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"Preparation and Identification of Chemical Constitute of Guava leaves by Maceration Process"

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Abstract: -

The American tropics' guava (Psidium guajava L.) leaf is used extensively for its flavour; odour, and taste. Its leaves have been reported to have antibacterial characteristics, and its fresh fruit and leaf tea have been used to treat a variety of medicinal conditions, including diabetes mellitus, diarrhoea, and dysentery. But given its rich composition, guavas may also be a good source of antioxidants for novel formulations intended for dermatological or cosmetic uses, which is the primary goal of this study. In this article, we outline the investigation of the antioxidant capacity and phytochemical makeup of a guava extract made with safe solvents with an eye towards biological uses. The findings demonstrate that the extract contains a wide variety of phenolic chemicals, including but not limited to quercetin, kaempferol, and schottenol. Based on data from the literature, every component of guava extract has biological effects, primarily related to antioxidant characteristics.

KEY WORDS: Plant Extract, Psidium Guajava

Introduction: -

Place of beginning the exotic leaf guava (Psidium guajava) is used. Its origin is a region that stretches from southern Mexico across Central and South America, although its exact location is unknown. Its cultivation has currently spread throughout a great deal of the world's tropical and subtropical regions. The primary natural source of many bioactive chemicals is plants. Thus, they are applied in the management of several illnesses. Psidium guajava, the botanical name for guava leaves, belongs to the Myrtaceae family, also known as the Myrtle family. Typically, guava trees are little, evergreen trees. Eastern medicine has long utilised guava leaves to treat diarrhoea and lessen food poisoning symptoms. It's a really traditional and unusual plant. It has medicinal uses. Guava leaves were utilised by people to treat wound healing, diabetes, stomach and intestinal conditions, and pain. Since ancient times, guava leaves have been utilised in traditional Eastern medicine. More recently, they have been well-known as a natural alternative medicine [1].

Plant leaves are the primary source of plant preparations used in food, drink, cosmetics, and medicine. The greatest accumulators of bioactive substances, such as secondary metabolites, among all plant parts are the leaves. The phytochemical profiles and biological activity of leaf extracts from a variety of grown plants were reported in a number of recent research. Owing to its many nutritional and therapeutic uses, guavas are a highly special and traditional plant. The advantages of guava leaf extracts, which are consumed as dietary supplements, have also been shown in numerous research. Additionally, research using animal models has demonstrated the effectiveness of guava leaf isolates as cytotoxic, antitumor, and anticancer drugs [2].

Consuming guava leaves at home can help reduce toothaches since they have anti-inflammatory and antibacterial properties that help fight infections and kill germs. It's also claimed that the juice of guava leaves can treat mouth ulcers and inflamed gums. Psidium guajava is an evergreen tree that can grow to a height of 6 to 25 feet. Vitamin A and C concentrations are higher in guavas. Additionally, guavas are an excellent source of pectin, a crucial dietary fibre. It is rich in carotenoids, fructose sugar, and flavonoids. Considering the basic information, key components, and typical applications of Psidium guajava (guava), Present research focuses on the phytochemistry and therapeutic potential of this helpful plant.

This tropical fruit is a great complement to a balanced diet because it is low in calories, high in fibre [3].
Adding guava leaves to boiling water to make a tea can have several health benefits, including decreasing cholesterol, avoiding diabetes, curing diarrhoea, the flu, and even showing signs of anti-cancer potential. The plant's branch network is widely dispersed. Its branches are primarily bent and have oppositely oriented leaves with tiny, 3–16 cm petioles. The leaves have distinct veins and are broad, with a vivid green hue. The shrub yields fragrant white blooms with incurved petals [4].
One of the main natural sources of many bioactive chemicals is plants. Since ancient times, folk medicine has used a range of plant preparations to treat a number of illnesses. Today, the cosmetic, pharmaceutical, and nutraceutical sectors are giving plant preparations and pure phytochemicals more attention [5].
Therefore, even though they are regarded as agricultural waste, plant leaves are a rich source of valuable non-pharmaceutical chemicals. Guava leaves (Pasidium guajava folium; GL) have an obtuse-type apex and are oval, elliptic, and dark green in colour. Guava leaves, pulp, and seeds are used to treat respiratory and gastrointestinal disorders, as well as to increase platelets in people suffering from dengue fever. The antispasmodic, cough sedative, anti-inflammatory, antidiarrheic, antihypertensive, anti-obesity, and antidiabetic qualities of GLs are also extensively utilised. Guavas are highly tasty and abundant in nutrients [6].

Definition of Guava :-

Any of a number of myrtle-family tropical American shrubs or small trees (genus Psidium) in particular: A shrubby trees (P. guajava) are commonly grown for their sweet, acidic yellow or pink meat and yellow-skinned fruit. The guava fruit, which is shaped resembles a round to pear. Guava leaves are a rich source of bioactive chemicals and numerous kinds of macro and micronutrients which encourage health.

History of Guava leaves:

It is thought that guava trees originated in Mexico, Central America, and the Caribbean before spreading to tropical and subtropical areas of Australia, Asia, and the Americas. Guava trees are currently grown in Brazil, China, Mexico, India, Nigeria, the Philippines, Southeast Asia, Pakistan, Bangladesh, and Brazil. The leaves of these trees are sold in specialised shops and internet retailers all over the world.

Objective: -

1. Studies have been conducted on the potential health advantages of guava leaves.
2. The biological properties of guava leaf extract, such as its antioxidant, anticancer, and antidiabetic properties, have been investigated.
3. Since ancient times, a variety of plant preparations have been used to treat a number of ailments.
4. Guava leaves are therefore regarded as agricultural trash even though they contain a wealth of valuable medicinal ingredients.
5. The researchers came to the conclusion that guava leaf extracts and essential oil are powerful anti-S. aureus agents and so represent a significant avenue for the discovery of novel antibacterial compounds.
6. Stating that, given their recognised anti-inflammatory properties, leaf extracts may be helpful in the treatment of acne.
7. Guava leaves are used to treat diabetes, ulcers, stomach and intestinal disorders, and discomfort.
8. The leaves are applied to treat hypertension.

Contents of Guava leaves: -

1) Moisture 82.47%

2) Ash-3.64%

3) Fat-0.06%

4) Protein-18.35%

5) Carbohydrate-12.35%

6) Ascorbic acid - 103mg

7) Gallic acid - 1717m

8) The bark contains 12–30% tannin, and according to one source, it also contains polyphenols, resin, and calcium oxalate crystals (tannin 27.4%). Roots also contain tannin. Roots also include sterols, gallic acid, and leuko cyanidins. Salts and carbohydrates coexist in large quantities. Its component is likewise tannic acid.

Chemical Composition of Guava leaves:

Iron, calcium, phosphorus, vitamin C, and iron are all present in guava fruit. Compared to the orange, it has higher vitamin C. Flavonoids, guaijavarin, quercetin, lyxopyranoside, arabopyranoside, oleanolic acid, and saponin are all present in the fruit. The main components of guava, ascorbic acid and citric acid, have significant anti-mutagenic properties. Fruit skins have extremely high levels of ascorbic acid, but heat can destroy them.

The carbonyl chemicals are responsible for the fruit's overpoweringly sweet scent. Terpenes, caryophyllene oxide are found in great quantities in guava fruit, and these compounds have a calming effect. The guava methanolic extract has a greater flavonoid concentration. Guava contains nine aromatic compounds, 25 esters, 13 alcohols, and 41 hydrocarbons. Fruit possesses titratable acidity and total soluble solids.

Guava also contains guajadial. α-pinene, limonene, β-pinene, isopropyl alcohol, menthol, terpenyl acetate, caryophyllene, longicyclene, and β-bisabolene are all found in leaves that contain essential oil. The guava leaves also contain oleanolic acid. Leaf content is high in caryophyllene (approximately 21.3%) and limonene (about 42.1%). Guava leaves contain a large number of volatile substances.

Benefits of Guava leaves: -

1. May Assist in Relieving Excruciating Menstrual Pain.
2. It's also believed that guavas' greater potassium and soluble fibre content promote heart health.
3. Furthermore, there is evidence linking guava leaf extract to lowered blood pressure, an increase in “good” HDL cholesterol, and a decrease in “bad” LDL cholesterol.
4. Guava leaves have been shown to reduce cholesterol.
5. Guava Leaves used for Reduction of Weight.
6. Guava Leaves Help People with Diabetes.
7. Guava Leaves Can Help With Dysentery and Diarrhoea
8. Guava Leaves Promote Better Digestion
9. Guava Leaves Offer Benefits Against Ageing
10. Guava Leaves for Black Spots and Acne Treatment.
11. Might Assist in Reducing Blood Sugar.
12. Might Improve Heart Health.
13. May be advantageous to your digestive system.
14. It Could Promote Weight Loss
15. Potential Anticancer Impact
16. Might Stimulate an Immune Response
17. Consuming Guavas Could Benefit Your Skin
18. In addition to other advantages, guava fruit and leaf extracts may improve your immune system, digestion, and heart health.
19. It makes Complexion better.

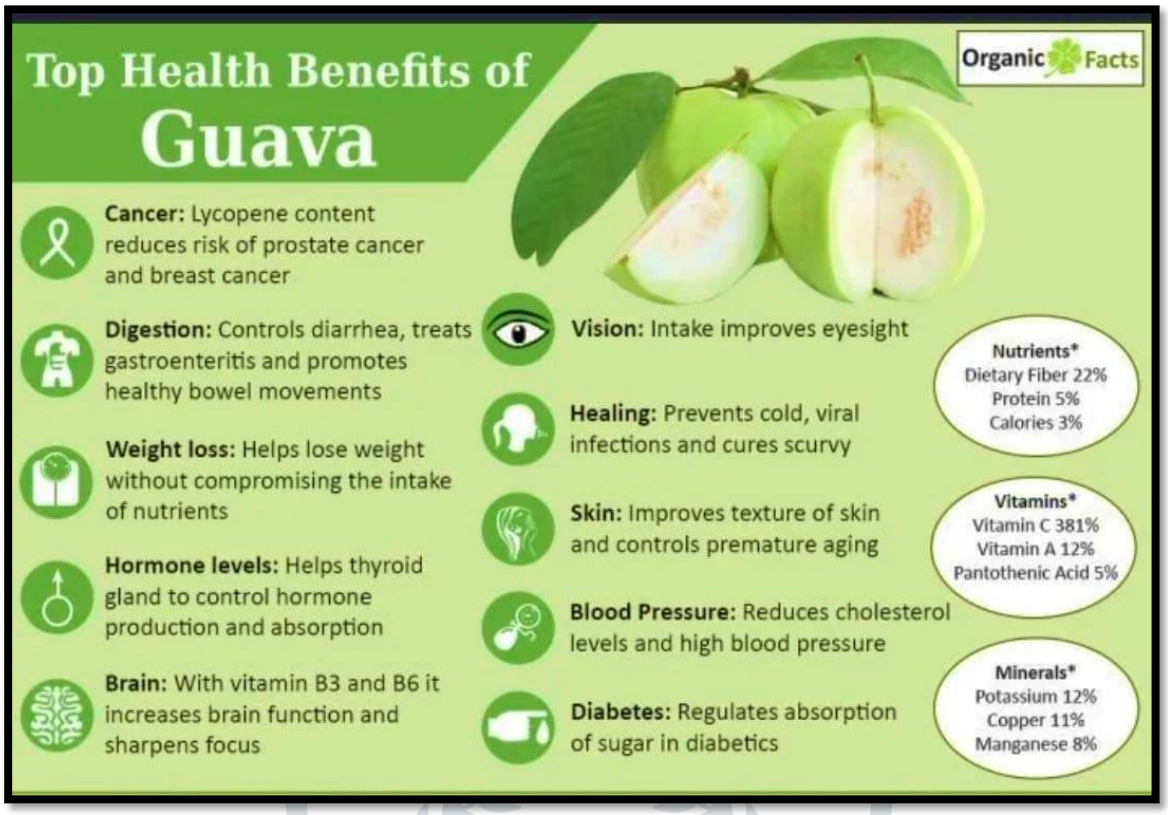

Fig 1. Benefits of Guava leaves

Uses of Guava leaves: -

1. Diarrhoea: Guava leaf extract does not appear to shorten the duration of diarrhoea or lessen pain when taken in conjunction with oral rehydration therapy (ORT). However, it might lessen the frequency of stomach ache.
2. Cramps during menstruation (dysmenorrhea). According to preliminary studies, guava leaf extract may somewhat lessen menstrual cramps.
3. A minor case of gingivitis, or gum disease. For gingivitis sufferers, rinsing the mouth with guava leaf extract may help lessen gum disease. However, it doesn't appear to lessen plaque.
4. Elevated blood pressure :- According to preliminary studies, individuals with high blood pressure can drop their blood pressure for 12 weeks by substituting high-fat diets with big amounts of guava every day.
5. Pain in the knees :- According to preliminary studies, individuals with knee pain may experience a slight reduction in pain and stiffness while consuming guava leaf extract.
6. Cancer: - A high licopene content lowers the risk of breast and prostate cancer.
7. Digestion: - Manage diarrhoea, treat gastrointestinal disorders, and encourage regular bowel movements.
8. Weight loss:- Aid in weight loss without sacrificing food intake.
9. Harmone level:- Assists the thyroid gland in regulating the synthesis and uptake of hormones.
10. Brain:- Focus is sharpened and blood functions are increased when combined with vitamins B3 and B6.
11. Vision:- Increased intake enhances vision.
12. Healing:- Scurvy is cured and viral infections are prevented.

13. Skin: - enhances skin structure and prevents early ageing.
14. Blood Pressure:- Lower blood pressure and cholesterol levels.
15. Diabetes:- Control the diabetic's sugar absorption.

Side Effects of Guava leaves: -

1. When consumed by mouth: Guava fruit is Probably safe to consume in food form.
2. When taken as a medication, guava fruit and leaf extract have the potential to be safe.
3. Regarding topical application: Guava leaf extract MAY BE SAFE when used as a skin or oral rinse. It could cause skin pain in certain persons.
4. Guava leaf extract may exacerbate eczema. Chemicals in guava leaf extract have the potential to irritate skin, particularly in those with eczema. Use guava leaf extract with caution if you have eczema.
5. Guava may help diabetics with blood sugar issues. If you use guava and have diabetes, be sure to closely monitor your blood sugar.
6. Surgery: The guava plant may reduce blood sugar. Theoretically, guava might make bleeding more likely or affect blood sugar regulation both before and after surgery. Give up taking guava medication at least two weeks prior to an anticipated surgery.

SCIENTIFIC CLASSIFICATION	
Kingdom	Plantae
Clade	Tracheophytes
Clade	Angiosperm
Clade	Eudicots
Clade	Rosids
Order	Myrtales
Family	Myrtaceae
Genus	Psidium
Species	P. Guajava
Binomial Name	
Psidium Guajava	

Table. Scientific Classification

Experimentation: -

a) Chemicals: -

Chloroform, Conc.H2SO4, ammonium hydroxide, methanol, formic acid, acetonitrile, ampicillin, glucose, phenol 5,5- dimethyl prolinr-N-oxide (DMPO), FeSO4, H2O2 , ethanol, acetoline.

Apparatus: -

Test tubes, conical flask, pipette, measuring cylinder, dropper, mortal pistal , sieve, filter paper,tripod stand, funnel.

b) Instruments: - The instrument used is Rotatory Evaporator.

c) Methods / Procedure Used :-

a. Gathering and processing sample
b. Preparation of Guava leaf extracts.
c. Phytochemical screening of Guava leaf extracts.
d. Determination of antibacterial activity.
e. Determination of antioxidants activity.

1) Sample Collection and Processing: -

The Specialty Plant House's guava trees provided the leaf samples that were taken from them. For extraction, random samples were gathered and placed in plastic zip-lock bags in the lab.

Fig No. 2. Sample Collection of Guava Leaves.

2) Extraction Methods Used on Guava:

After being cleaned with tap water and dried, the leaf samples were put in a blender to be ground into a powder. For the maceration extraction process, four solvents were organised in increasing polarity: boiling distilled water, n-hexane (>95%), methanol (>95%), and ethanol (>99.5%). A 20% concentration was achieved by adding the

leaf powder to each of the solvents. For three days at room temperature, the mixes were prepared in sterile 125 mL Erlenmeyer flasks covered in aluminium foil to prevent evaporation and light exposure.

3) Phytochemical Screening of Guava leaf Extract: -

Utilising the usual protocol, chemical assays were conducted with the extracts to screen for and identify bioactive chemical elements in the guava. With the exception of the saponin test, which used three millilitres of solvent extract, one millilitre of each solvent extract was used for analysis for each test.

4) Determination of Antibacterial activity: -

Antimicrobial susceptibility testing was done using the well-diffusion method according to standard of the National Committee for Clinical Laboratory Standards. Mueller Hinton II plates were used to analyse the plant extracts for the existence of antibacterial activity.

5) Determination of Antioxidants activity: -

The assays for reducing power, beta-carotene bleaching, and free radical (DPPH) scavenging were used to assess the antioxidant capacity of guava leaves essential oil (EO). In this case, ascorbic acid and butylated hydroxytoluene (BHT) were employed as common antioxidants. While suppressing DPPH radicals, guava leaf extract (EO) demonstrated concentration-dependent free radical scavenging activity while also acting as a mild antioxidant.

Procedure: -

Maceration of plant powder for three days. Filtration was done on the mixture after maceration. Plant material/crude plant extract (leaves) different solvent was used on the same plant sample. The same or different bioactive chemicals will be extracted using different solvents. Moreover, the solvent may have an impact on the quantity of these bioactive substances. The process of transferring solution into a clonal flask involves the use of a rotating evaporator. The heating bath, set at -40°, is the following phase. Next, activate the rotating evaporator. Next, attach the clonical flask to the rotating evaporator and activate the hoover cleaner. We must now gradually close the hoover. Next, activate Rotar. Then bubbling is seen. Next, the droplet is set. They transform into liquid by condensing. We can thus stop the rotavap as soon as your sample colour turns yellow or greenish.

Turn off the evaporator and halt the rotation. Open the hoover now and take out the clonal flask. Now that we have our plant extracts, we will load them into a coloured bottle, transfer them, and use HPLC and LC-MS to assist identify and quantify the molecule. We need to transfer them into amber vials after the rotary evaporator.

Preparation of guava leaf extract:

The 20 g of guava leaf powder had been prepared for 30 minutes at 90 °C in 100 mL of double-distilled water in a sterile 150 mL Erlenmeyer flask. For ten minutes, the mixture was centrifuged at 4000 rpm (Biswas et al. 2013). After being separated, the supernatant was kept for further research at 4 °C.

Fig 3. Organic Guava leaves

Fig. Process of guava leaves

Evaluation Parameters: -

1) Extraction procedure
2) Maceration
3) Extraction Yield
4) Total phenolic content (TCP) determination
5) Chromatographic separation and determination of phenolic compounds
6) Flavone and flavonol content

7) Flavanone and dihydroflavonol content

8) Stastical data

Extraction Procedure: -

20 gm of raw propolis were crushed while freezing. Double maceration (M1, M2), double microwave treatment (Mi1, Mi2), and double ultrasonication (U1, U2) were the methods used to carry out the extraction. Every extraction was carried out 3 times.

Maceration:

50 mL of 70% ethanol was used to dissolve 1 g of crushed propolis. An orbital shaker was used to agitate the solution for 24 hours at 250 rpm while it was at room temperature. Following that, the mixture was centrifuged for 10 minutes at 4000 rpm in order to separate the supernatant from the residue. Whatman 5 filter paper was used to filter the supernatant. Another extraction of the residue was performed using 50 millilitres of 70% ethanol. Following the collection of the two supernatants in a volumetric flask, the solution was diluted with 70% ethanol to a volume of 50 mL.

Extraction yield: -

The extraction yield was calculated in accordance with Popova et al. (2007) and expressed as balsam content (soluble ethanolic fraction). In an oven set at 60°C, 2 millilitres of ethanolic extract were evaporated to a constant weight.

Total Phenolic Content (TCP) Determination:

The TPC determination was performed using the Escriche and Juan-Borrás (2018) approach. After combining 100 µL of extract with 1900 µL of water in a tube, 100 µL of Folin-Ciocalteau reagent was added. 800 µL of 5% sodium carbonate (w/v) was added after two minutes. After keeping this solution in a water bath at 40 ° C for 20 minutes, the reaction was stopped by quickly chilling the tube with crushed ice. At 760 nm, the determination was made. The findings were reported as mg of pinocembrin: galangin (1:1, w / w) / g of propolis and mg of gallic acid / g of propolis. Methanol was added to the flask to create the ethanolic solution. At 486 nm, the determination was made. The content was stated as mg of propolis and mg of pinocembrin. Each experiment was performed 3 times.

Chromatographic Separation and Determination of phenolic compounds: -

Using the technique outlined by Coneac et al. (2008) and Oroian and Ropciuc (2017), the phenolic components were isolated and measured. Utilised was a High-Performance Liquid Chromatography (HPLC) system from Shimadzu in Kyoto, Japan, which included an SPD - M - 20A diode array detector, SIL 20A auto samples, CTO - 20AC auto sampler, and LC - 20 AD liquid chromatograph. The Zorbax SP - C18 column, measuring 150 mm in length, 4.6 mm in diameter, and 5 µm in particle size, was used for the separation process. To halt the reaction, the phenolics tube was quickly chilled using crushed ice. At 760 nm, the determination was made. The findings were reported as mg of pinocembrin: galangin (1:1, w / w) / g of propolis and mg of gallic acid / g of propolis. Each experiment was run in triplicate, with the ethanolic solution serving as a blank.

Flavone and flavanol content: -

The Popova et al. (2007) method was used to measure the flavone and flavanol content. In a 50 mL volumetric flask, 2 mL of extract, 20 mL of methanol, and 1 mL of AlCl3 (5%), were combined. The flask was then filled with methanol following a previous homogenization step. At 425 nm, the determination was made. The information was presented as mg propolis / g galangin. Each experiment was performed three times.

Flavanol and di hydroflavonal content: -

The method developed by Popova et al. (2017) was utilised to quantify the content of flavanone and dihydroflavonol. 1 mL of extract was combined with 2 mL of DNP solution (one gramme of dinitrophenylhydrazine (DNP) in two millilitres of 96% H2SO4, diluted to one hundred millilitres with methanol); the mixture was then heated to 50 °C for fifty minutes. Following cooling, 10% KOH in methanol was used to dilute the liquid to 10 mL. In a 50 mL flask, 1 mL of the solution was combined with 20 mL of methanol, and the flask was then filled with methanol following homogenization. For every constituent, (n = 5) displayed correlation coefficients (R2) greater than 0.99. The quantitative findings were given as milligrammes of the chemical per gramme of propolis.

Statistical analysis: -

ANOVA and Principal Component Analysis (PCA) were the statistical analyses carried out with the Unscrambler X 10.1 software system (Camo, Norway). ANOVA, or one-factor analysis of variance, was used to evaluate the data related to each variable. Statistical significance was set at a = 0.05, and multiple comparisons were carried out using the Fisher ratio (F) and the least significant difference test (LSD).

Result: -

It is normal practice to extract polyphenols from various goods using hot or cold solvents through liquid extraction; aqueous mixes containing ethanol, methanol, acetone, etc. are the most commonly used solvents for this purpose (Antolovich et al., 2000). Because propolis is used as a macerated product in ethanol solutions in "herbal medicine," the traditional method of preparing propolis was compared to two faster extraction methods in this article.

Conclusion: -

According to our research, Psidium guajava L.'s hydroethanolic extract contains a range of compounds that have been shown to have potential health benefits. It should be noted that substances found in guava extract prepared with safe solvents showed antioxidant activity similar to that of the enzyme superoxide dismutase. Moreover, it is believed that the extract maintains its stability at temperatures below 60 ºC, which makes it a good alternative for antioxidant components included in cosmetics and other health-care products that aim to protect the skin from the sun.

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