses. Nowadays the estimated average requirement and recommended dietary allowance of ascorbic acid are 100 mg per day and 120 mg per day, respectively.[2]

The best way to get the daily requirement of essential vitamins, including vitamin C, is to eat a balanced diet that contains a variety of foods from the food guide pyramid. Vitamin C should be consumed every day because it is not fat-soluble and, therefore, cannot be stored for later use. The Food and Nutrition Board at the Institute of Medicine recommends the following amounts of vitamin C:

Infants and Children

• 0 - 6 months: 40 milligrams/day (mg/day)

• 7 - 12 months: 50 mg/day

• 1 - 3 years: 15 mg/day

• 4 - 8 years: 25 mg/day

• 9 - 13 years: 45 mg/day

Adolescents

• Girls 14 - 18 years: 65 mg/day

• Boys 14 - 18 years: 75 mg/day

Adults

• Men age 19 and older: 90 mg/day

• Women age 19 year and older: 75 mg/day

Women who are pregnant or breastfeeding and those who smoke need higher amounts.[5]

1-1-7 Content in foods:

AA can be mostly found in fruits and vegetables. The main sources of AA are citrus fruits, hips, strawberries, peppers, tomatoes, cabbage, spinach and others . If one wants to uptake AA from animal sources, liver and kidney are the tissues with highest contents of this molecule, but in comparison with plant sources the amount of AA is very low . The

content of AA in food can be affected by many factors such as clime, method of harvest, storing and processing. Thus, there is a need of analytical procedures able to not only monitor AA content in agricultural and food products, but also in body liquids, tissues and in blood serum. [2]

1-1-8The effect of low & high amount:

Lack of ascorbic acid in the daily diet leads to a disease called scurvy, a form of avitaminosis that is characterized by: loose teeth, superficial bleeding, fragility of blood vessels, poor healing, compromised immunity, mild anaemia.

High doses (thousands of mg) are used but may result in diarrhea. Any excess of vitamin C is generally excreted in the urine. Vitamin C is needed in the diet to prevent scurvy. It also has a reputation for being useful in the treatment of colds and flu.[1]

1-1-9 Synthesis pathway :

1-1-9-1 Biosynthesis:

Ascorbic acid is synthesized by many vertebrates. The biosynthetic capacity has, however, subsequently been lost in a number of species, such as teleost fishes, passeriform birds, bats, guinea pigs and primates including humans, for whom ascorbic acid has thus become a vitamin . Fish, amphibians and reptiles synthesize ascorbic acid in the kidney, whereas mammals produce it in the liver. Vitamin C is also formed by all the plant species studied so far . Interestingly, different pathways have evolved for vitamin C biosynthesis in animals, plants and fungi.

- *Biosynthesis in animals:*

The biosynthesis of L-ascorbic acid in animals followed the glucuronic acid metabolic pathway which is crucial in the metabolism of sugars under both normal and disease states. The glucuronic acid pathway is regulated by other physiological functions of the body. It is an important mechanism during detoxification processes in the body and varies from species to species . In animals, Dglucuronate, derived from UDP-glucuronate, is reduced to L-gulonate. The latter is converted to its lactone which in turn, oxidized to L-ascorbic acid, catalyzed by L-gulono-1,4-lactone oxidase (GLO). The complete biosynthetic pathways are shown in (Figure **2-1**) and (Table1-1).

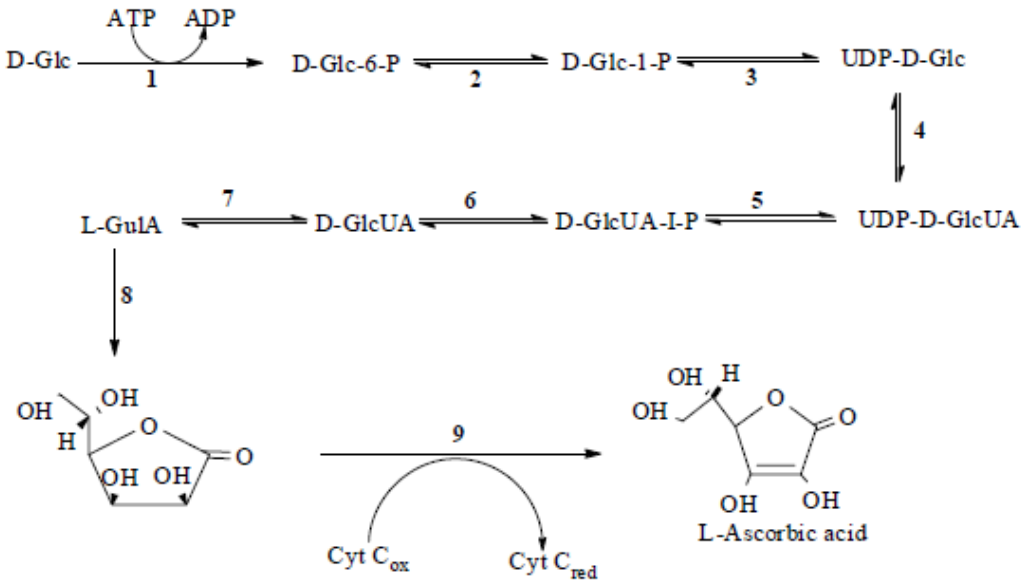


Figure2-1: Biosynthetic Pathway of L-ascorbic acid in animals.

Catalytic step	Enzyme	Substrate
1	Hexokinase	D-Glucose
2	Phosphoglucomutase	D-Glucose-6-phosphate
3	UDP-D-Glucose pyrophosphorylase	D-Glucose-1-phosphate
4	UDP-D-Glucose dehydrogenase	UDP-D-Glucose

5	D-Glucuronate-1-phosphate	UDP-D-Glucuronic acid
6	D-Glucurono kinase	UDP-D-Glucuronic acid-1-phosphate
7	D-Glucuronate reductase	D-Glucuroinc acid
8	Aldonolatonase	L-Galacturonic acid
9	L-Gulono-1,4-lactone dehydrogenase	L-Gulono-1,4-lactone

Table1-1: Biosynthetic Pathway of L-ascorbic acid in animals.

The deficiency of L-ascorbic acid biosynthesis in certain animals and humans is due to the lack of the terminal flavor-enzyme, L-gulono-1,4-lactone oxidase (GLO), which completely blocks the production of L-ascorbic acid in the liver of human beings . This oxidizing enzyme is required in the last step of the conversion of L-gulono-γ-lactone to 2-oxo-L-gulono-γ-lactone, which is a tautomer of L-ascorbic acid that is spontaneously transformed into vitamin C.

- *Biosynthesis in plants:*

The biosynthesis of L-ascorbic acid in plants is not clearly understood as compared to that in animals. But recent advances helped to understand its biosynthesis in plants and resolved the several past contradictions. Biosynthetic pathways generally proceed *via* GDP-D-mannose and GDP-L-galactose , which was proposed by the Smirnoff group . The Smirnoff-Wheeler-L-ascorbic acid biosynthetic pathway represents the major route of L-ascorbic acid biosynthesis in plants (Figure **3**-1) and (Table2-1). The initial step of L-ascorbic acid biosynthesis in plants is also utilized for the synthesis of cell wall polysaccharide precursors, while later steps following GDP-L-galactose are solely dedicated to plant biosynthesis of Lascorbic acid.[6]

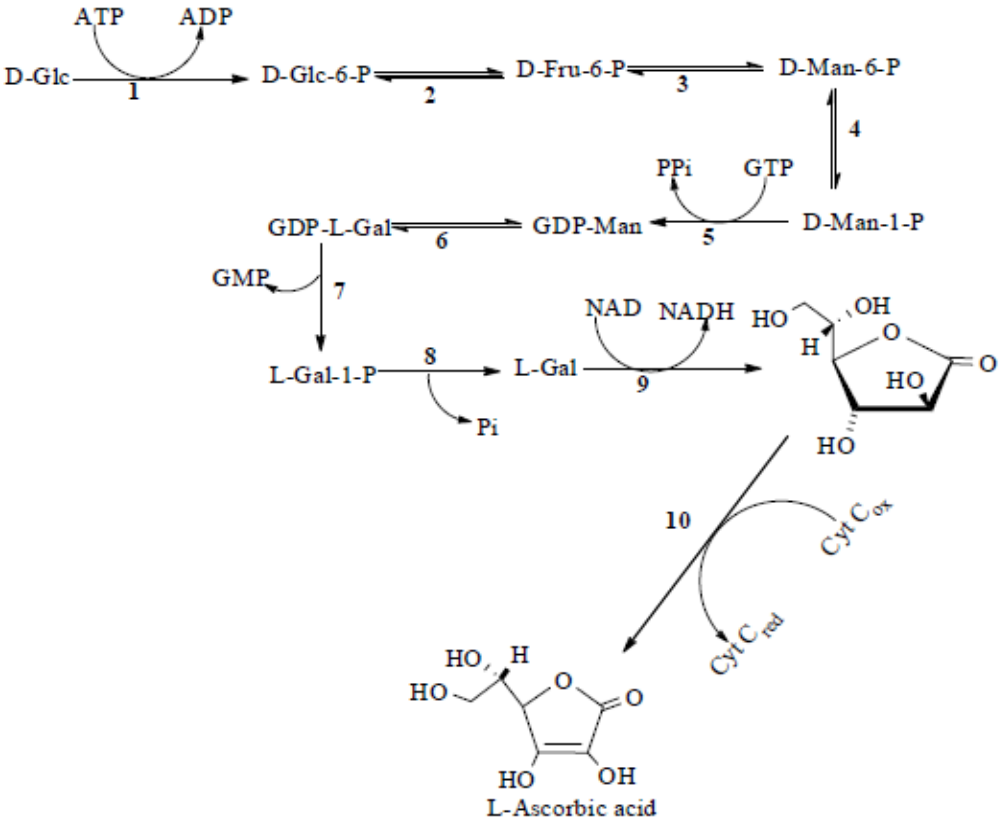


Figure3-1 Biosynthetic Pathway of L-Ascorbic acid in plants

Catalytic step	Enzyme	Substrate
1	Hexokinase	D-Glucose
2	Phosphoglucose isomerase	D-Glucose-6-phosphate
3	Phophomannose isomerase	D-Fructose-6-phosphate
4	Phophomannose mutase	D-Mannose-6-phosphate
5	GDP-Mannose pyrophoshorylase	D-Mannose-1-phosphate
6	GDP-Mannose-3,5-epimerase	GDP-D-Mannose
7	GDP-L-Galactose	GDP-L-Galactose
8	L-Galactose-1-phosphate phosphatase	L-Galactose-1-phosphate
9	L-Galactose dehydrogenase	L-Galactose
10	L-Galactono-1,4-lactone dehydrogenase	L-Galactono-1,4-lactone

Table2-1 Biosynthetic Pathway of L-Ascorbic acid in plants

1-1-9-2 Chemical synthesis:

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 *Acetobactersuboxydans* to sorbose. Only one of the six hydroxy 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 carboxyli cacid 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%.

A more biotechnological process, first developed in China in the 1960s, but further developed in the 1990s, bypasses 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, bypassing both the need for a second fermentation and the need to reduce glucose to sorbitol.[7]

1-1-10 Methods of determination:

Many analytical techniques including sensors and biosensors have been suggested for a detection of ascorbic acid in very varied types of samples. Hyphenated instruments consisting of flow injection analysis , high performance liquid chromatography or capillary electrophoresis instruments and a detector are mostly utilized for the determination of AA. However, some of these methods are time-consuming, some are costly, some need special training operators, or they suffer from the insufficient sensitivity or selectivity. Limits of detection ranged from μm to nm and lower .

Electrochemical detection is an attractive alternative method for detection of electroactive species, because of its inherent advantages of simplicity, ease of miniaturization, high sensitivity and relatively low cost. Electrochemical detection typically worked in amperometric or coulometric mode can be coupled with liquid chromatography to provide high sensitivity to electroactive species.

The more sensitive technique is further applied on analysis of real samples (pharmaceutical preparation, oranges and apples fruits, and human blood serum). [2]

1-2 The Baobab:

1-2-1 Definition:

The Baobab(Adansonia digitata L). is a multipurpose tree species widely used for food and medicine. The baobab fruit pulp is probably the most important food stuff. It can be dissolved in water or milk. The liquid is then used as a drink, a sauce, a fermenting agent in local brewing, or as a substitute for cream of tartar in baking. The fruit pulp has a very high vitamin C content and is a rich source of calcium. The acidic pulp is rich in pectin, contains a high amount of carbohydrate, is low in protein, and extremely low in fat. Nevertheless, the fruit pulp can be considered as a rich source of amino acids and linoleic acid. It contains a very low amount of iron.

Baobab seeds can be eaten fresh, or may be dried and ground into a flour which can either be added to soups and stews as a thickener, or roasted and ground into a paste, or boiled for a long time, fermented and then dried for use. The seeds can be classified as both protein- and oil-rich. They contain appreciable quantities of crude protein, digestible carbohydrates and oil, whereas they have high levels of lysine, thiamine, Ca, Mg and Fe.

The leaves of the baobab tree are a staple for many populations in Africa. Young leaves are widely used, cooked like spinach, and frequently dried, often powdered and used for sauces over porridges, thick gruels of grains, or boiled rice. Baobab leaves are superior to fruit pulp in nutritional quality, and contain interesting levels of vitamin A.

They appear to be a good source of protein, and contain particularly significant amounts of the amino acid tryptophan. Baobab leaves are a significant source of Fe, Ca, K, Mg, Mn, P and Zn.

Baobab bark is mainly used for its medicinal properties and for its fibres. The alkaloid ‘adansonin’ in the bark is thought to be the active principle for treatment of malaria and other fevers, as a substitute for quinine. Several plant parts have interesting anti-oxidant, anti-viral and anti-inflammatory properties, and baobab has been used extensively since ancient times in traditional medicine.
However, for baobab, the nutritional and medicinal data are widely scattered and research is fragmentary.

1-2-2 Phytochemistry:

In general and regardless the variation in reported data, one can conclude that baobab pulp is rich in vitamin C, the leaves are rich in good quality proteins – most essential amino acids are present in the leaves – and minerals, and the seeds in fat. Moreover, pulp and leaves exhibit antioxidant activity . A variety of chemicals have been isolated and characterised from A. digitata. They belong to the classes of terpenoids, flavonoids, steroids, vitamins, amino acids, carbohydrates and lipids.

1-2-3 Baobab fruit:

The baobab fruit is composed of an outer shell (epicarp) (45%), fruit pulp (15%) and seeds (40%) . The woody epicarp or pod contains the internal fruit pulp (endocarp) which is split in small floury, dehydrated and powdery slides that enclose multiple seeds and filaments, the red fibres, that subdivide the pulp in segments (Figure 4-1).

Figure 4-1 Baobab fruit.

1-2-4 Vitamins in baobab fruit:

Baobab fruit pulp has among the highest vitamin C or ascorbic acid content found in any fruit . Umoh reports 373 mg/100 g wet weight, which is more than six times the level of vitamin C in citrus fruits (30-50 mg/100 g wet weight) and the highest known in natural fruits.

The exact vitamin C content depends on the individual tree. With baobab fruit powder, a drink with a vitamin C content equal to that of orange juice is easily obtained. However, to retain vitamin C in soft drinks it is important not to boil the pulp but rather to add the powder to previously boiled water.

1-2-5 The Seeds:

The vernacular name for Adansonia digitata L, baobab, means ‘fruit with many seeds’ (Figure 5-1).

Figure 5-1 Baobab seeds

The seeds are eaten raw or are roasted and have a pleasant nutty flavour . baobab seed flour is an important source of energy and protein. The nutritious seeds have high values for proteins, fats (oils), fibre and most minerals . The baobab seed contains appreciable quantities of oil (29.7%, expressed on a dry weight basis). Besides, baobab seeds have high levels of lysine, thiamine, calcium, and iron . Baobab seed can be classified as both protein- and oil-rich. It is also a very rich source of energy and has a relatively low fat value .

Fermentation of baobab seeds decreases protein and carbohydrate but increases fat levels. Fermentation has varied effects on the mineral concentrations of the baobab seeds.

- *Amino Acid Profile:*

The seed contains a relatively high amount of essential amino acids . (Table 3-1) shows the amino acid composition of baobab seeds.

Amino Acid		A	B	C
Crude protein (Total protein)		196.00	-	-
Aspartic acid	ASP	21.10	-	10.30
Glutamic acid	GLU	48.90	2.10	23.70
Serine	SER	11.40	-	6.10
Glycine	GLY	10.40	-	8.60
Histidine	HIS	5.05	1.43	2.20
Arginine	ARG	2.21	8.62	8.00
Threonine	THR	6.98	1.64	3.80
Alanine	ALA	10.60	-	7.10
Proline	PRO	9.55	0.62	6.90
Tyrosine	TYR	5.59	3.62	1.50
Valine	VAL	11.60	0.76	5.90
Methionine	MET	2.29	5.94	1.00
Isoleucine	ILE	8.27	7.10	3.60
Leucine	LEU	14.00	7.48	7.00
Phenylalanine	PHE	10.30	5.18	4.00
Lysine	LYS	11.20	17.36	5.00
Cysteine	CYS	3.60	12.63	1.50
Tryptophan	TRP	2.81	2.64	-

Table3-1 Amino Acid Composition of Baobab Seeds

- *Fatty Acid Profile:*

The oil of baobab seeds contained high proportions of linoleic and oleic acid as well as palmitic and □-linolenic acid. ,baobab seed oil is an excellent source of mono-and polyunsaturated fatty acids. The principal fatty acids in baobab oil are linoleic and oleic acid, 39.42% and 26.07% respectively. Of the total fatty acids 73.11% is unsaturated while 26.89% is saturated . Polyunsaturated fatty acids play an important role in modulating human metabolism, therefore, the high linoleic acid content is of nutritive significance. The ability of some unsaturated vegetable oils to

reduce serum cholesterol level may focus attention on the seed oil of baobab . This high content of mono- and polyunsaturated fatty acids suggests that baobab seed oil would be useful as a food oil . The saponification value is high, suggesting that baobab oil may be suitable for soap making.

• *Mineral Composition:*

The most important minerals in baobab seeds are calcium and magnesium reported phosphorous, calcium and potassium as the major mineral elements present in the seeds. At the same time, the seeds are a poor source of iron, zinc and copper . The huge differences found may be due to the use of different methods, but may also have other causes.

• *Anti-nutritional Factors:*

The acceptability and optimal utilization of baobab seed as a protein source is limited by the presence of anti-nutritional factors such as trypsin inhibitors, protease inhibitors, tannins, phytic acid, oxalate, alkaloids, phytate and amylase inhibitors .The investigation about anti-nutritional factors in baobab seeds found that they contain a Trypsin Inhibitor Activity of 5.7 TIU/mg sample, 73 mg/100 g of phytic acid and 23% catechin equivalent of tannin. While processing techniques may ‘rob’ a food item of some nutrients, processing systems may also enhance food nutritional quality by reducing or destroying the anti-nutrients present. Some of the commonly used processing techniques include soaking in water, boiling in alkaline or acidic solutions. A decrease in tannin contents of baobab seeds was observed after a cold-water, hot-water and hot-alkali treatment. This can be explained by several possibilities: tannins are water soluble, tannin molecules are degraded when heated,

heating may cause the formation of water-soluble complexes with other macromolecules of the seeds or reaction between tannic acid and the base. Dehulling and cold-water treatment of seeds greatly reduced the activity of amylase inhibitors . Sun drying, roasting and fermentation are traditional processing techniques. Fermentation of the seeds for 6 days offered much advantages over roasting as shown by crude protein, moisture and minerals contents. A 6-day fermentation appeared to be the most promising method for producing nutritious food from baobab seed.

1-2-6 Uses in Traditional Medicine:

Baobab leaves, bark, pulp and seeds are used as food and for multiple medicinal purposes in many parts of Africa . Ethnomedicine has been an intensive area of research, with several authors discussing the main ethnomedicinal uses of baobab products.

- ***Fruit:***

Baobab is used in folk medicine as an antipyretic or febrifuge to overcome fevers. Both leaves and fruit pulp are used for this purpose. Fruit pulp and powdered seeds are used in cases of dysentery and to promote perspiration . Baobab fruit pulp has traditionally been used as an immunostimulant , anti-inflammatory, analgesic , pesticide , antipyretic, febrifuge, and astringent in the treatment of diarrhoea and dysentery . The fruit pulp has been evaluated as a substitute for improved western drugs . The aqueous extract of baobab fruit pulp exhibited significant hepatoprotective activity and, as a consequence, consumption of the pulp may play an important part in human resistance to liver damage in areas where baobab is consumed . Medicinally, baobab fruit pulp is used as a febrifuge and as an anti-dysenteric, and in the treatment of smallpox and

measles as an eye instillation . In Indian medicine, baobab pulp is used internally with buttermilk in cases of diarrhoea and dysentery. Externally, use is made of young baobab leaves, crushed into a poultice, for painful swellings.

- **Seeds:**

Seeds are used in cases of diarrhoea, and hiccough. Oil extracted from seeds is used for inflamed gums and to ease diseased teeth . Since seed oil is used to also treat skin complaints, it can be considered to have cosmetic applications as well.

- **Leaves:**

Powdered leaves are used as an anti-asthmatic and known to have antihistamine and anti-tension properties. The leaves are also used to treat a wide variety of conditions including fatigue, as a tonic and for insect bites, Guinea worm and internal pains, and dysentery ; and diseases of the urinary tract, opthalmia and otitis . In Indian medicine, powdered leaves are similarly used to check excessive perspiration . Baobab leaves are used medicinally as a diaphoretic, an expectorant, and as a prophylactic against fever, to check excessive perspiration, and as an astringent . The leaves also have hyposensitive and antihistamine properties. Leaves are used to treat kidney and bladder diseases, asthma, general fatigue, diarrhoea, inflammations, insect bites and guinea worm .

- **Bark:**

The widest use in tradition medicine comes from the baobab bark as a substitute for quinine in case of fever or as a prophylactic. A decoction of the bark deteriorates rapidly due to the mucilaginous substances present. This process can be prevented by adding alcohol or a small quantity of sulphuric acid to the decoction . Baobab bark is used in Europe as a

febrifuge (antipyretic). In the Gold Coast (Ghana), the bark is used instead of quinine for curing fever . In Indian medicine, baobab bark is used internally as a refrigerant, antipyretic and antiperiodic. It is used as a decoction, 30 g/l of water, boiled down to two thirds . The activity of baobab bark as a febrifuge, however, has not been detected in experimental malaria treatments, although it is both diaphoretic and antiperiodic. The bark, however, is certainly used for the treatment of fever in Nigeria . Moreover, the bark contains a white, semi-fluid gum that can be obtained obtainable from bark wounds and is used for cleansing sores . There are no alkaloids present in the bark, and accounts from Nigeria are inconclusive . However, the bark contains the alkaloid ‘adansonin’, which has a strophanthus-like action. In East Africa, the bark is used as an antidote to strophanthus poisoning. In Congo Brazzaville, a bark decoction is used to bathe rickety children and in Tanzania as a mouthwash for toothache . Furthermore, a new flavanonol glycoside was reported in the root bark .

Baobab bark, fruit pulp and seeds appear to contain an antidote to poisoning by a number of Strophanthus species. The juice of these species has been widely used as an arrow poison especially in East Africa. In Malawi, a baobab extract is poured onto the wound of an animal killed in this way to neutralize the poison before the meat is eaten. An infusion of roots is used in Zimbabwe to bathe babies to promote smooth skin.[8]

1-3 Guava leaves:

1-3-1 Definition & Botany:

P. guajava is a large evergreen shrub or small tree that grows up to 15 m in height. It is native to and widely distributed in Mexico and Central America and is common throughout all warm areas of tropical America and the West Indies. Today, the plant is cultivated from Asia to the west coast of Africa, with varieties originally introduced over the past 300 years from the United States. The guava berry, also known as guava, is an important tropical fruit that is primarily consumed fresh. The berry contains several small seeds and consists of a fleshy pericarp and seed cavity with pulp.[9]

Guava tree (psidium guajava) is basically from the Meso Americam area. It can also find in tropical and subtropical areas. Guava tree is member of myrtaceae family, all the parts of this tree widely use in curing many health problems. A lot of work on Pharmalogical researches has been done to demonstrate the use of extract from guava leaves which proved that guava leaves extracts are such a useful medicine, widely using by doctors and pharmacist. WHO (world health organization) also says that plants would be the best source for obtaining different types of medicines and drugs. These natural products are widely used by human with its effective results. Extraction from guava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C. Guava leaf extracts introduces many biological activities i.e. Antibacterial, antioxidant, and analgesic, anti inflammatory, antimicrobial, phytotoxic, hepatoprotection, and anti hyperglycaemic and anti cancer activities .[10]

Figure 6-1 Guava leaves

1-3-2 History:

The young leaves of the plant have been used as a tonic to treat digestive conditions such as dysentery and diarrhea in the indigenous medical systems of Brazil and Mexico. Mexican medicinal data document the treatment of acute diarrhea, flatulence, and gastric pain by using a guava leaf water decoction for oral administration 3 times daily. A decoction of young leaves and shoots has been prescribed as a febrifuge and a spasmolytic. In Bolivia and Egypt, guava leaves have been used to treat cough and pulmonary diseases; they have also been used to treat cough in India and as an anti-inflammatory and hemostatic agent in China.

Guava bark has been used medically as an astringent and to treat diarrhea in children, while the flowers have been used to treat bronchitis and eye sores and to cool the body. The fruit has been used as a tonic and laxative and for treatment of bleeding gums. The plant has been used in Africa and Asia to prevent and treat scurvy and to treat hypertension in western

Africa. Ethnomedicinal reports document use of the plant in treating malaria. Scientific investigations of the medicinal properties of guava leaf products date back to the 1940s.

Pakistan, India, Brazil, and Mexico are the major commercial producers of guava fruit. Hawaii is the largest producer in the United States. Processed guava products include beverages, cheese, ice cream, jams, jellies, juice, syrup, toffee, wine, and dehydrated and canned products.

1-3-3 Chemistry:

Phytochemical analyses of guava leaf reveal alkaloids, anthocyanins, carotenoids, essential oils, fatty acids, flavonoids (especially quercetin), lectins, phenols, saponins, tannins, triterpenes, and vitamin C (80 mg per 100 g of guava).

The essential oil contains alpha pinene, caryophyllene, cineol, D-limonene, eugenol, and myrcene. The major constituents of the volatile acids include (E)-cinnamic acid and (Z)-3-hexenoic acid. The guava fruit has a high water content with lesser amounts of carbohydrates, proteins, and fats. The fruit also contains iron, vitamins A and C, thiamine, riboflavin, niacin, and manganese. The characteristic fruit odor is attributed to carbonyl compounds. Unripe fruits are high in tannins. The major constituent of the fruit skin is ascorbic acid, largely destroyed by canning and processing.

1-3-4 Uses and Pharmacology:-

1-3-4-1 Diarrhea:-

Animal data:

Guava leaf extracts decreased spasms associated with induced diarrhea in rodents. Reduced defecation, severity of diarrhea, and intestinal fluid secretion reductions have also been demonstrated. Activity is generally associated with the ability of quercetin and its derivatives to affect smooth muscle fibers via calcium antagonism, inhibit intestinal movement, and reduce capillary permeability in the abdominal cavity.

Clinical data:

In vitro studies suggest leaf and bark extracts are bactericidal against a range of pathogens causative of diarrhea ; however, data from controlled clinical trials are limited, and few of the trials have been published in peer-reviewed journals. Trials have evaluated guava leaf extract in infantile viral enteritis, infectious gastroenteritis, and acute diarrhea with improvement in outcome measures including number of daily stools, time to cessation, stool composition, and abdominal pain and spasms for P. guava -treated patients.

1-3-4-2 Diabetes:

Animal data:

Extracts of guava bark, leaves, and fruit containing tannins, flavonoids, triterpenes, and quercetin have been evaluated in induced-diabetic rats. In some, but not all, experiments, no effect was observed in either normal rats or normal glucose-loaded rats. A polyphenolic effect may be responsible for the observed inhibition of low-density lipoprotein glycation. Inhibition of protein tyrosine phosphatase 1B as well as increased glucose uptake in rat hepatocytes has also been described.

Clinical data:

Limited evidence from a few clinical trials suggests guava fruit and leaf tea extracts may be of benefit in type 2 diabetes. Reductions in postprandial serum glucose levels of a lesser size than chlorpropamide and metformin have been demonstrated, and inhibition of alpha-glucosidase enzymes has been suggested to play a role in the mechanism of action.

1-3-4-3 Dysmenorrhea:-

Animal data:

An in vitro study using uterine tissue from rats demonstrated a spasmolytic effect of guava leaf extract. Activity is postulated to be due to an estrogenic effect of the flavonoids or to anti-inflammatory effects.

Clinical data:

Decreases in dysmenorrheal pain intensity were reported after 4 months of daily guava leaf extract standardized at 6 mg of flavonoid content per day.

1-3-4-4 Cancer:-

Leaf extracts, leaf oil, guava seed, and whole plant extracts have been evaluated for potential chemotherapeutic applications. Activity against various human cancer cell lines have been demonstrated including prostate, colon, and epidermal cancers, as well as leukemia and melanoma. Data from in vivo animal or clinical studies are lacking.

1-3-5 Toxicology:

Acute toxicity tests in rats and mice have found the median lethal dose of guava leaf extracts to be more than 5 g/kg. In vitro genotoxicity and

mutagenicity tests on P. guajava in human peripheral blood lymphocytes found no disturbances in cell division or hemolysis.

Despite experiments suggesting hepatoprotective effects, intraperitoneal administration of ethanolic leaf extracts in rats has resulted in increases in serum liver enzymes, an effect that may be dose dependent. No histological evidence of hepatotoxicity has been observed.[9]

1-4 High performance liquid chromatography:-

1-4-1 Definition:

High performance liquid chromatography (HPLC) is a form of liquid chromatography to separate compounds that are dissolved in solution. HPLC instruments consist of a reservoir of mobile phase, a pump, an injector, a separation column, and a detector. Compounds are separated by injecting a plug of the sample mixture onto the column. The different components in the mixture pass through the column at different rates due to differences in their partitioning behavior between the mobile liquid phase and the stationary phase.[11]

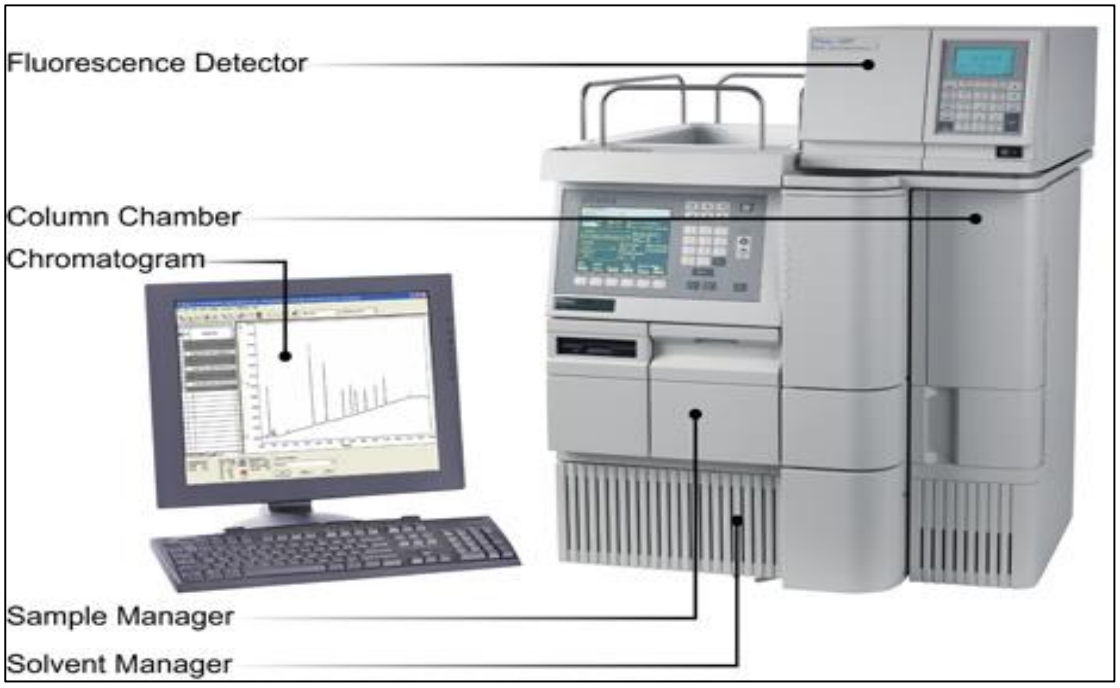


Figure 7-1 HPLC instrument

1-4-2 The Liquid Chromatography:-

In chromatography a liquid is pumped through a bed of particles. The liquid is called the mobile phase and the particles the stationary phase. A mixture of the molecules that shall be separated is introduced into the mobile phase.

The heart of a HPLC system is the column. The column contains the particles that contains the stationary phase. The mobile phase is pumped through the column by a pump.

The mixture to be separated is injected into the flowing mobile phase by an injector.

When the mobile phase passes through the column that contains the stationary phase, the molecules that adsorbed most to the stationary phase migrates slowest through the column. When the mobile phase has passed through the column it enters into the detector that detects the different molecules as they have pass through it. A signal goes from the detector to a printer that presents the separation graphically.[12]

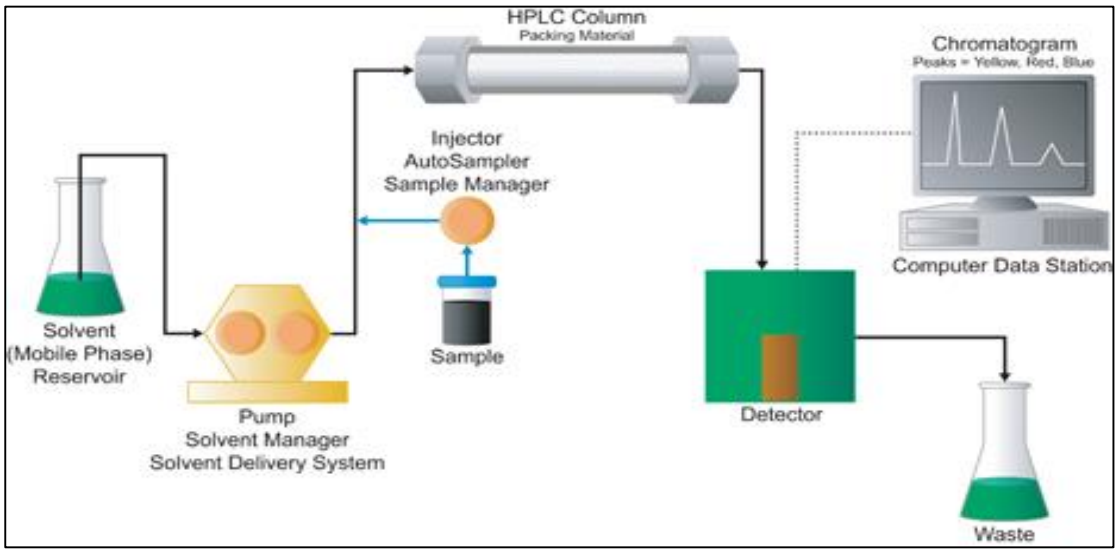


Figure 8-1 The sample path

Chapter two

Experiment

2-1 Chemicals:-

- Potassium iodide (Assay 98.5% ,M.wt=166 ,ALPHA CHEMIKA)
- Iodine (M.wt=253.81 ,Techno pharmchem)
- Starch Maize (M.wt=$(162.14)_n$,ALPHA CHEMIKA)
- Sodium thiosulphate (M.wt=248.17 ,ALPHA CHEMIKA)
- Ascorbic acid (stander)
- Methanol (Assay 99.8% ,M.wt=23.04 ,Chem-lab NV)
- Ortho phosphoric acid (Density =1.75g/cm ^{3}, M.wt= 98, Asaay 88% , Central drug house)
- Oxalic acid (M.wt=126.7 ,ALPHA CHEMICA)
- De ionized water
- Distilled water

2-2 Apparatus:-

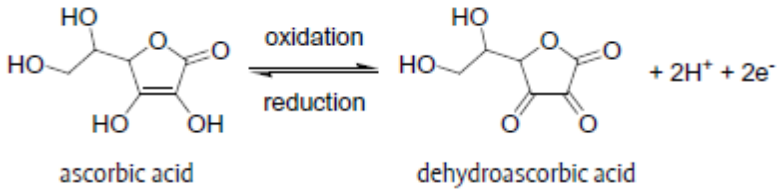
- Burette (25ml)
- Pipette (5ml and 10ml)
- Volumetric flask (25ml ,50 ml,100 ml,250 ml,1L)
- Measuring cylinder (10ml,50ml)
- Conical flask (100ml, 250ml)
- Beaker (100ml,250ml and 500ml)
- Mortar and pestle
- Filter paper

- Cheesecloth
- Funnel
- Glass rod
- Dropper

2-3 Instruments:-

- High performance liquid chromatography (SHIMADUZ, DGU-20A3 prominence degasser, LC-20AD prominence liquid chromatography, SPD-20A prominence uv/vis detector)
- Heater
- Balance

2-4 Theory:-



ascorbic acid + I_2 → 2 I^- + dehydroascorbic acid

2-5 Method:-

2-5-1 Determination of ascorbic acid by titration:-

- *Iodine solution(0.005M) preparation:-*

2 g of potassium iodide was weighted into a 100 ml beaker. 1.3 g of iodine was weighted and added into the same beaker. A few ml of distilled water was added and swirled for a few minutes until iodine was dissolved. Iodine solution was transferred to a 1 L volumetric flask, all traces of solution was rinsed into the volumetric flask by used distilled water. Make the solution up to the 1 L mark with distilled water.

- *Starch indicator solution(0.5%) preparation:-*

0.25 g of soluble starch was weighted and added to 50 ml of near boiling water in a 100 ml conical flask. Stirred to dissolve and cooled before using.

- *Sodium thiosulphate solution(0.005M) preparation:-*

0.125 g of sodium thiosulphate was weighted in beaker (100ml),dissolved by distilled water then transferred to volumetric flask (100ml) and volume was completed to the mark by distilled water, then shacked well and transferred to beaker.

- *Sample preparation:-*

10 g of sample (10.007g of Guava leaves – 10.009 g of new Baobab – 10.003 g of old Baobab) grinded in a mortar and pestle. 100 ml of distilled water was added to the sample ,the ground sample pulp strained through cheesecloth, rinsing the pulp with a few 10 ml of water . Finally, the liquid extract was decanted off into(250 ml) volumetric flask. The

volume was completed to the mark by distilled water, then shacked well and transferred to beaker.

- *Titration:-*

20 ml of sample solution was transferred by pipette to a conical flask , 3ml of starch indicator added to it, then the content of conical flask titrated against Iodine solution (0.005 M) from burette , until end point and the concentration was calculated

2-5-2 Determination of ascorbic acid by HPLC:-

- *Oxalic acid(9.5%) preparation:-*

0.4261 g of oxalic acid was weighted in a beaker (100 ml) , then dissolved by distilled water , and the solution was transferred into volumetric flask (50ml), and the volume completed to the mark , then the solution transferred to a beaker(100ml).

- *Mobile phase(0.02M) preparation:-*

13 ml of 88% ortho phosphoric acid was diluted to 1L with de ionized water. Then 100 ml of this stock solution was diluted to 1L with de ionized water.

- *Standard ascorbic acid(0.005M) preparation:-*

0.0517 g of ascorbic acid (std.) was weighted in a beaker (100 ml) and dissolved by ortho phosphoric acid(6%) then transferred to a volumetric flask (25 ml) and the volume completed to the mark by ortho phosphoric acid(6%) ,then 1ml of the solution was transferred by pipette two

volumetric fiask (25ml) and volume completed to the mark by ortho phosphoric acid(6%), then the solution transferred to a beaker(100ml).

- *Sample preparation:-*

10 g of sample(10.012g of Guava leaves, 10.095g of new Baobab, 10.085g of old Baobab) were weighted in beaker and diluted with 10 ml of 9.5% oxalic acid solution, 10 ml of methanol and 60 ml de ionized water, homogenized and filtered by cheesecloth and then by filter paper. Then extracted solution transferred to beaker (100ml) . 1 ml of Baobab solutions (new and old) transferred by pipette to volumetric flask (25 ml) and volume completed to the mark by water, then solution was transferred to beaker.

- *Determination by HPLC:-*

The orthophosphoric acid 0.02M (mobile phase) was injected at a flow rate of 0.7 ml/min and UV detection at 254 nm .Then standard solution of ascorbic acid was injected and waited until the peak appear . after this the first sample was injected and then wait for the peak. The same step was repeated with other samples. Chromatographic peak in the samples was identified by comparing the retention time with the standard.

Chapter three

3-1 Result:-

- *Titration*

Baobab "new"

Initial volume	Final volume	Used volume
0.00	1.90	1.90

Baobab "old"

Initial volume	Final volume	Used volume
0.00	1.20	1.20

Guava leaves

Initial volume	Final volume	Used volume
0.00	0.30	0.30

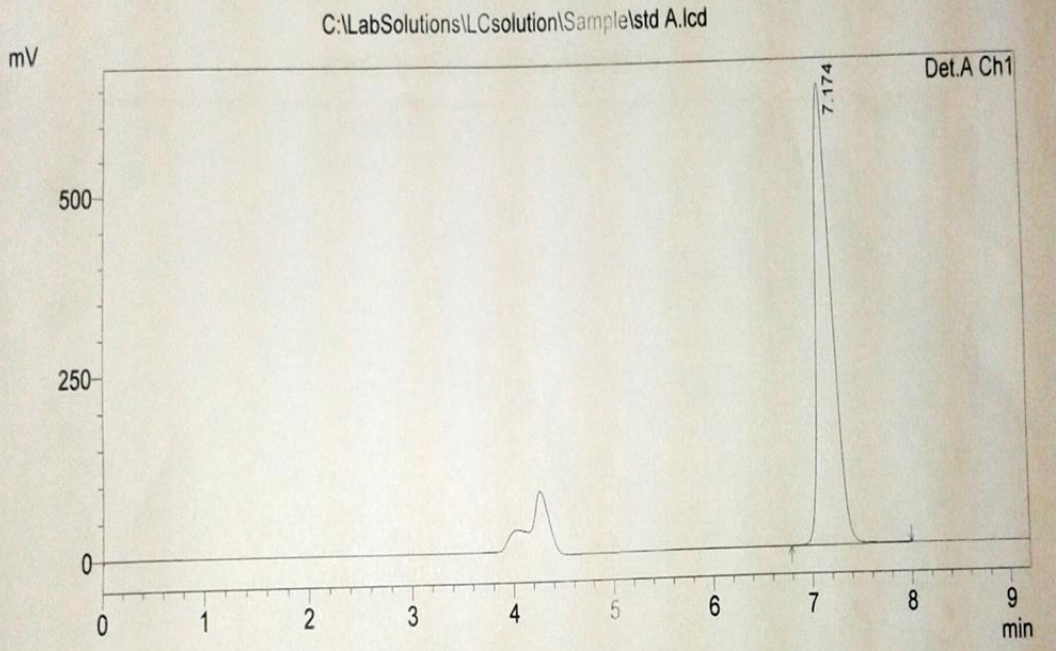
- *HPLC*

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\LCsolution\Sample\std A.lcd

Acquired by : Admin
Sample Name : ascorbic acid
Sample ID : std
Tray# : 1
Vail # : -1
Injection Volume : 5 uL

<Chromatogram>



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

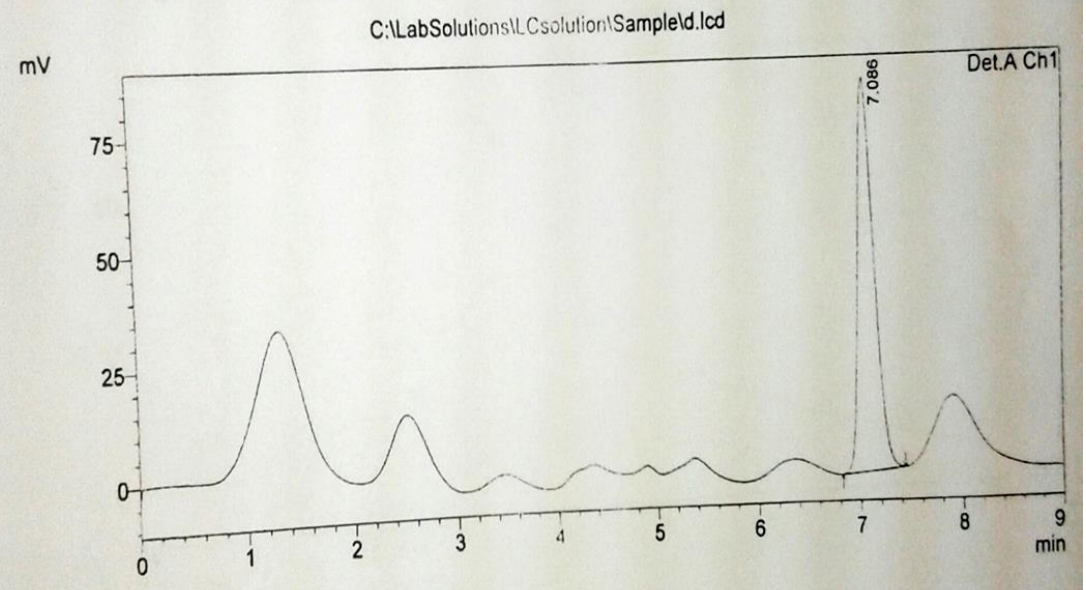
Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.174	7080576	648145	100.000	100.000
Total		7080576	648145	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\LCsolution\Sample\d.lcd

Acquired by	: Admin
Sample Name	: ascorbic acid
Sample ID	: sample - Baobab " new "
Tray#	: 1
Vail #	: -1
Injection Volume	: 5 uL

<Chromatogram>



1 Det.A Ch1/254nm

PeakTable

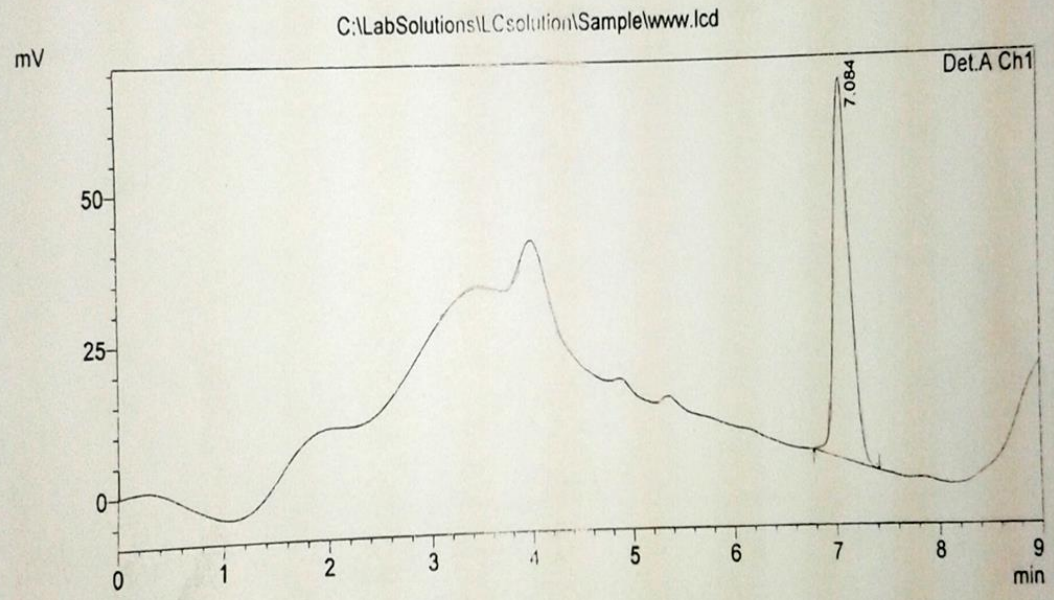
Detector A Ch1 254nm					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.086	964741	88971	100.000	100.000
Total		964741	88971	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\LCsolution\Sample\www.lcd

Acquired by	: Admin
Sample Name	: ascorbic acid
Sample ID	: sample - Baobab " old "
Tray#	: 1
Vail #	: -1
Injection Volume	: 5 uL

<Chromatogram>



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

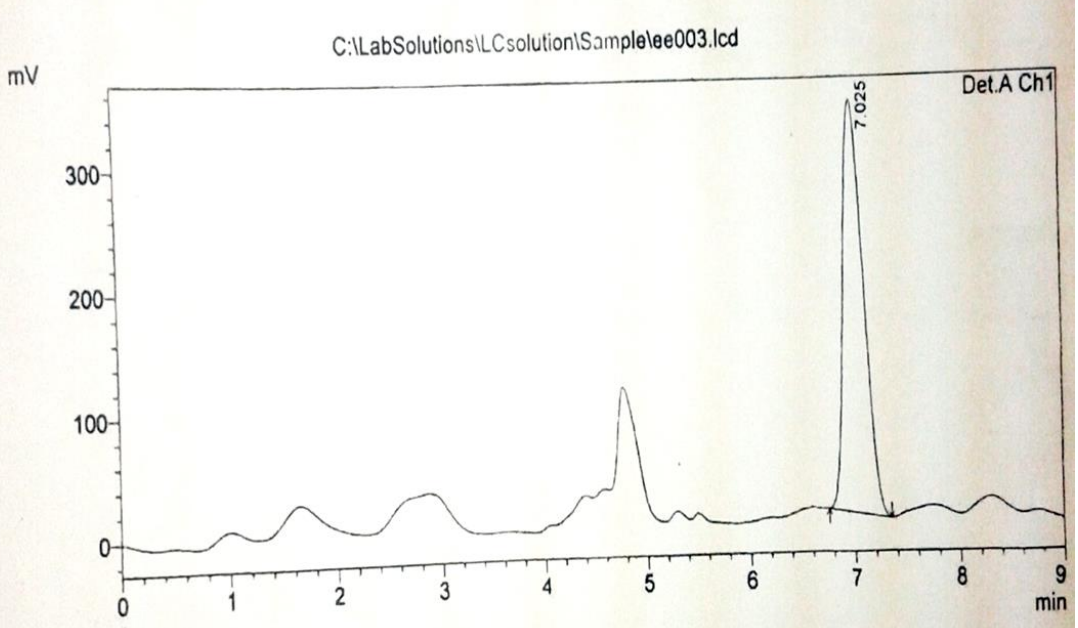
Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.084	693845	64370	100.000	100.000
Total		693845	64370	100.000	100.000

==== Shimadzu LCsolution Analysis Report ====

C:\LabSolutions\LCsolution\Sample\ee003.lcd

Acquired by	: Admin
Sample Name	: ascorbic acid
Sample ID	: sample - Guava
Tray#	: 1
Vail #	: -1
Injection Volume	: 5 uL

<Chromatogram>



1 Det.A Ch1/254nm

PeakTable

Detector A Ch1 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.025	4559724	343100	100.000	100.000
Total		4559724	343100	100.000	100.000

3-2 Calculation:-

- Titration

Baobab "new"

$\left(\frac{M\times V}{n}\right) A.A = \left(\frac{M\times V}{n}\right) I_2$

$\frac{M \times 20}{1} = \frac{0.0046 \times 1.9}{1}$

M = 0.000437 M

$^{g}/_{l} = M \times M.wt$

$^{g}/_{l} = 0.000437 \times 176.1$

$^{g}/_{l} = 0.07854\ ^{g}/_{l}$

ppm $= {}^{g}/_{l} \times 1000$

ppm = 78.54 ppm

wt= d × v

wt= 1.65 × 1000 = 1650g

78.54mg/1650g

X=(78.54×100)÷1650 =4.76mg

mg/g = 4.76mg/100g

Baobab "old"

$\left(\frac{M\times V}{n}\right) A.A = \left(\frac{M\times V}{n}\right) I_2$

$$\frac{M \times 20}{1} = \frac{0.0046 \times 1.2}{1}$$

M = 0.000276 M

$^{g}/_{l} = M \times M.wt$

$^{g}/_{l} = 0.000276 \times 176.1$

$^{g}/_{l} = 0.048603 \ ^{g}/_{l}$

ppm $= {}^{g}/_{l} \times 1000$

ppm = 48.6036 ppm

48.6036mg/1650g

X=(48.6036×100)÷1650 =2.95mg

mg/g = 2.95mg/100g

Guava leaves

$\left(\frac{M\times V}{n}\right) a.a = \left(\frac{M\times V}{n}\right) I_2$

$$\frac{M \times 20}{1} = \frac{0.0046 \times 0.3}{1}$$

M = 0.000069 M

$^{g}/_{l} = M \times M.wt$

$^{g}/_{l} = 0.000069 \times 176.1$

$^{g}/_{l} = 0.01215\ \ ^{g}/_{l}$

ppm $= {}^{g}/_{l} \times 1000$

ppm = 12.1509ppm

12.1509mg/1650g

X=(12.1509×100)÷1650 =0.736mg

mg/g = 0.736mg/100g

- *HPLC*

Std:

$$\frac{wt}{M.wt} = \frac{M \times V}{1000}$$

$$\frac{0.0517}{176.1} = \frac{M \times 25}{1000}$$

M =0.01174 M

(M×V)a =(M×V)b

M= $\frac{0.01174 \times 1}{25}$

M = 0.0004697

Baobab "new":

$$\frac{P.A.(st.)}{P.A(samp.)} = \frac{C(st.)}{C(samp.)}$$

$$\frac{(7080576)}{(964741)} = \frac{0.0004697}{C(samp.)}$$

C =0.00006399 M

(M×V)a=(M×V)b

(0.00006399×25)=(M×1)

M= 0.001599 M

$$^{g}/_{\mathrm{l}} = \mathrm{M} \times \ \mathrm{M.wt}$$

$$^{g}/_{\mathrm{l}} = 0.001599 \times \ 176.1$$

$$^{g}/_{\mathrm{l}} = 0.28158$$

Ppm = $^{g}/_{l} \times 1000$

Ppm =281.0583 ppm

281.0583mg/1650g

X=(281.0583×100)÷1650 =17.03mg

mg/g = 17.03mg/100g

Baobab "old":

$$\frac{(7080576)}{(693845)} = \frac{0.0004697}{C(samp.)}$$

C =0.000046027 M

(M×V)a=(M×V)b

(0.000046027×25)=(M×1)

M= 0.0011506 M

$^{g}/_{\mathrm{l}} = \mathrm{M} \times \mathrm{M.wt}$

$^{g}/_{\mathrm{l}} = 0.0011506 \times 176.1$

$^{g}/_{\mathrm{l}} = 0.20262$

Ppm = $^{g}/_{l} \times 1000$

Ppm =202.62 ppm

202.62mg/1650g

X=(202.62×100)÷1650 =12.28mg

mg/g = 12.28mg/100g

Guava leaves

$$\frac{(7080576)}{(4559724)} = \frac{0.0004697}{C(samp.)}$$

C =0.0003024 M

$^{g}/_{\mathrm{l}} = \mathrm{M} \times \mathrm{M.wt}$

$^{g}/_{\mathrm{l}} = 0.0003024 \times 176.1$

$^{g}/_{\mathrm{l}} = 0.053256$

Ppm = $^{g}/_{l} \times 1000$

Ppm =53.256 ppm

53.256mg/1650g

X=(53.256×100)÷1650 =3.23mg

mg/g = 3.23mg/100g

Discussion

The Ascorbic acid was determinate in Guava leaves and Baobab "new & old" ,it is shows different concentration for our samples in the two methods. The concentration in the Titration method for samples by mg/100g was (4.76mg/100g) for new baobab, (2.95mg/100g)for old baobab and (0.736mg/100g) for guava leaves, and also for High

Performance Liquid Chromatography by mg/100g was (17.04mg/100g) for new baobab, (12.28mg/100g)for old baobab, and (3.23mg/100g) for guava leaves. The results present the huge difference in concentration between methods. High Performance Liquid Chromatography is modern, accurate, selective instrument so it best than Titration (traditional method).That mean the results of the High Performance Liquid Chromatography is the accurate.

Then we compare the results with the standard concentration of guava leaves and the standard concentration of baobab(373mg/100g), we found that the concentration of our samples was low so that can be due to mistake in sample preparation, effect of high temperature, exposed to the air or impure in materials.

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