TOTAL PHENOLIC CONTENT AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *PSIDIUM GUAJAVA L.* FRUIT

A project report submitted to

Mahatma Gandhi University, Kottayam

In partial fulfillment of the requirements for the award of

Master Degree in CHEMISTRY

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Under the supervision of

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BHARATA MATA COLLEGE, THRIKKAKARA

(Affiliated to Mahatma Gandhi University, Kottayam)

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BHARATA MATA COLLEGE

THRIKKAKARA

CERTIFICATE

This is to certify that the project report entitled "**TOTAL PHENOLIC CONTENT AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *PSIDIUM GUAJAVA L.* FRUIT**" is a bonafied work carried out by Ms. **ANN MARY SAJI,** M.Sc. Chemistry student, under my supervision and guidance and that no part of this has been submitted for any degree, diploma or other similar titles of recognition under any university.

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DECLARATION

I **ANN MARY SAJI** hereby declares that this project report entitled "**TOTAL PHENOLIC CONTENT AND PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *PSIDIUM GUAJAVA L.* FRUIT**" is an authentic work carried out during my course of study under the guidance of Dr. JINSA MARY JACOB, Department of Chemistry, Bharata Mata College, Thrikkakara.

Place: Thrikkakara

Date:

ACKNOWLEDGMENT

I thank God Almighty for showering his blessings on me for the successful completion of the project work undertaken by me.

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ANN MARY SAJI

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CHAPTER 1

INTRODUCTION

Every day we depend upon plants to keep us alive and healthy. For air, water, food and medications we take, we owe to nature. For happiness and well-being, a better health is very much important to human beings. Health of the people gives a great contribution to economic progress of nation because only a healthy population can be more productive. So countries give very importance to the health of their people.

Many medicines are derived from plants. This medicinal practice is widely used in India, China, Pakistan and Srilanka. An innovative discovery in this field was in 1953, by the isolation of serpentine from Indian plant Rauwolfia serpentine root. About 70% of medicinal plants in India are found in tropical forest in Eastern and Western Ghat, Chota Nagpur plateau, Aravalli range and The Himalayas. The geographical position and the climatic conditions are very useful for the growth of medicinal plants in India.

Aloe Vera is used to treat external bums, bites, cuts or scrapes. Chamomile is used to treat external bites, stings and internally it soothes stress. Cranberries cure urinary tract infection, heart diseases and gum diseases. Mint boosts immunity, expels cough from the body, benefits respiratory health and enhances mood. Adathoda vasica cures gonorrhea, heart diseases and bronchitis. Tulsi is used for treating cough, indigestion, hair loss and diabetes. Fennel is used internally and it helps in digestion, cramps and asthma. Some medicinal plants are a rich source of antioxidant compounds. These antioxidant compounds can neutralize harmful free radicals formed in our body.

1.1. ANTIOXIDANTS

Antioxidants are a type of substances which inhibits oxidation. Oxidation can harm the cells of the organism by producing free radicals. Our body itself has the ability to produce some antioxidants. Even though oxygen is needed for our existence its high reactivity can damage living organisms. Our body itself has an antioxidant system which can manage the reactive oxygen species. Certain plant based foods are thought to be rich in antioxidants. These plant antioxidants are group of compounds with structural variations. Since they are obtained from an external source they are called exogenous natural antioxidants. These dietary antioxidants include vitamin C, vitamin E, beta carotene, flavonoids, polyphenols etc. Guava is a natural source of different antioxidants like ascorbic acid, tannins, phenols, saponin, fibers and sugars. Lycopene and flavonoids are important antioxidants in guava. The phytochemicals present in the guava prevents humans from getting diabetes, high blood pressure and obesity. The antioxidants in guava also decrease the risk of many degenerative diseases like heart disease, cancer, arthritis, inflammation etc. Polyphenols and ascorbic acid are the two important antioxidants present in the fruits. The polyphenols are mostly flavonoids. It was observed that white flesh guava contain more amounts of antioxidants. This is due to the presence of phenolic and flavonoid compounds. While comparing a non-peeled fruit with a peeled one, the phenolic and flavonoid compounds were more in a non-peeled fruit.

Solvent extraction is the technique of separating soluble components into a solvent. The antioxidant compounds in the guava are extracted using four different solvents. The antioxidant potential of these guava extracts has various therapeutic applications.

1.2. PHENOLIC CONTENT IN GUAVA

The anti-hyperglycemic and anti-hyperlipidemic properties of guava are mainly due to the phenolic compounds present in it. The total phenolic content

in guava is determined using Folin-Ciocalteu (F.C) assay. This is an in-vitro assay. Phosphomolybdate and phosphotungstate mixture is F.C. reagent. This assay is done to compare the phenolic compounds present in various extracts of guava. Presence of phenolic type compounds is an indication of the antioxidant activity.

1.3. PHYTOCHEMICALS

Medicinal value of plants is due to the presence of phytochemicals in it. Phytochemicals are biologically active compounds present in plants which are derived from roots, stem, leaves, flowers, seeds, fruits etc. These phytochemicals help plants to survive in unfavorable conditions. They are the primary and secondary metabolites. Phytochemicals vary from plant to plant based on the growing environment, varieties, processing methods and all. About thousands of phytochemicals are known and many remain unknown. These are not essential for the life of humans but studies have shown that they have the property to cure diseases. Anticancer, antioxidant and antimicrobial properties are a few among their medicinal properties.

From a recent study of World Health Organization (WHO) about 80% of the populations of developing countries rely on medicinal plants for their primary medical needs. Phytochemicals act as link between modern and traditional medicines. While using plant derived medicines the risk of side effects is much lesser. These plant compounds can be used as alternative medicines. The results of using these in chronic diseases are long run. Phytochemicals are also consumed as supplements in need of nutrients. But it is not sure whether the effects are same while using supplements.

1.4. PHYTOCHEMISTRY

The field of chemistry which is concerned of phytochemicals is called phytochemistry. It is also a subfield of botany since it involves plant derived compounds. There are a wide range of applications for phytochemistry. The phytochemicals are classified as; carotenoids and polyphenols which include phenolic acids, stilbenes/lignans and flavonoids which are further classified into flavones, anthocyanins, isoflavones and flavonols.

Major classification of phytochemicals is as primary and secondary metabolites. Primary metabolites are concerned with the basic growth factors of plants like sugars, amino acids , proteins etc. Secondary metabolites help plant to survive during harsh conditions. These include diseases, pollution, UV and stress. Flavonoids, saponins, tannins, alkaloids, steroids, phytosterols come under secondary metabolites. These have many commercial applications too.

Traditionally newer drug synthesis was based on natural plants which contain polyphenolic compounds. Primitive medicines used plants not only to treat diseases but also to kill animals. Bearberry was used to treat bladder and urinary tract related diseases, raspberry in digestive system disorders, hawthorn as a diuretic and to treat high blood pressure and rose bay willow herb used as an intestinal astringent, anti-spasmodic in asthma and in ointments to cure cutaneous infections.

Plants having secondary metabolites have potential anti–cancer properties. Currently available therapeutic drugs are phytochemical derivatives. But the exact mechanism of action of the phytochemicals remains unclear. By understanding action of phytochemicals as anti-cancer drugs scientists will be able to develop next generation drugs. Even though many studies are going on

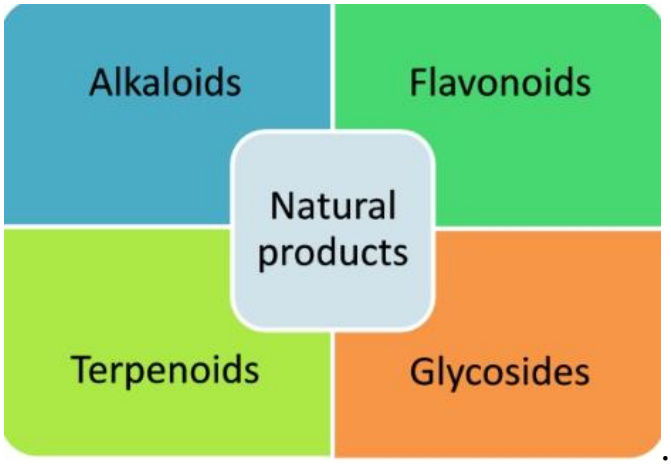
phytochemicals due to their therapeutic potential and structural properties, in most cases no further proceedings are reported.

Plant physiology is the study of behavior and functioning of various parts of the plant including stem, root etc. As we have already mentioned phytochemicals are present in various parts of the plant. A better knowledge on plant phytochemistry can lead to understanding of physiology of plant. From this analysis five class of plant hormones are identified, they are; auxins, cytokinins, gibberillins, abscisic acid and ethylene. These plant hormones can be either growth promoters or growth inhibitors.

The interaction of phytochemicals with the ecology may be of two types either between plants or with animals .But a detailed study of this is a time consuming process for a phytochemist since it is complicated. Alkaloids, terpenes, sterols etc. involve in these processes. Phytochemistry is also used to study about fossils. According to a plant pathologist this phytochemical analysis is very important. Various phytotoxins can be identified even though they have complex structures. Lycomarasmin and fusaric acid are the most familiar phytotoxins which are found in tomato. Glycopeptides, naphthaquinones have also been isolated. Special precautions should be taken while handling these phytotoxins because of their high chemical reactivity.

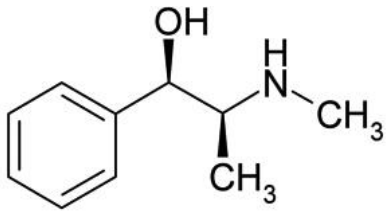
1.5. CLASSIFICATION OF PHYTOCHEMICALS

Phytochemicals are natural products derived from plants. They are classified into alkaloids, flavonoids, terpenoids, and glycosides.



ALKALOIDS

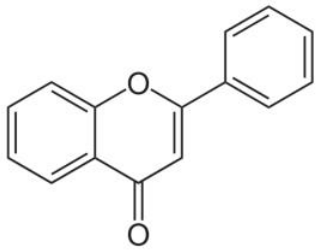
Alkaloids are a class of organic compounds that contain nitrogen atom in an amine type structure – a hydrogen atom in ammonia replaced by a hydrocarbon. Alkaloids are found mainly in plants and play an important role during seed formation. The nitrogen atom in alkaloids acts as a base. Their name ends with suffix 'ine'. Most alkaloids are colorless and volatile. They are optically active. Almost all the alkaloids have a bitter taste. For example, quinine is a bitter tasting alkaloid. Alkaloids can be grouped based on the type of heterocyclic ring system present in the molecule. Nomenclature of alkaloid is based on the type of plant in which they occur and their physiological characteristics. Various classes of alkaloids with different heterocyclic rings are pyrrolidine alkaloids, pyridine alkaloids, pyrrolidine-pyridine alkaloids, pyridine- piperidine alkaloids, quinolone alkaloids, isoquinoline alkaloids. Pharmacological activities of alkaloids are antihypertensive effects, antiarrhythmic effects, antimalarial activity and anticancer activity. Quinine is used as antimalarial drug. Caffeine, nicotine and morphine are used as analgesics.



FLAVONOIDS

Flavonoids are polyphenolic compounds which occur in fruits, vegetables and beverages like tea and coffee. More than 4000 flavonoids have been recognized so far. Flavonoids have broad biological and pharmacological activities including antimicrobial, cytotoxicity and anti-inflammatory activities. Flavonoids can act as powerful antioxidant. This property depends upon their molecular structure. The position of hydroxyl group and other features are very important for flavonoid's antioxidant behavior. Flavonoids are better antioxidants than nutrients antioxidants. They contain a wide range of substances which play an important role in protecting biological systems. Flavonoids have enzyme inhibition, antimicrobial, estrogenic activity, anti-allergic and vascular activity.

They are used as dyes. Malvidine is responsible for the red color in wine. Anthocyanidins is used for yellow, red and blue color.

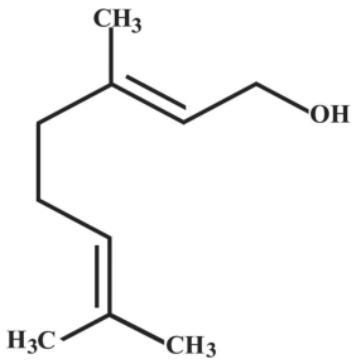


TERPENOIDS

Terpenoids are a class of natural products which are lipids derived from five- carbon isoprene units. They can be found in every class of living things so they are considered as the largest group of natural products. These are commercially useful in foods and cosmetics as flavors and fragrances. Their

building block is the hydrocarbon isoprene units. Terpenoids are classified into hemiterpenoids, monoterpenoids, sesquiterpenoids, diterpenes, triterpenes, and tetraterpenoids according to the number of isoprene units present.

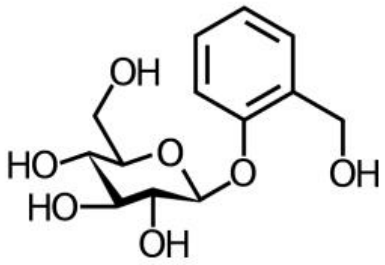
Terpenoids are structurally more diverse group among plant secondary metabolites. Volatile terpenes produced by plants help them to attract specific insects for pollination. They play a role as signal compounds and growth regulators. They have medicinal properties like anti-carcinogenic, antimalarial, anti-ulcer, hepaticidal and antimicrobial.



Geraniol

GLYCOSIDES

Glycosides contain a carbohydrate component linked by an ester bond to a noncarbohydrate nucleus. Many plants store chemicals in the form of inactive glycosides which can be activated by enzyme hydrolysis. Saponins, glycosinolates, glycoalkaloids etc. are some important glycosides. They are formed by some biochemical reactions which makes a water insoluble compound more polar than a water soluble molecule. In man these are formed in liver. Glycosides show therapeutic properties in low doses but they are toxic in higher doses. Many of plant glycosides are used in medical treatments. The first glycoside identified was amygdalin.



1.6. PSIDIUM GUAVAJAVA L.FRUIT

Many plants have medicinal properties. *Psidium guavajava L*. is an example. Guava in the family Myrtaceae was originated in the tropical South America. It grows widely in India, Brazil, Thailand, West Indies, California etc. The common guava has quadrangular branchlets, oval shaped leaves about 7.6cm (3 inches) in length and four petaled white flowers about 2.5cm (1 inch) broad. The fruits are round pear shaped and measure up to 7.6cm in diameter, their pulp contains many small hard seeds more abundant in wild forms than cultivated varieties. According to the color of flesh, guavas are either pink or white. Pink guavas are more aromatic and their color is due to carotenoid, a naturally occurring organic pigment.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Genus	Psidium
Species	P. guajava

Guava can adapt in variety of climates and soils so its cultivation is quite easy. Guava plant grows mostly in the tropic areas since that soil is suitable for it. Apart from using it as a food it has medicinal properties also. Guava is rich in antioxidant compounds. It contains high level of ascorbic acid. Essential oils, flavonoids, carotenoids, polyphenolic compounds, pentacyclic triterpenoids, esters and aldehydes etc. are present in it. Guava is used to overcome vitamin C deficiency. Since its wood is hard and tough it is used in house buildings in rural areas. Guava fruit contains vitamin A, C, calcium, iron and phosphorous. The phenolic compounds present in the fruit prevent skin aging before time and also shows anticancer p properties. The leaves of guava contain many bacteriostatic and fungi static compounds. Antiviral, anti-inflammatory, antiplaque and antimutagenic activities are also shown by guava. Guava leaf extract has anticough activity. The antiplaque activity is due to the presence of flavonoid. These flavonoids can be extracted from guava. These extracted flavonoids can inhibit the growth of certain bacteria. This bacteriostatic effect makes it useful against cough, diarrhea and oral ulcers. The guava extracts has protein degradation properties so it is an antiviral. The presence of compounds like Gallic acid, galangin, kaempferol is more in seeds than fruits and leaves. It is the presence of all these compounds that make guava more medicinally useful.

Guava can be used to treat diarrhea which is a well-recognized disease. Guava has high cytotoxicity. Ethyl acetate extract of guava can stop infections and antigens. While ethanol extract has anti-inflammatory action. Ethanolic extract can be used for the treatment of infertile males. The methanolic extract shows high antimicrobial activity or minimum inhibitory concentration. So this high activity methanolic extract is very effective and shows anti hemolytic activity. Its antibacterial activity is more against gram positive bacteria than gram negative bacteria.

Guava also contains high amount of phytochemicals which helps to prevent diabetes and obesity. Water and other solvent extracts contain many antioxidant compounds in it. Due to the presence of these compounds guava has a major importance.

CHAPTER 2

AIMS AND OBJECTIVES

AIM

To determine the Total Phenolic Content and the phytochemical analysis of different extracts of Psidium *guavajava L.* fruit.

OBJECTIVES OF THE PRESENT WORK

1. To extract the phytochemicals present in Psidium *guajava L.* fruit using methanol, chloroform, ethyl acetate, and hexane as solvents.
2. To determine the Total Phenolic Content of different guava extracts spectrophotometrically using Folin-Ciocalteu reagent.
3. To do the phytochemical analysis of all the guava extracts.

CHAPTER 3

EXPERIMENTAL METHOD

MATERIAL

About 1.5 kg of *Psidium guajava* (guava) was collected from Ernakulam district. The powdered form of the dried fruit is used for further processes.

GENERAL PROCEDURES

All chemicals are purchased from Merck. Solvents from the extract were removed by Superfit rotary evaporator. Phytochemical analysis was done using eight standard tests.

EXTRACTION OF *PSIDIUM GUAJAVA L.*

1.5 Kg of *Psidium guajava L.* was collected from Ernakulam district. The material was chopped into small pieces and allowed to sundry for four days. The dried material was crushed into fine particles using a mixer grinder, to make the extraction more easier. The powdered material was weighed (175g) and was subjected to extraction using methanol, chloroform, ethyl acetate and hexane as solvents. For this, 35g of dried guava powder was added into four different conical flasks. About 150mL of each solvent was added to each conical flask and subjected to stirring in a magnetic stirrer for six hours. It is then allowed to settle. The supernatant liquid in each conical flask is filtered out using filter paper and funnel into RB flasks. The solvents were removed under pressure on a rotary evaporator. The crude extracts were weighed.

Weight of methanol extract = 2.7514g

Weight of chloroform extract = 1.4818g

Weight of ethyl acetate extract = 1.5326g

Weight of hexane extract = 1.3471g

These weighed extracts were used for further works.

EXPERIMENTAL PROCEDURE

1. PHYTOCHEMICAL ANALYSIS

Phytochemistry is the branch of chemistry that deals with the study of phytochemicals, which are chemicals derived from plants. Phytochemical analysis refers to the extraction, analysis and identification of medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, tannin, saponins, sterol etc.

1. TEST FOR TANNINS

Ferric chloride test: A few drops of 1% neutral $FeCl_3$ solution were added to the extract. Identification is blackish blue color.

2. TEST FOR SAPONINS

Foam test: A small amount of extract was shaken with little quantity of distilled water. Identification is foam produced persist for 10 min.

3. TEST FOR FLAVONOIDS

Lead acetate test: To the extract, a few drops of aqueous basic lead acetate solution were added. Yellow precipitate is the indication for the reaction.

4. TEST FOR STEROLS

Liebermann Burchard test: To the extract, few drops of acetic anhydride was added and mixed well. 1 mL of conc. H_2SO_4 was added from sides of the test tube. Appearance of reddish brown ring is the indication of the reaction.

5. TEST FOR GLYCOSIDES

Keller-Killiani test: 2 mL of the extract, 3 mL of glacial acetic acid and one drop of 5% $FeCl_3$ was added. This solution was transferred to surface of 2 mL conc.H_2SO_4 and observation was noted down. Identification is coloured ring at the interphase.

6. TEST FOR ALKALOIDS

Mayer's test: The extract was treated with Mayer's reagent. Potassium mercuric iodide solution is the Mayer's reagent. Formation of a whitish yellow or cream colored precipitate or creamy white precipitate is the indication of the reaction.

7. TEST FOR CARBOHYDRATES

Fehling's test: Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling solutions A and B. Fehling solution A is a blue aqueous solution of copper(II) sulfate pentahydrate crystals, while Fehling solution B is a clear solution of aqueous potassium sodium tartrate (also known as Rochelle salt) and a strong alkali (commonly sodium hydroxide). Formation of precipitate indicates the presence of carbohydrates.

8. TEST FOR RESINS

To the extract 50% HNO_3 is added. Greenish color is indication of the reaction.

2. TOTAL PHENOLIC CONTENT

Folin – Ciocalteu reagent (0.5mL) previously double diluted with water in the ratio 1:2, was added to extracts of different concentrations and mixed thoroughly. To the mixture, 1mL saturated sodium carbonate solution was added and made up to 10ml with distilled water. The mixture was allowed to stand at ambient temperature for 45 minutes and the test tubes were centrifuged if precipitated. The absorbance of the supernatant was measured at 760nm using a spectrophotometer.

A standard curve was obtained by plotting absorbance along Y-axis and concentration along X-axis. From the graph, the unknown concentration of the extract has been calculated as ascorbic acid equivalent.

CHAPTER 4

RESULT AND DISCUSSIONS

1. TOTAL PHENOLIC CONTENT (TPC)

CALCULATIONS:

0.025g of Ascorbic acid in 50 mL of water is prepared as stock solution.

For making different concentrations of ascorbic acid, the following equation is used.

$$M_1V_1=M_2V_2$$

For making a solution of concentration 0.02 mg/mL,

Where M_1 = 0.02 mg/mL

V_1 = 50 mL

M_2 = 0.5 mg/mL

V_2 = X mL

$M_1V_1=M_2V_2$

0.02×50ml = 0.5× X mL

X = 0.02×50/0.5

= 2 mL

Similarly for,

0.04, X = 4 mL

0.06, X = 6 mL

0.08, X = 8 mL

0.1, X = 10 mL

So for making the different concentrations of ascorbic acid 0.02 mg/mL, 0.04 mg/mL, 0.06 mg/mL, 0.08 mg/mL, 0.1 mg/mL, we should take the volumes about 2 mL, 4 mL, 6 mL, 8 mL, 10 mL from the stock solution respectively and made up to 50 mL.

Volume of the extract = 3 mL

Volume of FC reagent =0.5 mL

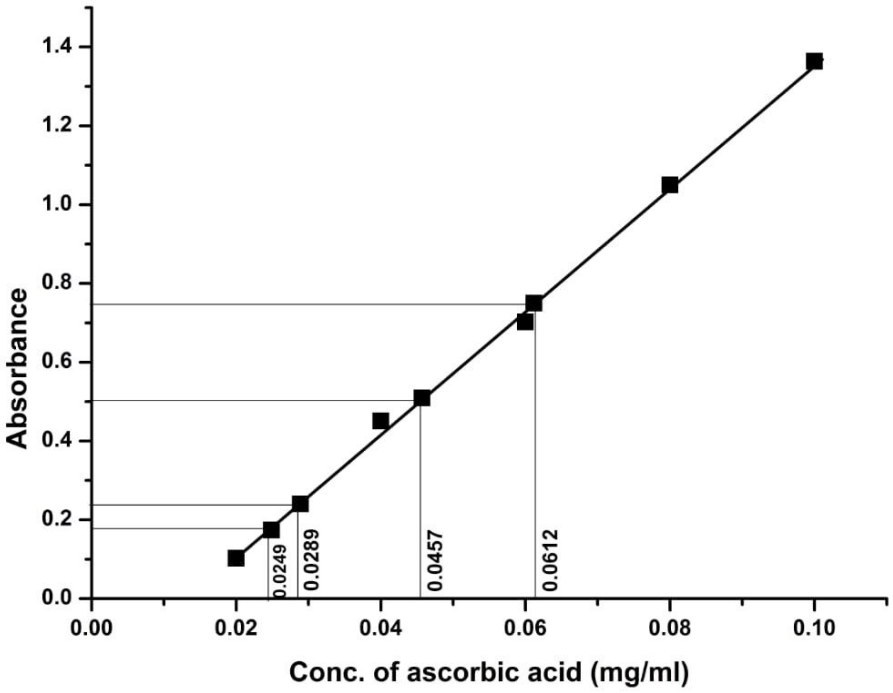
Volume of saturated sodium carbonate solution = 2 mL

When the colour develops, the absorbance was taken using spectrophotometer. The absorbance are measured at 760 nm. The absorbance of corresponding concentrations of the ascorbic acid and extracts were obtained from spectrophotometer. The unknown concentrations of the extracts are determined in terms of ascorbic acid equivalence.

DATA ANALYSIS OF TOTAL PHENOLIC CONTENT

CONCENTRATION OF ASCORBIC ACID	ABSORBANCE
0.02mg/Ml	0.103
0.04mg/mL	0.165
0.06mg/mL	0.314
0.08mg/mL	0.794
0.1mg/mL	1.364
Extract , Methanol(mg/mL)	0.509
Extract , Ethyl acetate(mg/mL)	0.240
Extract ,Chloroform(mg/mL)	0.750
Extract ,Hexane(mg/mL)	0.173

Using the data, a graph is plotted with concentration of ascorbic acid along the X-axis and the absorbance along the Y-axis.



From the graph it is clear that the concentrations of the extracts in equivalent to ascorbic acid are;

Methanol extract = 0.0457 mg/mL

Ethyl Acetate extract = 0.0289 mg/mL

Chloroform extract = 0.0612 mg/mL

Hexane extract = 0.0249 mg/mL

2. PHYTOCHEMICAL ANALYSIS

A. METHANOL EXTRACT

TEST	RESULT
1.Test for tannins	Presence of tannins
2.Test for saponins	Absence of saponins
3.Test for flavonoids	Presence of flavonoids
4.Test for sterols	Presence of phytosterols
5.Test for glycosides	Presence of glycosides
6.Test for alkaloids	Presence of alkaloids
7.Test for carbohydrates	Presence of carbohydrates
8.Test for resin	Absence of resins

B. ETHYL ACETATE EXTRACT

TEST	RESULT
1.Test for tannins	Presence of tannins
2.Test for saponins	Presence of saponins
3.Test for flavonoids	Presence of flavonoids
4.Test for sterols	Presence of phytosterols
5.Test for glycosides	Presence of glycosides
6.Test for alkaloids	Presence of alkaloids
7.Test for carbohydrates	Presence of carbohydrates
8.Test for resin	Absence of resins

C. CHLOROFORM EXTRACT

TEST	RESULT
1.Test for tannins	Presence of tannins
2.Test for saponins	Presence of saponins
3.Test for flavonoids	Presence of flavonoids
4.Test for sterols	Presence of phytosterols
5.Test for glycosides	Presence of glycosides
6.Test for alkaloids	Presence of alkaloids
7.Test for carbohydrates	Presence of carbohydrates
8.Test for resin	Absence of resins

D. n-HEXANE EXTRACT

TEST	RESULT
1.Test for tannins	Presence of tannins
2.Test for saponins	Absence of saponins
3.Test for flavonoids	Absence of flavonoids
4.Test for sterols	Absence of phytosterols
5.Test for glycosides	Presence of glycosides
6.Test for alkaloids	Presence of alkaloids
7.Test for carbohydrates	Presence of carbohydrates
8.Test for resin	Presence of resins

CHAPTER 5

CONCLUSIONS

Extraction of guava fruit was done with four different solvents like methanol, hexane, ethyl acetate and chloroform. The nature of solvent affects the extraction process. Highest amount of extraction was obtained when methanol was used as the solvent. From this we can conclude that extraction of phytochemicals depends on the solubility of the antioxidant compounds in the solvents used for extraction.

The total phenolic content of each extract was determined spectrophotometrically and standard curve was obtained by plotting concentration of ascorbic acid v/s absorbance. It is observed that they have remarkable antioxidant activities, the extent of which depends on solvents used for extraction. The total phenolic content (TPC) is found to be higher for chloroform extract.

From the phytochemical analysis, it is clear that *Psidium guajava L* .fruit is a good source of flavonoids, phytosterols, glycosides and alkaloids. So it could be utilized as a source of natural antioxidants and as a crude drug in the Ayurveda treatment. It improves eye sight, boosts the immune system, promotes healthy skin, reduces the risk of several cancers and supports weight loss.

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