بسم الله الرحمن الرحيم

جامعة السودان
للعلوم والتكنولوجيا
كلية العلوم – قسم المختبرات العلمية – كيمياء

بحث تكميلي لنيل درجة البكلاريوس بعنوان :

EXTRACTION OF ASCORBIE ACID FROM PSIDIUM GUAJAVA LEAFES

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٢٠١٤ م

الإستهـــلال

قال تعـالى:ـ

(أَفَمَنْ أَسَّسَ بُنْيَانَهُ عَلَىٰ تَقْوَىٰ مِنَ اللَّهِ
وَرِضْوَانٍ خَيْرٌ أَمْ مَنْ أَسَّسَ بُنْيَانَهُ عَلَىٰ شَفَا
جُرُفٍ هَارٍ فَانْهَارَ بِهِ فِي نَارِ جَهَنَّمَ ۗ وَاللَّهُ لَا
يَهْدِي الْقَوْمَ الظَّالِمِينَ)

صدق الله

العظيم

سورة التوبة الاية ١٠٩

الإهـــداء

بدانا بأكثر من يد وقاسينا أكثر من هم وعانينا الكثير من الصعوبات وهانحن اليوم والحمد لله نطوي سهر الليالي وتعب الإيام وخلاصة مشوارنا بين دفتي هذا العمل المتواضع.

إلى منارة العلم والامام المصطفي إلى الأمي الذي علم التعلمين إلى

سيد الخلق إلى رسولنا الكريم سيدنا محمد صلى الله عليه وسلم .

إلى الينبوع الذي لا يمل العطاء إلى من حاكت سعادتي بخيوط منسوجة من قلبها إلى

والدتي العزيزة.

إلى من سعى وشقى لأنعم بالراحة والهناء الذي لم يبخل بشئ من أجل دفعي في طريق النجاح الذي علمني أن أرتقي سلم الحياة بحكمة وصبر إلى

والدي العزيز.

إلى من حبهم يجري في عروقي ويلهج بذكراهم فؤادي إلى

أخواتي وأخواني .

إلى من علمونا حروفا من ذهب وكلمات من درر وعبارات من أسمى وأجلى عبارات في العلم إلى من صاغوالنا علمهم حروفا ومن فكرهم منارة تنير لنا سيرة العلم والنجاح

إلى **أساتذتنا الكرام.**

الشكر و التقدير

الشكر أولاً و أخيراً لله عزّ و جل
أن وفقنا في أكمال هذا البحث و سهل لنا الكثير من الصعوبات
و الشكر موصول إلي كل الأساتذة

بجامعة السودان للعلوم
و التكنولوجيا

الذين لم يبخلو علينا بمعلومة في سبيل مساعدتنا
لما قدموه لنا من مساعدة و معلومات

Abstract

The antioxidant such as ascorbic acid (AA) in psidium guajava leafs has attracted a lot of interest from the public and herbal industries because of the antioxidant can inhibit the oxidation of body cells that can lead to health problem. The work done in this research investigates the most suitable operating parameter to extract the ascorbic acid from psidium gua java leafes using soxhlet extraction. The process has been done at boiling temperature of solvent and extraction Has been done in ٢٥٠ ml ethanol for ٣.٥hour. the extraction yield was Analyzed using IR to identify and Red ox Titration to determinate quantity Of a ascorbic acid The extracted amount of ascorbic acid found equal to be ٢٢.٥١%

Chapter (١)

بسم الله الرحمن الرحيم

Introduction

١-١ history of vitamin C:-

From the middle of the ١٨th century, it was noted that lemon juice could help prevent sailors from getting scurvy. At first, it was supposed that the acid properties were responsible for this benefit; however, it soon became clear that other dietary acids, such as vinegar, had no such Benfits. In ١٩٠٧, two Norwegian physicians reported an essential Disease preventing compound in foods that was distinct from the one That prevented beriberi.

These physicians were investigating dietary-deficiency diseases using the New animal model of guinea pigs , which are susceptible to scurvy. The newly discovered food-factor was eventually called vitamin C .From ١٩٢٨ to ١٩٣٢, the Hungarian research team led by Albert Szent-Györgyi, As well as that of the American researcher Charles Glen King, identified The ant scorbutic factor as a particular single chemical substance.

Mayo clinic, Szent-Györgyi had isolated the chemical hexuronic acid From animal adrenal glands.e suspected it to be the ant scorbutic factor but could not prove it Without a biological assay. This assay was finally conducted at the University of Pittsburgh in the laboratory of King, which had been Working on the problem for years, using guinea pigs. In late ١٩٣١, King's lab obtained adrenal hexuronic acid indirectly from Szent-Györgyi and, using their animal model, proved that it is vitamin C by early ١٩٣٢.This was the last of the compound from animal sources, But , later that year, Szent-Györgyi's group discover that paprika peppe, a common spice in the Hungarian diet, is a rich source of hexuronic acid. He sent some of the now-more-available chemical to Walter NormanHaworth , a British sugar chemist . In ١٩٣٣, working with the then-Assistant Director of Research (later Sir) Edmund Hirstand their research teams, Haworth deduced the correct structure and optical-isomeric nature of vitaminC.in ١٩٣٤ reported the first synthesis of the vitaminC In honor of the compound's antiscorbutic properties, Haworth and Szent-Györgyi now proposed the new name of "a-scorbic acid" for the compound. It was named L-ascorbic acid by Haworth and Szent-Györgyi when its structure was finally proven by synthesis.In ١٩٣٧, the Nobel Prize for chemistry was awarded to Norman Haworth for his work in determining the Structure of ascorbic acid (shared with Paul Karrer, who received his Award for work on vitamins) , and the prize for Physiology or Medicine That year went to Albert Szent-Györgyi for his studies of the

biological functions of L-ascorbic acid. The American physician Fred Kenner M.D. Promoted vitamin C as a cure for many diseases in the ١٩٥٠s by elevating The dosages greatly to as much as tens of grams vitamin C daily by Injection. From ١٩٦٧ on, Nobel Prizewinner LinusPauling recommended High doses of ascorbic acid , (he himself took ١٨ grams daily) as A prevention against cold and cancer. The results of Klenner have been Controversial as yet, since his investigations do not meet the modern Method ological standards.

١-٢ Definition of Ascorbic acid:-

Ascorbic acid is a 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 acid 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 a vitamin Activity, not then a specific substance), the suggestion was made to rename L -hexuronic acid. The new name for L-hexuronic acid 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 ascorbic acid include other primates, guinea pigs, Tallest fishes, bats, and some micronutrient (that is, in vitamin form).

There exists an Ascorbic acid, which does not occur in nature. It may be Synthesized artificially. It has identical antioxidant properties to L-Scorbic acid yet has far less vitamin C activity (although not quite zero).

This fact is taken as evidence that the antioxidant properties of ascorbic Acid 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-acerbate and not D-

Ascorbate).

١-٣-sources of vitamin C:-

١-٣-١-Plant sources:-

While plants are generally a good source of vitamin C, the amount in foodsOf plant origin depends on the precise variety of the plant, soil condition,Climate where It grew , length of time since it was picked, storage conditions, and method of preparation.

the data are subject to potential variation and difficulties for comparison.The amount is given in milligrams per ١٠٠ grams of fruit or vegetable and is a rounded average from multiple authoritative sources:

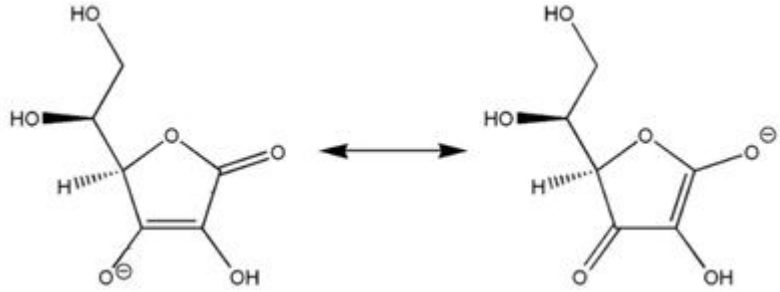
١-٣-٢-Animal sources:-

Goats, like almost all animals, make their own vitamin C. An adult goat, weighing approx. ٧٠ kg, will manufacture more than ١٣,٠٠٠ mg of vitamin C per day in normal health, and levels many fold higher when faced with stress.

The overwhelming majority of species of animals (but *not* humans or guinea pigs) and plants synthesize their own vitamin C .Therefore, some animal products can be used as sources of dietary vitamin C.Vitamin C is most present in the liver and least present in the muscle. Since muscle provides the majority of meat consumed in the western human diet, animal products are

not a reliable source of the vitamin. Vitamin C is present in human breast milk, but only in limited quantity in raw cow's milk. All excess vitamin C is disposed of through the urinary system.

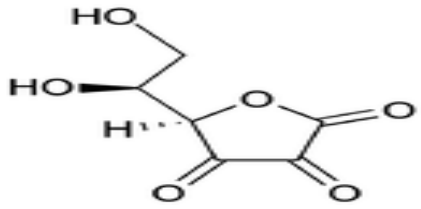
١-٤ Acidity:-



Canonical structures for the ascorbate anion.

Ascorbic acid is classed as a reductone. The ascorbate anion is stabilized by electron delocalization, as shown above in terms of resonance between two canonical forms. For r this reason, ascorbic acid is much more acidic than would be expected if the compound contained only isolated hydroxyl groups.

١-٥-Antioxidant mechanism:-



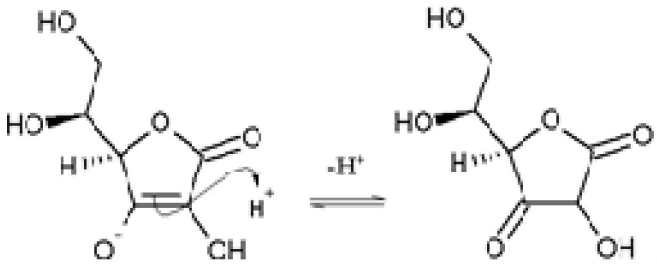
The ascorbate ion is the predominant species at typical biological PH values. It is a mild reducing agent and antioxidant. It is oxidized with Loss ofone electron to form a radical cation and then with loss of a second.

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 radicals initiate chain reactions. Ascorbate can terminate These Chain radical reactions by electron**.** Ascorbic acid is special Because it can transfer a single electron, owing to the resonance- Stabilized nature of its own radical ion called, semidehydroascorbate.

The net reaction is:

$RO\bullet + C_6H_7O_6^- \rightarrow ROH + C_6H_6O_6\bullet$ -

The oxidized forms of ascorbate are relatively unreactive and do Not 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 .



١-٦-Food chemistry:-

Ascorbic acid and its sodium, potassium, and calcium salts are Commonly used as antioxidant additives. These compounds are water-soluble and, thus , cannot 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 the world's supply of ascorbic acid is produced in China.

The relevant European food additive E numbers are:-

i. E٣٠٠ ascorbic acid (approved for use as a food additive in the EU- USA-and Australia and New Zealand)

ii. E٣٠١ sodium ascorbate (approved for use as a food additive in the EU USA and Australia and New Zealand)E٣٠٢ calcium ascorbate (approved for use as a food additive in the EU- USA- and Australia and New Zealand)

iii. E٣٠٣ potassium ascorbate.

iv. E٣٠٤ fatty acid esters of ascorbic acid:-

(i) ascorbyl palmitate (ii) ascorbyl stearate.

It creates volatile compounds when mixed with glucose and amino acids in ٩٠°C.

١-٧-Biosynthesis :-

Ascorbic acid is found in plants and animals where it is produced From glucose. 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 a genetic Mutation and are, therefore, unable to make ascorbic acid. Synthesis and Signaling properties are still under investigation.

١-٧-١ Animal ascorbic acid biosynthesis pathway

The biosynthesis of ascorbic acid starts with the formation of UDP-Glucuroni c acid. UDP-glucuronic acid is formed when UDP-glucose Undergoes two oxidations catalyzed by the enzyme UDP-glucose ٦-Dehydrogenase . UDP-glucose ٦-dehydrogenase uses the co-factor NAD+ as the electron acceptor. Pyrophosphorylase removes a UMP and glucuronokinase, with the cofactor ADP, removes the final phosphate leading to D -glucuronic acid. The aldehyde group of this is reduced to a primary alcohol using the Enzymee glucuronate reductase and the cofactor NADPH, yielding L-gulonic acid. This is followed by lactone formation with the hydrolase gluconolactonase between the carbonyl on C١ and hydroxyl group on the C٤. L- gulonolactone then reacts with oxygen, catalyzed by the enzyme L-gulonolactone oxidase (which is nonfunctional in humans and

other primates) and the cofactor FAD+ . This reaction produces ٢oxogulonolactone , which spontaneously undergoes enolization to form ascorbic acid.

١-٧-٢Plant ascorbic acid biosynthesis pathway:-

There are many different biosynthesis pathways fo r 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, whereby the lactone ring opens and forms again but with between the carbonyl on C١and hydroxyl group on the C٤, resulting in L-galactonolactone. L-galactonolactone then reacts with the mitochondrial flavoenzyme L- galactonolactone dehydrogenaseto produce ascorbic acid.

An interesting fact about L-ascorbic acid is that it has shown to have a negative feedback on L-galactose dehydrogenas e in spinach. Ascorbic acid efflux by embryo of dicots plants is a well-established mechanism of iron reduction, and an step obligatory for iron uptake.

١-٨-Industrial preparation:-

Ascorbic acid is prepared in industrial from glucose in a method Based on the historical Reichstein process. In the first of a five-step process, glucose isthe microorganism Acetobacter suboxydans to sorbose. Only one ofthe 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.) 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 ٩٠%.More biotechnological process, first developed in China in the ١٩٦٠s but further developed in the ١٩٩٠s, bypasses the use of acetone-protecting groups . Asecond genetically-modified microbe species (such asmutant Erwinia, among others) oxidizes sorbose into ٢- ketogluconic acid (٢-KGA), which can then undergo ring-closing lactonization videhydration. This method is used in the predominant process used by the ascorbic acid industry in China, which supplies ٨٠% 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 ٢-KGA, bypassingboth the need for a second fermentation and theneed to reduce glucose to sorbitol.

١-٩-Properties :-

Molecular formula	$C_٦H_٨O_٦$
Molar mass	١٧٦.١٢ g $mol^{-١}$
Appearanc	White or light yellow solid
Density	١.٦٥ $g/cm^{٣}$
Melting point	١٩٠ to ١٩٢ °C (٣٧٤ to ٣٧٨ °F; ٤٦٣ to ٤٦٥ K) decomposes
Solubility in water	٣٣٠ g/L
Solubility inethanol	٢٠ g/L
Solubility inglycerol	١٠ g/L
Solubility inpropylene glycol	٥٠ g/L
Solubility in other solvents	Insoluble in benzene ,petroleum ether, diethyl ether,chloroform, , oils, fats
Acidity (**pKa**)	٤.١٠ (first), ١١.٦ (second)

*source:-**From Wikipedia, the free encyclopedia***

١-١٠- ASCORBIC ACID ORAL USE:-

Ascorbic acid (vitamin C) 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 normaldiet 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, joint pain, Tiredness, or tooth loss.

Vitamin C plays an important role in the body. It is needed to maintain The health of skin , cartilage, teeth, bone, and blood vessels. 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 vitamin C 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 researchers think C supplements will help treat or

prevent OA. What the evidence does show is 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.

١-١٠-١-Age-related Macular Degeneration

Vitamin C (٥٠٠ mg) appears to work with other antioxidants, including zinc (٨٠ mg), beta-carotene (١٥ mg), and vitamin E (٤٠٠ IU) to protect the eyes against developing macular degeneration (AMD), the leading cause of legal blindness in people over ٥٥ 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 should be taken only under a doctor's supervision.

١-١٠-٢-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.

١-١٠-٣-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.

(Vitamin C (Ascorbic acid) | University of Maryland Medical)

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١-١١-ASCORBIC ACID IN PSIDIUM GUAJAVA LEAFES

Psidium guajava leaf is an important part of guava tree which is useful in Curing many health problems. Guava leaves having properties like antibacteria l, anti-oxidant , anti-cancer , anti ulcer etc .

used in many diseases. In this article different extraction methods are discussed and yield of these methods represented . Extraction processes with different solvent such as ethanol, methanol, ethyl acetate and water are discussed in this article. The purpose of this article is to introduce the different extraction processes for different compounds for curing different health problems. In this study, aqueous extract shows it's widely use in medical as compare to other solvents extract. Aqueous extract of guajava

leaf have antiglycation activity and prevent neurodegenerative and cardiovascular disease and also shows antiprostate cancer activity phytotoxic, hepatoprotection, and anti hyperglycaemic and anti cancer activities.

١-١١-١-Chemical composition:-

The leaves contain essential oil 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 [Zakaria]. 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 ٦%, and volatile oil ٠.٣٦٥% .٣.١٥ % resin , ٨.٥% tannin, and a number of other fixed substances.

The essential oil contains eugenol [confirmed Nadkarni &Nadkarni], mallic acid and tannin from ٨-١٥%.the leaves contain an essential oil rich in cineol and four triterpenic acids as well as three flavonoids Leaves contain resin, fat, cellulose, tannin, volatile oil, chlorophyll land mineral salts .

(*Begum et al).*

١-١١-٢ Properties and Composition of psidium

guajava Guava Leafes:-

Guava leaf extract has analgesic, anti-inflammatory , antimicrobial, Hepatoprotective and antioxidant activities. These effects are probably due to the presence of phenolic compounds (*Jiménez-Escri et al).*

reported the presence of higher amounts of phenolic compounds with antioxidant activity in the leaves of white (Psidium guajava var. pyrifera L.) and red guava (Psidium guajava var. pomifera L.) when compared with other vegetable species. (Chen et al ٢٠٠٠ *).* found gallic acid,

catechins,epicatechins, rutin, naringenin and kaempferol in the leaves.

Studies have shown that gallic acid, catechin, and epicatechin inhibit pancreatic cholesterol esterase, which decreases cholesterol levels.

Catechins are important as a preventive treatment for diabetes type ٢ and obesity. Quercetin has been associated to decreased mortality from heart disease and decreased incidence of stroke.

Quartet in presents hypocholesterolemic and antioxidant activity. Rutin is effective in the inhibition of triglyceride accumulation in adipocytes.

Naringenin and kaempferol can promote moderate cytostatic activity Against all cell lines and kaempferol can be useful as anti cancer .

(Fu et al). elucidated the structure of three novel sesquiterpenoid-based meroterpenoids of psidials A-C found in guava leaves. (Matsuzak et al).

isolated two new benzophenone galloyl glycosides, guavinosides A and B, and a quercetin galloyl glycoside, guavinosideC as well as five known quercetin glycosides from guava leaves. The structures of the novel glycosides were elucidated to be ٢,٤,٦-trihydroxy benzophenonetrihydroxy benzop hen one ٤-O-(٦''-O-galloyl)-beta-D: - glucopyranoside (١, guavinoside A); ٢,٤,٦ - trihydroxy-٣,٥-dimethylbenzophenone

٤-O-(٦''- O-galloyl)-beta-D: -glucopyranoside (٢, guavinoside B), and quercetin٣-O-(٥'-O-galloyl- alpha-L -arabinofuranoside (٣, guavinosideC) *Kim et al.*

١-١١-٣-medicinal use of psidium guajava leafes:-

-Antibacterial activity:

The extract of guava leafes also showed invitro antimicrobial activity against Escherichia coli, Salmonella typhi, Staphylococcus aureus, Proteus mirabilis and Shigella dysenteria [Iwu]. Another paper showed the effectiveness of the leaf extract against Staphylococcus aureus [Gnan and Demello]. It was shown to antibacterial inanother study and in addition to Staphylococcus aureus was also useful againstStreptococcus spp [Pranee]. The

leaves are rich in tannin, and have antisepticproperties [Hernandez]. Astrong antimicrobial action of guava leave son Gram-positive and Gram -negative organisms has been reported (Sarcina lutea and Staphylococcus aureus) and also noted action on Mycobacterium phlei . The flavone derivatives isolated were reportedto inhibit the growth of Staph. aureus in a dilution of ١:١٠,٠٠٠[Oliver-Bever

Anti-inflammatory effect:

The essential oil has also been proven to have antiinflammatory effect.

The essential oil, steam-distilled from leaves of P. guajava leaves, was given orally rats to study its effectson the exudative and proliferative phases of the inflammatory reaction(carrageenan-induced pawoedema and cotton pellet-induced granuloma models). The essential oil (٠.٨ mg/kg) significantly reduced oedema formation induced by carrageenan. The essential oil (٠.٤ and٠.٨ mg/kg) significantly reduced granuloma formation induced by cotton pellets. [Kavimani et al]. Another paper confirmed the anti-inflammatory activity and also showed significant antipyretic activity and potent anti-arthritic activity in rats [Sen et al]. In Peru it is said to be good for oedema[Raintree]

Coughs:-

Boiled with lemon grass (*Cymbopogon citratus*) to make a decoction that

Is drunk for coughs. Adecoction is also taken in Senegal for

tracheobronchitis(*Wyk et al)*

Malaria:-

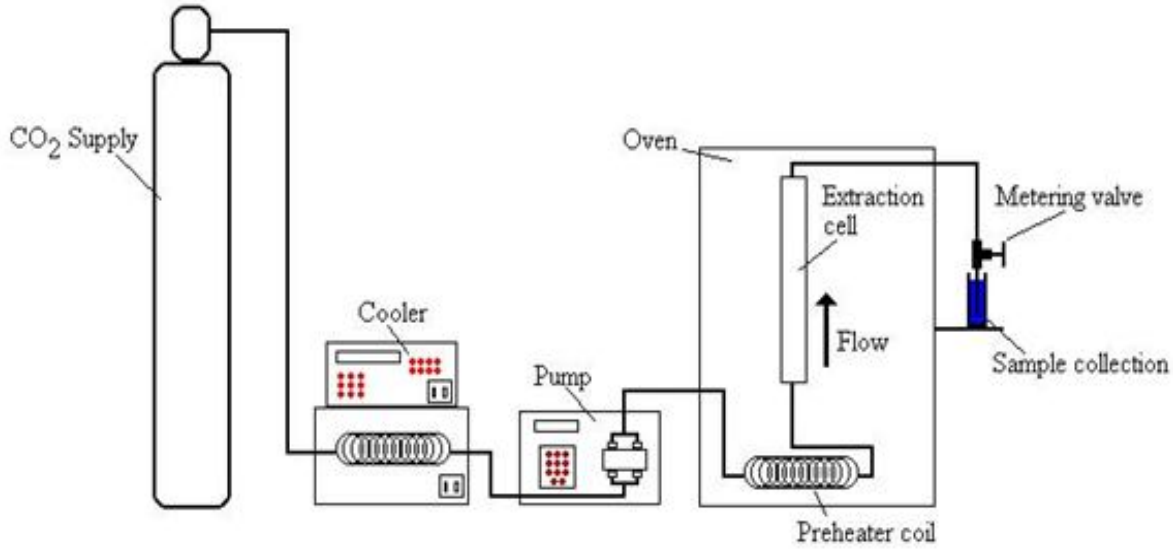
The leaves are used as an ingredient in the preparation of fever "teas".

They are also used as part of the pot herb used in steam treatment for malaria.

Indeed, the main ethno therapeutic use in Africa is said to be for malaria.

١-١٢- Methods Of Extraction ascorbic acid from psidium guajava leafes:

A. Super critical fluid extraction:-



Extraction process is basically for separation of one component from other component by using extracting solvent. In SFE, we use supercritical fluid as extracting solvent with some co-solvent to increase its capacity to separate. For this type of extraction, we take sample of guava leaves ١ mg in a cell column. In SFE CO٢ is mostly taken as super critical fluid with ethanol and methanol as co-solvent. Fig.١ showing different components:

a pressure cell, pressure controller, collecting vessel, heating and cooling system and pump. First liquid is converted into supercritical condition then

pass it to the extraction vessel where it could easily diffuse into solidmatrix of sample and dissolve the material which we have to extract.

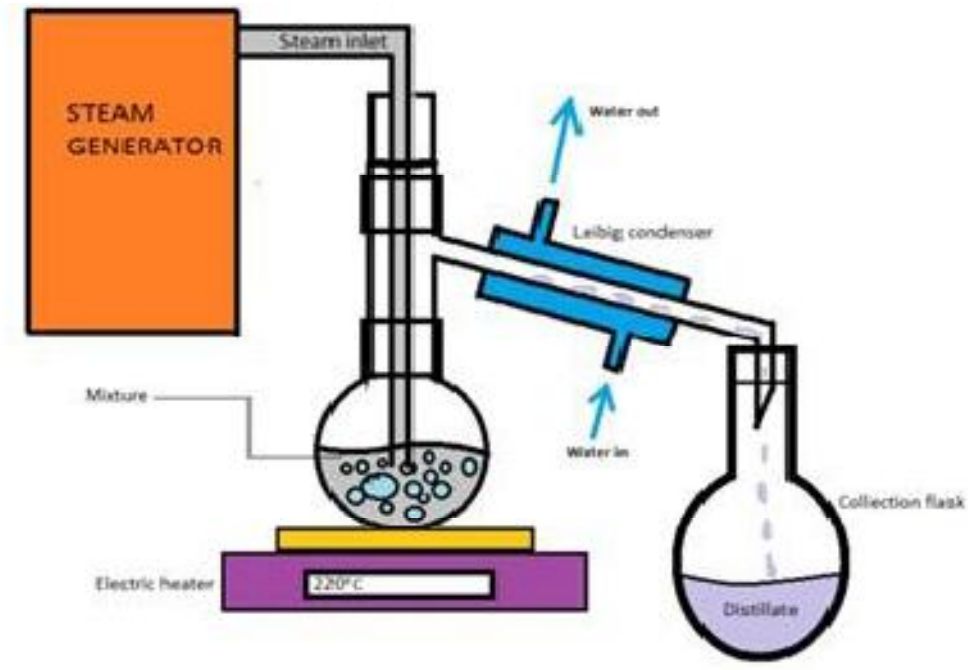
The dissolved material will swept away from cell column at lower pressure and extracted material settle out.CO_2 can be recycled. Temperature andpressure conditions should be ٤٥-٥٥ C and ٢٠٠-٣٠٠ bar.

B. Ultrasound extraction:-

We will take ١.٥ mg of solid sample in ultrasound apparatus. Ultrasound apparatus consist of water tank, ultrasound generator, digital timer, rotary gear and others (shown in fig.٥).process temperature should be ٤٠-٥٠ C and process time should be ١-٢ hours. After this process, material was filtered using a vacuum evaporator**.**

C. Steam distillation :-

Steam distillation extraction apparatus:-



This process is basically for natural aromatic compounds used for temperature sensitive compounds which decompose at high temperature i.e. ٢٥ mg of dried leaf material distilled in ٣٠٠ ml water. Process carried out for ٣-٤ hours at boiling temperature of solvent and after distillation, volatile compounds and water separated by methyl chlorate

D. Soxhlet extraction

Different components which used in Soxhlet extraction like thimble, water cooling system, and reservoir, by pass tube, siphon tube and condenser can be seen in figure ٢. We will take ١٠ mg of solid material of leaves keep in thimble which is loaded into soxhlet vessel having flask containing extractor solvent. Solvent vapor moves up to the colum and floods into the chamber housing the thimble of solid. Some part of non volatile compounds dissolves in solvent. Process repeats many times until we get desired concentrated compounds in flask. Process has been done at boiling temperature of solvent and extraction has been done in ١٠٠ ml ethanol

for ٣.٥ hours *(Vibha Porwal, November- December ٢٠١٢)*

Chapter (٢)

EXPERIMENT

٢-١Chemical's:-

Ethanol (٧٧.٨%)-

Starch(١g/٥٠ml)-

Iodine(Assay ٩٨.٥% ,M.wt=٢٥٣.٨١ ,ALPHACHIMIKA)|-

Potassium Iodide(Assay ٩٨.٥% ,M.wt =١٦٦ ,ALPHA CHEMIKA)-

Potassium permanganate(Assay ٩٨% ,M.wt =١٥٨.٠٣ ,CDH laboratory)-

Sulphuric acid(Assay ٩٨% ,M.wt =٩٨.٠٨ ,OXOFORD laboratory)-

Di ethyl ether(Assay ٩٨%,, M.wt=٧٤.١٢ , ALPHA CHEMIKA)-

٢-٢ Apparatus:-

Soxhlet-

Volumetric flask (١٠٠ml)-

Volumetric flask (١L)-

Burate (٥٠ml)-

Pipette (١٠ml)-

Flask (٢٥٠ml) conical-

٢-٣Instruments:-

-Infra red photometer.

-Balance.

٢-٤ Method :-

Extraction:-

The sample of guava leaves was dried and prepared in small shape by grined with mortar. ١٠g of dried guava leaves was placed in thimble which was loaded into soxhlet vessel having flask containing extractorsolvent. The sample was extracted with ٢٥٠ml of ethanol. The extraction has been done within ٤ hours.

The abstract was collected and transfered into beaker (٢٥٠ml) ,the solvent Was separated from extraction used rotary vaccum distillation.

Then the abstract was put for two days without covering ,after that ,the Precipitate Was washed with five portions (٢٠ml) of di ethyl ether with filtrationThen the precipitate was dried , weight ,and the yield percentage was

determinate .

Then the extraction yield was analyzed using IR to identify, and Redox titration was used to measure the quantity of ascorbic acid.

Determination:-

Potassium permanganate titration-٠.٠٥٧g of potassium permanganate was weight into a ١٠٠ ml beaker, added distilled water and swirl for afew minutes until dissolved, the solution was transfered to ١٠٠ ml volumetric flask and solution was made up to ١٠٠ ml mark with distilled water.٠.١ g of sample was dissolved in distilled water and transfered into ١٠٠ml volumetric flask, the solution made up to١٠٠ml mark with distilled water ,then ٢ml of solution was taken to conical flsk, ٢ml of sulphuric acid(١٤N) added to it, the content of flask was titrated potassium permanganate from burate until end point.

-Iodine titration١.٠٠g of potassium iodine was weight into a١٠٠ml beaker, then ٠.٦٥g of iodine was weight and added to the same beaker. few ml of distilled water was added ,and swirl for a few minutes until Iodine was dissolved . Iodine solution was transferred to ٥٠٠ ml volumetric flask, the Solution was made up to the mark with distilled water.

١.٠٠ g of soluble starch was weight, then added it to ٥٠ ml of near boiling Water in ١٠٠ ml conical flask, and stirred to dissolved and cooled before Using.

٢٥ ml of unknown sample which was prepared before was taken to Conical Flask, then titrated with iodine solution from burette ,with used ١ml from Starch indicator, until ending point.

Chapter(٣)

Result

Table(١)shows the weight of extract:--

Weight of beaker/g	Weight of beaker&extrac/g	Weight of extract/g
٣٣.٠٤٨٤	٣٥.٣٠٠١	٢.٢٥١٧

Table(٢) shows,the permanganate titration volume:-

Intential volume\ml	volume\ml final	Used volume\ml
٠.٠٠	٠.٥	٠.٥
٠.٥	١	٠.٥

Table (٣) shows ,the iodine titration volume(unknown) :-

Intential volume/ml	Final volume/ml	Used volume/ml
٠.٠٠	٧.٠٠	٧.٠٠
٧.٠٠	١٤.٠٠	٧.٠٠

Table(٤) shows the iodine titration volume (stander):--

Intential volume/ml	Final volume/ml	Used volume/ml
١٠.٠٠	٢٨.٠٠	١٨.٠٠
٢٨.٠٠	٤٦.٠٠	١٨.٠٠

Calculation:-

Yield percentage =- $\frac{\text{Weight of extract}}{\text{Weight of sample}} \times ١٠٠$

$$= \frac{٢.٢٥١٧}{١٠} \times ١٠٠$$

$$= ٢٢.٥١\%$$

Titration:- potassium permanganate

$$\frac{M \times V}{n} = \frac{M \times V}{n}$$

$$\frac{٠.٠١ \times ٠.٥}{٢} = \frac{M \times ٢}{٥}$$

$M = ٠.٠٠٦٢٥ mol/L$

$٠.٠٠٦٢٥ \rightarrow ١٠٠٠ ml$

$X \rightarrow ١٠٠ ml$

$X = ٠.٠٠٠٦٢٥ mol/١٠٠ ml$

Iodine titration :-

M×V/n =M×V/n

٠.٠٠٥× ٧ /١ = M×٢٥/١

M = ٠.٠٠١٤mol/l

٠.٠٠١٤ → ١٠٠٠

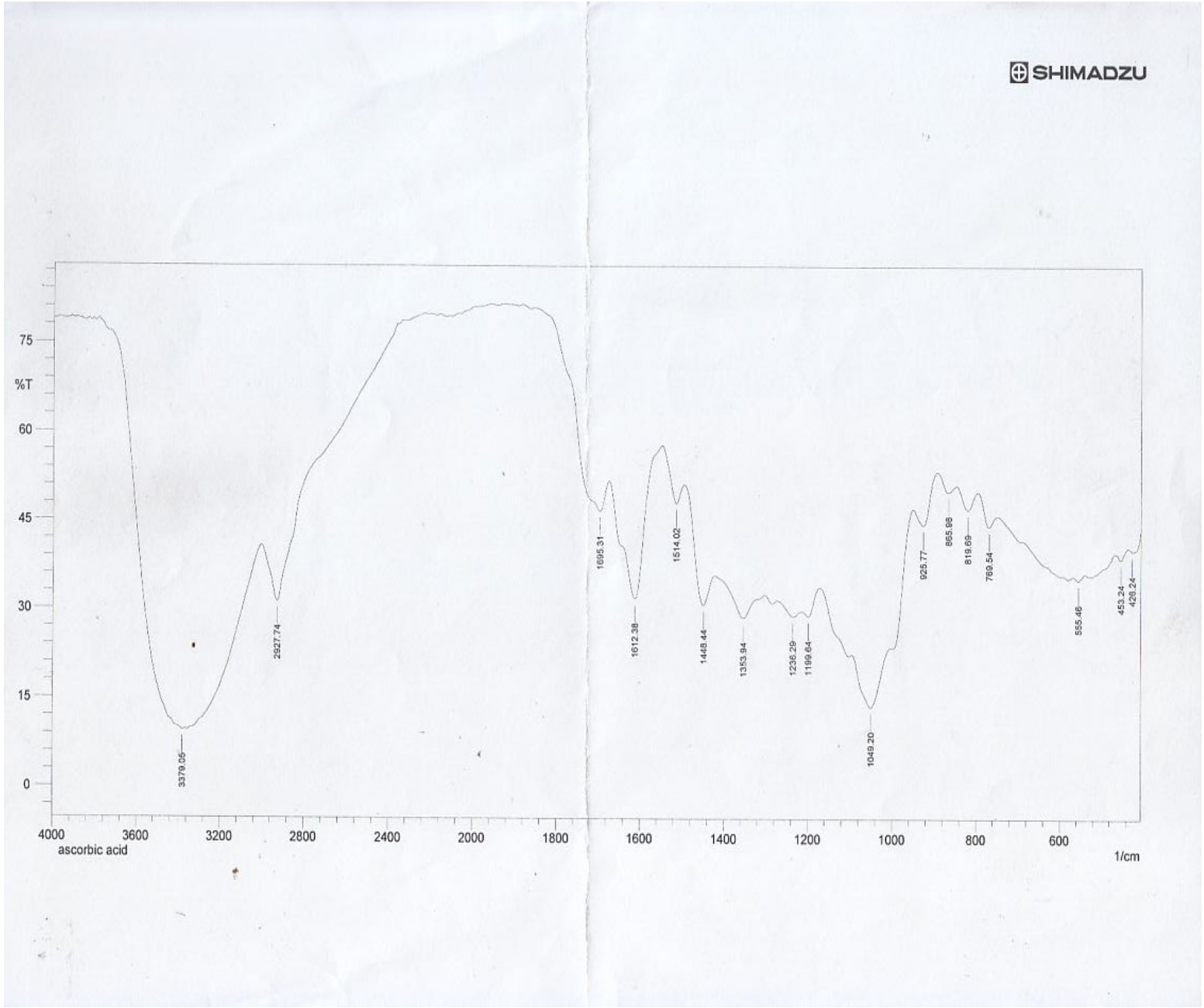
X → ١٠٠

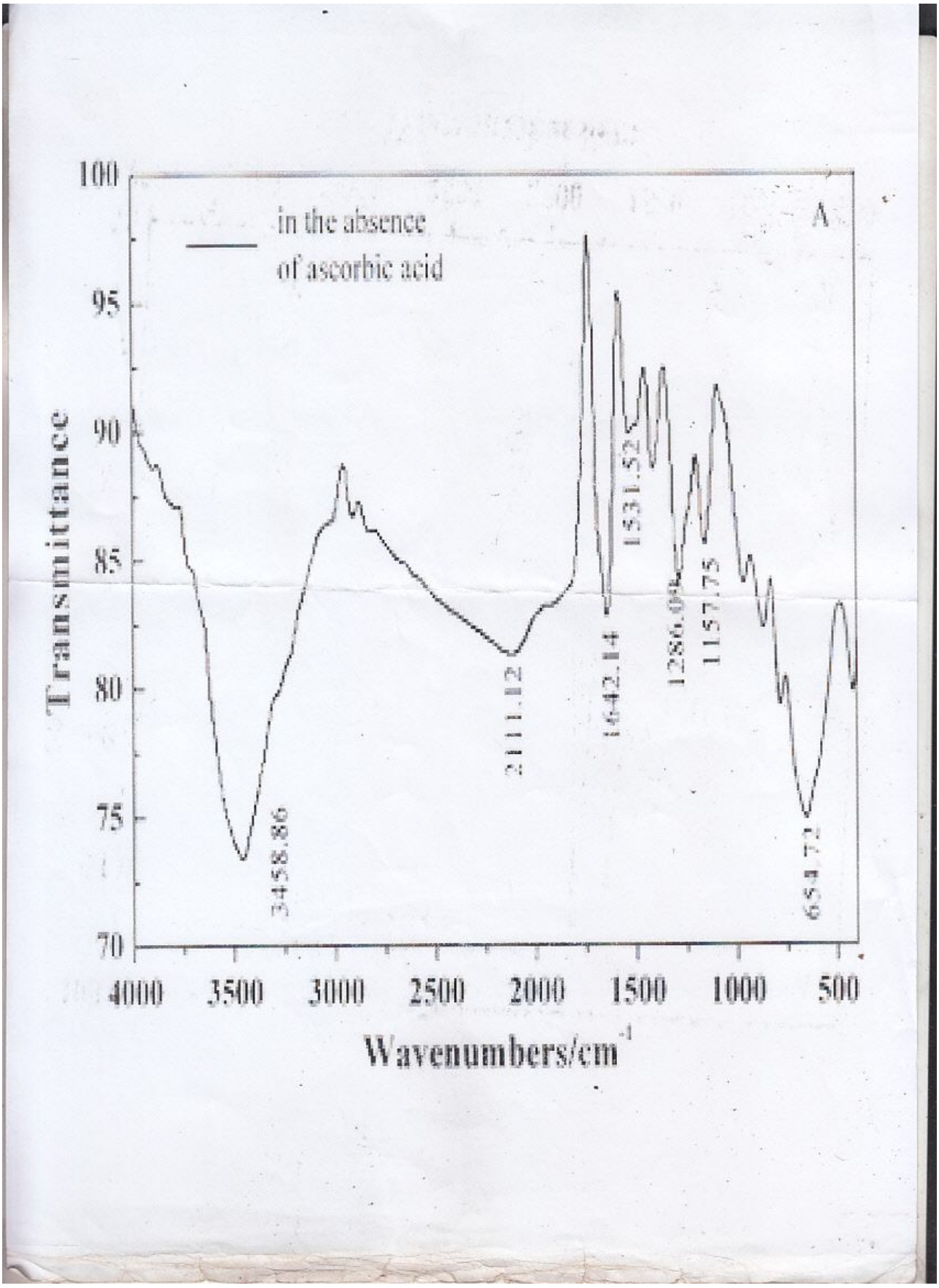
X = ٠.٠٠٠١٤mol/١٠٠ml

٠.١ g/١٠٠ml(standerd)→ ٠.٠٠٠٣mol/١٠٠ml

٠.١g/١٠٠ml(unknown)→X

X= ٠.٠٠٠٣mol/١٠٠ml


SHIMADZU
%T
75
60
45
30
15
0
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
1/cm
ascorbic acid
3379.05
2927.74
1695.31
1612.38
1514.02
1448.44
1353.94
1236.29
1199.64
1049.20
925.77
865.66
819.69
769.54
555.46
453.24
428.24


A
in the absence
of ascorbic acid
Transmittance
100
95
90
85
80
75
70
3458.86
2111.12
1642.14
1531.52
1286.09
1157.75
654.72
4000
3500
3000
2500
2000
1500
1000
500
Wavenumbers/cm-1

Chapter(٤)

Discussion

Ascorbic acid was extracted from psidium guajava leafes. It Identification with IR.and was sure that it actually ascorbic acid bycompared the peaks of sample with peaks of standard. Found that there is

a mismatch in the range between:-

(٣٣٧٩&٣٤٥٨this range represent hydroxyl faction group),

(٢٩٢٧.٧٤&٢١١١.١٢this range represent C-H stretching vib)

,(١٦٤٢.١٤&١٦٩٥.٣this range represent skeletal)

,(١٥٣١.٥٢&١٥١٤.٠٢this range represent carbonyl faction group)

Was to make sure that the sample containing these factional group.

If faction group ascorbic acid sample that the learned ascorbic acid.

And the emergence of some peaks in the difference span and it's may be due to the Presence of some impurities with sample or due to that non-sellective solvent used for ascorbic acidOxidizes ascorbic acid by the permanganate and turns to the DeHydro Ascorbic Acid , It works the role of the reducing agent . In the iodine titration the iodine formed is immediately reduced to iodide as long as there is any ascorbic acid present. Once all the ascorbic acid has been oxidized , the excess iodine is free to react with the starch indicator, forming the blue-black starch-iodine complex. This is the endpoint of the titration. Also turned into dehydroascorbic acid.

Percentage of extraction was calculated and found to be equal to ٢٢.٥١%.low percentage may be due to mistake in sample preparation or because Guavaleave's was dried under the sun, so the ascorbic acid was broken down.

Suggestion

In sample preparation ,firstly the drying leave's must be in the shade or in adring oven at alow temperature,because the high temperature will break the vitamin C.Muste take mature guava leave's not withered ,in extraction must be used a suitable solvent ,selective and high purity such as ethanol ٩٩%.

Employment accuracy technique to extraction .to estimate the amount of sample must resort to high accuracy methode such as HPLC not traditional method.

After extraction must take advantage of abstract use in cosmetic product or some therapeutic uses,such as in flammation.

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This article is about the molecular aspects of ascorbic acid.