PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *PSIDIUM GUAJAVA L.*FRUIT

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CERTIFICATE

This is to certify that the project report entitled "PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF PSIDIUM GUAJAVA L.FRUIT" is a bonafied work carried out by SANDRA VIJAYAN, B.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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I SANDRA VIJAYAN hereby declare that this project report entitled "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.

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CHAPTER 1

INTRODUCTION

Human beings are dependent upon nature for many of their requirements such as medicine, shelter, food stuffs, fragrances, clothing, flavours, fertilizers etc. throughout the ages. Medicinal plants continue to play a dominant role in the health care system and this is mainly true in developing countries, where herbal medicine has continuous history of long use.

The plants are a source of many new medicines. The traditional medicinal practice is widespread in China, India, Pakistan, Sri Lanka and Thailand in cosmetics, pharmaceuticals, drugs etc. For the development of new drugs, medicinal plants perform a dynamic part. Medicinal plants have proved their sole role in coping with a number of deadly diseases including cancer and diseases associated with viral infections like hepatitis, AIDS etc. The isolation of serpentine in 1953 from the Indian plant Rauwolfia Serpentine root was an innovatory episode in the treatment of hypertension and lowering of blood pressure. The Vinblastine is used for the treatment of leukemia in children.

Nearly about 70% of the medicinal plants in India are found in tropical forest in Eastern and Western Ghats, Chota Nagpur plateau, Aravalli Range, Vindhyas and The Himalayas. India is rich in medicinal and aromatic plants covering an extensive area with different environmental conditions. The geographical position of India and the presence of abiotic factors defined it as a region of high plant diversity.

Medicinal plants are a gift to us from the nature as they provide a number of health benefits to us. In India these medicinal plants are used for about centuries for their properties and are still used to this date. India has a variety of traditional medical systems like Ayurveda, siddha, unani and a huge class of ethnomedicine. This knowledge of medicine was disappeared due to the western culture that has been on us on the past and is reappearing again as their importance have been realized and lack of side effects are also an important aspect in these types of traditional medicine.

The extensively used medicinal plants in India are as follows. Aloe vera is used for constipation, digestive distress, acne and poor body immunity. Ocimum tenuiflorum (Tulsi), is an aromatic perennial plant in the family Lamiaceae which is used for treating cough, indigestion, hair loss, heart diseases and diabetes. Mentha (Mint) boost immunity, expels cough from the body, benefits respiratory health and enhances mood. Adhathoda vasica (vasaka) is helpful for curing gonorrhea, heart diseases and bronchitis.

1.1 PHYTOCHEMICALS

Plants are used as a source of medicine for about thousands of years for curing simple ailments like common cold to devastating diseases like cancer. These plants can work miracles because they contain a set of compounds called phytochemicals. These phytochemicals are the primary and secondary metabolites of the plants that help them survive in unfavourable conditions. Researchers are keen on identifying and analysing these phytochemicals and to find their applications in various fields of science.

Plants are a very important source of nutrients and have a very important part in human diet. They provide us carbohydrates, proteins, vitamins, cholesterol lowering compounds, antioxidants and other important sources of biologically active substances. Many nutritional values of plants have been discussed in the literature but there is very limited research in the biologically active compounds that are present in

them. These biologically active compounds are called phytochemicals. These phytochemicals are derived from every part of the plant including roots, stem, leaves, flowers, fruits, seeds etc.

As per a report by World Health Organization (WHO), over 80% of the people of developing countries are relying on the traditional medicines that are extracted from the plants for their primary health needs. Use of these traditional medicines for the preparation of modern medical preparations is indispensable and thus 'Phytomedicines' are a link between the traditional and modern medicine.

Medicinal plants are very important in health care of individuals and communities in many developing countries. Medicinal plants are believed to be much safer and are used in treatment of various ailments . The plants provide the basic nutrients needed for the growth of animals and humans like proteins, carbohydrates, fats, vitamins and minerals. These plant compounds are used as alternative medicine and have become popular in western culture. They are also used in everyday medicines that we take in our daily life and are also used as nutraceutical supplements for improving nutritional intake.

1.2 PHYTOCHEMISTRY

Phytochemistry is a branch of chemistry which deals with the study of phytochemicals and is often considered as the subfields of botany and chemistry. The subject is also called as plant chemistry and has been developed as a discipline that is closely related to organic chemistry and plant bio chemistry. In other words, it is the chemistry of plant metabolites.

The chemicals that are produced by plants are called phytochemicals. These are produced by the plant's primary and secondary metabolism. These phytochemicals are important for the plants to thrive or thwart other plants, animals, insects and microbial

pests and pathogens. They also protect them from disease and damage caused by environmental hazards like pollution, UV, stress and draught. They are used as traditional medicine and as poisons from ancient days. It is known that they have roles in the protection of human health. More than 4,000 phytochemicals have been catalogued and are classified by protective function, physical characteristics and chemical characteristics.

The phytochemicals are generally classified into the following types; they include carotenoids and polyphenols which include phenolic acids, stilbenes/ lignans, and flavonoids which are further classified into flavones, anthocyanins, isoflavones and flavanols.

The phytochemicals are majorly classified as primary and secondary metabolites. The primary metabolites are responsible for the basic development of the plant which includes the sugars, amino acids, proteins, nucleic acids, chlorophyll, etc.

Secondary metabolites are those which are needed for the survival of the plants in a harsh environment. They form the smell, colour and taste of the plants and secondary metabolites such as flavonoids, tannins, saponins, alkaloids, steroids, phytosterols are found to have other commercial applications like they can be used as colouring agents, drugs, flavouring agents, insecticides, pesticides, anti-bacterial and antifungal products. Moreover they can also be used to protect humans from many diseases like cancer, diabetes, cardiovascular diseases, arthritis and aging etc.

1.3 CLASSIFICATION OF NATURAL PRODUCTS

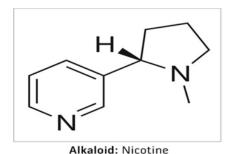
Phytochemicals come under the category of Natural Products which are compounds that are derived from natural sources such as plants, animals and micro-organisms. Due to the structural diversity, natural products are mainly classified into alkaloids, flavonoids,

terpenoids and glycosides.

1.3.1 ALKALOIDS

Alkaloids are the cyclic natural compounds in which basic nitrogen is present in the molecule. They are a very diverse class of secondary metabolites with more than 20000 known molecules. Alkaloids are present in plant tissues as water soluble salts of organic acids (tartaric, acetic, oxalic, citric, malic, and lactic acids), esters (e.g., cocaine, atropine), or combined with tannins or sugars (e.g., the glycoalkaloids of *Solanium* species) rather than as free bases. Most alkaloids are isolated from plant matrices in the form of crystalline, amorphous, nonodorous, and nonvolatile compounds. However, low molecular weight alkaloids, such as arecoline and pilocarpine, and alkaloids with no oxygen atom in their structure (e.g., nicotine) occur in the liquid form. Majority of alkaloids are colourless with a bitter principle in tonic water. Many alkaloids are optically active. The salts of alkaloids are soluble in water or dilute acids, whereas they are insoluble or sparingly soluble in organic solvents. These differences in the solubility of alkaloids, depending on

their form, are used in the pharmaceutical industry for their purification from complex plant matrices and for the production of pharmaceutically acceptable products.



1.3.2. FLAVONOIDS

The flavonoids are a group within the secondary natural products. They occur in the plant kingdom normally as glycosides as well as in free state. Flavonoids are present in various herbal drugs and extracts, such as in chamomile flowers and lime blossom. Flavonoids have a strong affinity towards the divalent ions of heavy metals. The flavonoids, especially the anthocyanidins, are able to form complexes with ions, which lead to the formation of pigments. Such a complex is protocyanin, which is responsible for the blue colour of the cornflower.

They are used as dyes. Substances such as anthocyanidins is used for yellow, red, and blue colour. Malvidine is partly responsible for the red colour of red wine.

1.3.3. TERPENOIDS

The terpenoids sometimes called isoprenoids, are a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from isoprene units. About 60% of known natural

products are terpenoids. Plant terpenoids are used extensively for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, the yellow colour in sunflowers, and the red colour in tomatoes. Some important terpenoids are citral, menthol, camphor etc. The steroids and sterols in animals are biologically produced from terpenoid precursors.

1.3.4. GLYCOSIDES

Glycosides are plant secondary metabolites containing a carbohydrate component linked by an ester bond to a noncarbohydrate nucleus (the aglycone). They are usually bitter substances and are important in human and animal nutrition and they are therapeutic in low doses, toxic in excess. Saponins, glycosinolates, glycoalkaloids etc. are some important glycosides. They occur in higher plant tissues in very small amounts, also in fungal, bacterial cells and animals. They are formed by the biochemical reaction that makes a water insoluble compound more polar than a water soluble molecule and hence they can be removed from an organic system. In man, glycosides are formed in the liver as part of the process of detoxification and they are excreted via

urine.

1.4. APPLICATIONS OF PHYTOCHEMISTRY

Generally speaking, the phytochemical analysis finds applications in many fields of biological sciences, phytogeography, ecology and palaeobotany. Phytochemical methods have become important for solving certain types of problems and will undoubtedly be used with increasing frequency in the future.

1.4.1 Plant Physiology

Phytochemicals determine the plant physiology by determining the chemical structures, the biosynthetic pathways and the action of natural growth hormones. As a result, five classes of plant hormones, also known as growth regulators are recognized: they include, auxins, cytokinins, gibberellins, abscisic acid and ethylene. These plant growth regulators can be of diverse composition of chemicals like terpenes (gibberellic acid) or carotenoid derivative (abscisic acid). They can be classified as growth promoters which induce cell elongation, cell division, fruiting, flowering and formation of seeds (Examples -Auxins, Cytokinins and gibberellins) and plant growth inhibitors which cause the inhibition of growth in plants or cause abscissions or induce dormancy. Example abscisic acid. These hormones find application in plant tissue culture and horticulture sectors.

1.4.2 Plant Pathology

Phytochemical techniques are primarily important to the pathologist for the chemical characterization of phytotoxins and of

phytoalexins (products of higher plant metabolism formed in response to microbial attack). A range of different chemical structures are involved in both cases. The most familiar phytotoxins are lycomarasmin and fusaric acid, amino acid derivatives which are wilting agents in the tomato. Other toxins that have been isolated are glycopeptides, naphthaquinones or sesquiterpenoids. Some phytotoxins are chemically labile so that special precautions have to be taken during their isolation and identification.

1.4.3 Drug Discovery

Phytochemicals are a very important source of drugs, there are a number of phytochemicals that have medicinal values. Thousands of secondary metabolites were isolated and used as drugs such as digoxin which is used as cardiotonic, isolated from Digitalis purpurea (purple or common foxglove), Aescin which is used as anti-inflammatory, venotonic, and as anti-eadematous drug, isolated from Aesculus hippocastanum (horse chestnut). Ajmalicine, the indole alkaloids used for treatment of circulatory disorders was first isolated from Rauwolfia serpentina.

1.4.4 Plant Ecology

The interaction of the secondary metabolites in the ecosystem is of two types, plant-plant interaction and plant- animal interaction. In both cases there are analytical problems and difficulties because of the very limited amounts of biological material at the disposal of the phytochemist for instance, the analysing of an insect's different organs for the estimation of the phytochemicals that are stored in its body by feeding on a plant is often complicated and time consuming. The important compounds that are found to have been involved in these processes are alkaloids, cardiac glycosides, steroids and terpenes etc.

1.4.5 Palaeobotany

Phytochemistry is been used in the study of fossil plants and has been used to identify the partially degraded chlorophyll pigments in lignite deposits 50 million years old. Identification of terpenes in fossil resins and ambers has also yielded new data of considerable phylogenetic interest. There are many researches taken place in this field from early 1970's.

1.4.6 Plant Genetics

Phytochemistry has been providing the means of identifying anthocyanin, carotene pigments in different genotypes of the plants and has indicated the probable pathway of pigment synthesis in these organisms and can be mapped out by phytochemical analysis.

1.4.7 Plant Systematics

It is a hybrid between chemistry and taxonomy known as chemotaxonomy or biochemical systematics. It involves the analysis of the plant secondary metabolites and uses the information for the classification of the plants.

1.5. PSIDIUM GUAJAVA L. FRUIT

There are many plants which are locally available having medicinal properties. One such important medicinal plant is **Psidium guajava L**. It is a tropical tree that is native to South America and Brazil. It is popularly known as guava and in Malayalam it is called as 'perakka' and has been used traditionally as a medicinal plant. It belongs to the family of Myrtaceae, cultivated for its edible fruits. The common guava has quadrangular branchlets, oval shaped leaves about 7.6 cm (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. Guavas tend to be classified according to the colour of their flesh; either pink or white. The pink guavas are a rouge-fleshed,

tropical variety of psidium guajava. The pink guavas are the most aromatic and can range from pale pink to deep orange-magenta. Their colour is due to a naturally occurring organic pigment called carotenoid.





Kingdom:	<u>Plantae</u>
Division:	Magnoliophyta
Class:	Magnoliopsida
Subclass:	Rosidae
Order:	Myrtales
Family:	Myrtaceae
Subfamily:	Myrtoideae
Genus:	<i>Psidium</i> L.

The cultivation of guava is relatively easy as it thrives in a variety of soil and adapts to different climatic conditions. The fruits are also borne in a short period of time. All parts of this tree including leaves, bark and root have been used for treating many health related problems. *P. guajava* L. is consumed not only as food but also as folk medicine in subtropical areas all over the world due to its pharmacologic activities. It is well known that guava is frequently employed in numerous parts of the world for the cure of a lot of sickness like diarrhea, reducing fever,

dysentery, gastroenteritis, hypertension, diabetes, caries, pain relief and wounds. The countries which have a long history of using medicinal plants are also using guava at big level like Mexico, Africa, Asia and Central America. With its medicinal uses, it is also used as food and in the preparation of food products. Guava contains high content of organic and inorganic compounds like secondary metabolites e.g. antioxidant, polyphenols, antiviral compounds and anti-inflammatory compounds. Guava has a lot of compounds which have anti cancerous activities. It has a higher number of vitamins and minerals. Phenolic compounds like flavonoids also find an important place in guava. Lycopene and flavonoids are important antioxidants. They help in the cure of cancerous cells and help to prevent skin aging before time.

Guava contains high amount of antioxidants and anti-providing nutrients which are essential not only for life but also help to control the free radical activities. It also has a variety of phytochemicals which are beneficial for human health like diabetes, obesity and high blood pressure. Extracts of guava in water and organic solvents have a large quantity of antioxidants which can stop the oxidation reaction. Guava is highly rich in antioxidants which are helpful in decreasing the incidences of degenerative diseases such as brain dysfunction, inflammation, heart disease, cancer, arteriosclerosis and arthritis. In fruits, the most abundant antioxidants are polyphenols and ascorbic acid. The polyphenols are mostly flavonoids and are mainly present in glycoside and ester forms. The free elagic acid and glycosides of apigenin and myricetin are found to be present in guava.

The plant materials must be dried to remove the water content and thus after the removal of water they can be stored. This process should be done immediately as soon as the plants are collected so that it is prevented from spoilage. There are two methods in drying the plants. Natural process and artificial process. Natural process involves sun drying and shade drying. These processes take few weeks for complete drying of the moisture. This time depends on the temperature and humidity. Sun-drying involves the drying of plant samples under direct sunlight; they are left in sun light for few days until they get dried and brittle. The temperature is usually about 33±4°C and may take few days for complete drying.

Artificial process involves hot air oven drying and freeze drying. Artificial drying is done using the help of artificial driers. This process will reduce the time consumed to few hours or minutes. The common method used for the drying of medicinal plants is warm-air drying. This is done using the hot air oven on which warm air is blown. The drying must be done in lower temperature to prevent the thermolabile compounds being disintegrated. In freeze drying, an instrument called lyophilisor or freeze dryer is used to dry the plant samples. Both fresh and dried samples can be lyophilised and it is one of the modern methods and has very high advantages like, freezing the sample inactivates all enzymes and microbial activity, as well as hydrolytic compounds can also be stable.

After complete drying of moisture the plant samples are to be powdered for further analysis. There are different types of powdering. Grinding can be done by milling in an electric grinder or mixie or by a spice mill or can also be in mortar or pestle. Grinding increases the efficiency of the extraction due to increased surface area of the plants. Milling the plants into a fine powder is always ideal but if they are too fine this affects the solvent's flow and also produces more heat which could degrade some thermolabile compounds.

The solvent that is being used for the extraction process is very important in determining the biologically active phytochemicals from the

plants. These solvents must be less toxic, easy to evaporate in less heat, should preserve the compounds in it and should not dissociate it. The various solvents commonly used for extraction include water, alcohol, acetone, chloroform and ether.

There are several types of extraction processes involved in phytochemical analysis and some of the important extraction processes are homogenization, serial exhaustive extraction, soxhlet extraction, maceration, decoction, infusion, digestion, percolation and sonication.

Solvent extraction has been widely used to extract bioactive components from plants. Solvent extraction is a process designed to separate soluble antioxidant compounds by diffusion from a solid matrix (plant tissue) using a liquid matrix (solvent).

In this project, we have extracted powdered guava fruit using the solvents methanol, chloroform, ethyl acetate and hexane using homogenization method and phytochemical analysis was done using standard tests.

CHAPTER 2 AIM AND OBJECTIVES

AIM

To do the phytochemical analysis of Psidium *guajava L.* fruit.

OBJECTIVES OF THE PRESENT WORK

- 1. To extract the phytochemicals present in Psidium *guajava L.* fruit using methanol, chloroform, ethylacetate and hexane as solvents.
- 2. To do the phytochemical analysis of all the guava extracts.

CHAPTER 3

EXPERIMENTAL METHOD

MATERIAL

About 1 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 Kg of *Psidium guajava L.* was collected from Ernakulam district. The material was chopped into small pieces and allowed to sundry for a week. The dried material was crushed into fine particles using a mixer grinder, to make the extraction more easier. The powdered material was weighed (183g) and was subjected to extraction using methanol, chloroform, ethyl acetate and hexane as solvents. For this, 40g 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 shaking in a mechanical shaker for two 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 = 2.2547g

Weight of chloroform extract = 1.4609g

Weight of ethyl acetate extract = 1.5662g

Weight of hexane extract = 1.4439g

These weighed extracts were used for phytochemical analysis.

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 FeCl₃ solution were added to the extract. Identification is blackish blue colour.

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

Leibermann Burchard test: To the extract, few drops of acetic anhydride was added and mixed well. 1 mL of conc. H₂SO₄ 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 A and B. Fehling's A is a blue aqueous solution of copper(II) sulfate pentahydrate crystals, while Fehling's 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% HNO₃ is added. Greenish color is indication of the reaction.

CHAPTER 4

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS

A. METHANOL EXTRACT

<u>TEST</u>	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

<u>TEST</u>	<u>RESULT</u>
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

<u>TEST</u>	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

<u>TEST</u>	<u>RESULT</u>

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

Solvents like methanol, chloroform, ethyl acetate and hexane are used for the extraction of guava fruit. It is observed that the solvent used for extraction significantly influenced the amount of extract obtained from *Psidium guajava L.* fruit. The highest amount of guava extract is obtained when methanol is 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.

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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