Monograph of *Psidium guajava* L. leaves

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**ABSTRACT**

The following article is a detailed monograph of *Psidium guajava* L. leaves containing all description of the leaves concerning its botany, chemistry and activity.

**Key words:** *Psidium guajava* L. Leaf, Guava leaf, Guava monograph

**INTRODUCTION**

Guava leaf is a main ingredient in many herbal mixtures marketed in the Egyptian market and worldwide. It has been stated very often that the lack of standardization of herbal remedies and plant medicines is holding back the use of medicinal plants in the modern system of medicine, therefore a growing demand for the establishment of a system of standardization for every herbal preparation in the market is required and the buildup of a monograph containing all information required about the herbal drug is necessary.

**MATERIALS AND METHODS**

**Plant materials**

*Psidium guajava* L. leaf used for the phytochemical investigation and the antimicrobial studies was collected from El tahrir (Alexandria – Cairo road at kilo 47). Samples of *Psidium guajava* L. leaf used for the comparative studies were collected from different localities in Egypt as indicated in the appendices below.

**Reference materials**

Quercetin, glucose, galactose, L-arabinose and D-arabinose were supplied by E. Merck (Darmstadt, Germany). Quercetin-3-β-D-glucoside and quercetin-3-β-D-galactoside were supplied by Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

**Solvents**

The solvents used in this work; petroleum ether (40-60°C), ether, chloroform, ethyl acetate, butanol, methanol and ethanol were of analytical grade.

**Chromatographic requirements**

Precoated HPTLC plates (silica gel 60F-254) with adsorbent layer thickness 0.2 mm, E- Merck, Darmstadt, Germany.

**HPTLC equipments**

Sample solutions were applied by means of a Camag (Wilmington, NC) Linomat IV automated spray-on band applicator equipped with a 100-µl syringe. Zones were quantified by linear scanning at 254 nm with a Camag TLC Scanner 3 with a deuterium source in the reflection mode. The peak areas of the chromatograms’ spots were determined using CATS TLC software and winCATS TLC software (version 4.X).

**NOMENCLATURE**

**Botanical Nomenclature**

*Psidium guajava* L.

**Botanical Family**

Myrtaceae

**Definition**

Guava consists of the dried leaves of *Psidium guajava* L. family Myrtaceae.

**Common Names**

Guava (Egypt, USA, latin America, Asia, Africa), guyaba (Cuba), guayaba (Guatemala, Nicaragua, Paraguay), amrood (India).[^4]

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[^4]: Source: [Common Names of Plants](https://www.worldfloraonline.org/)

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HISTORY

Guava is native to the American tropics. The English name guava probably came from the Haitian name, guajaba. The Spanish explorers took the guava to the Philippines and the Portuguese disseminated it from the Philippines to India. Then it spreaded easily and rapidly throughout the tropics because of the abundance of seeds with long viability and became naturalized to the extent that people in different countries considered the guava to be indigenous to their own region. It is now also grown in the subtropics.\(^2\)

IDENTIFICATION

Botanical Identification

Macroscopic Identification (Figures 1, 2)

Habit: Medium sized tree with thin smooth, patchy, peeling whitish brown bark,\(^1,5\) but under high moisture conditions, grows to 6-9 m in height.\(^2\)

Root: Tap, branched.\(^1\)

Stem: Erect, aerial, woody, branched, cylindrical, solid, glabrous, white or brown.\(^1\)

Leaves: Simple, alternate, short-petiolate, extipulate, gland dotted, aromatic, entire, apex ovate.\(^1\)

The leaves are 10-12 cm in length, 5-7 cm in width. They have a green colour and leathery texture.

The lamina is green, simple with acute apex, entire margin, symmetric – asymmetric base. The venation is pinnate reticulate. The midrib is more prominent on the lower surface. The upper surface is slightly paler in colour than the lower surface. Both surfaces are pubescent.

The petiole is short (0.3-0.4 in length and 0.2-0.3 in diameter), green in colour, showing a groove on the upper surface and hairy.

Inflorescence: Cymose, solitary and axillary.\(^1\)

Flower: Flowers occur singly or in clusters of 2 to 3 at the leaf axils of current and preceding growth.\(^5\) Flowers are Pedicellate, bracteate, complete, hermaphrodite, actinomorphic, epigynous, pentamorous, cyclic, white.\(^1\)

Calyx: 4-5 sepals,\(^3,5\) gamosepalous, reduced, fused.\(^1\)

Corolla: 4-5 petals,\(^3,5\) gamopetalous, forming a corolla cap covered by calyx cap, the so formed calyptra falls off when flower opens, superior.\(^1\)

Androecium: Stamens indefinite, polyandrous, attached on the rim of calyx cap, folded inwards in bud condition,

anthers dorsifixed, versatile, bicelled, small, dehisce longitudinally.[1]

Gynoecium: 4-5 carpels, style, stigma minute, quadra-pentalocular, [3,5] syncarpous, ovary inferior, axile placentation.[1]

Fruit: The fruit is a many-seeded berry, varying in size from 2.5 to 10 cm in diameter. The shape can be globose, ovoid, elongated or pear-shaped. Skin colour is yellow when ripe but flesh colour may be pink, salmon, white or yellow. Skin texture may be smooth or rough. The inner wall of the carpels is fleshy and of varying thickness and seeds are embedded in the pulp. Flavour and aroma vary widely among seedling populations.[2]

Microscopic Identification (Figures 3, 4, 5, 6, 7)

1. The leaf lamina
A transverse section in the leaf (Figures 3, 4, 5) shows upper and lower epidermises, hypodermis and a dorsiventral mesophyll. The palisade tissue consists of two rows of columnar cells and is discontinuous in the midrib region. The midrib is more prominent in the lower side and shows bicollateral arc-shaped vascular bundle which is surrounded by two arcs of pericyclic fibers above and below the bundle.

The epidermises: The upper epidermis of the lamina (Figure 6 u. ep.) consists of polygonal, nearly isodiametric or slightly elongated cells with straight, slightly thick anticlinal walls covered with thin smooth cuticle and devoid of stomata. The lower epidermis (Figure 6 l. ep.) consists of polygonal, nearly isodiametric cells with straight walls and covered with thin, smooth cuticle. Stomata are present on the lower epidermis only. They are oval in shape and of the paracytic type.

Trichomes (Figure 6 t) are present in both epidermises, being more numerous in the upper epidermis. They are non-glandular unicellular wooly straight, curved or twisted, covered with thick smooth cuticle, arising from a cicatrix surrounded by radiating epidermal cells. Measurements of upper and lower epidermises are shown in table 1.

Figure 3: Diagram of a transverse section of Psidium guajava L. leaf (x).
c. collenchyma; c. cl. calcium oxalate cluster crystal; c. p. calcium oxalate prism; hyp. hypodermis; l. ep. lower epidermis; o. gl. oil gland; p. palisade; p. f. pericyclic fibres; ph. phloem; t. trichome; u. ep. upper epidermis; xy. xylem.
The hypodermis: The hypodermis consists of 2-3 layers of collenchymatous cells.

The mesophyll: It is dorsiventral, the palisade is discontinuous over the midrib region and is formed of 2-3 rows of columnar cells, having straight anticlinal walls and containing green plastids. The spongy tissue is formed of 5-8 rows of more or less spherical cells and containing green plastids. Small vascular bundles and shizogenous and schizolysigenous oil glands may be embedded within the spongy tissue.

The midrib: The cortical tissue of the midrib consists of 2-3 rows of collenchymatous cells beneath the upper epidermis and 3-4 rows abutting the lower epidermis followed by 3-4 layers of thin walled parenchyma with distinct intercellular spaces. The parenchyma cells contain few prisms and numerous clusters of calcium oxalate. Shizosenous and schizolysigenous oil glands are also present. The pericycle is formed of two arcs of lignified fibres above and below the vascular bundle. The fibres (Figure 6.f) are long (80.45-91.95 µm in length and 6.89-17.24 µm in width), fusiform with wavy lignified walls, rather wide lumen and more or less acute apices.

The vascular tissue: It consists of an arc-shaped bicolateral vascular tissue which is formed of xylem and two arcs of phloem above and below it. The xylem consists of lignified spiral, annular vessels and wood parenchyma. The phloem consists of sieve elements and phloem parenchyma.

2. The leaf petiole
A transverse section in the petiole (Figure 7) is planoconvex, slightly grooved on the upper part. It is formed of an epidermis followed by the cortex which is formed of parenchymatous tissue containing prisms

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**Figure 4:** Detailed transverse section of the midrib of *Psidium guajava* L. leaf (x).

- c. collenchyma; c. cl. calcium oxalate cluster crystal; c. p. calcium oxalate prism; hyp. hypodermis; l. ep. lower epidermis; o. gl. oil gland; p. palisade; p. f. pericyclic fibres; ph. phloem; t. trichome; u. ep. upper epidermis; xy. xylem.

**Figure 5:** Detailed transverse section of the lamina of *Psidium guajava* L. leaf (x).

- hyp. hypodermis; l. ep. lower epidermis; sp. t. spongy tissue; u. ep. upper epidermis.
and clusters of calcium oxalate and one row of shizogenous and shizolysogenous oil glands situated in the outer region of the cortex. The cortex is traversed by crescent shaped vascular tissue similar to that present in the leaf.

3. Powdered leaf (Figure 6)

**Characteristic Features** The dried powdered leaf is light green in colour with an aromatic taste and a characteristic odour. It is characterized microscopically by the following elements:

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**Figure 6:** Elements of *Psidium guajava* L. leaf (x).
- cic. cicatrix; c. cl. calcium oxalate cluster crystal; c. p. calcium oxalate prism; f. fibre; l. ep. lower epidermis; o. gl. oil gland; s. stomata; t. trichome; u. ep. upper epidermis; v. vessel.

**Figure 7:** Diagram of a transverse section of *Psidium guajava* L. leaf petiole(x).

c. cl. calcium oxalate cluster crystal; c. p. calcium oxalate prism; ep. epidermis; o. gl. oil gland; p. f. pericyclic fibers; ph. phloem; t. trichome; xy. xylem.

**Table 1: Measurements of upper and lower epidermises.**

<table>
<thead>
<tr>
<th></th>
<th>Length (µm)</th>
<th>Width (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper epidermis</td>
<td>17.2 - 23</td>
<td>16.1 - 17.2 - 18.4</td>
</tr>
<tr>
<td>Cells of cicatrix</td>
<td>13.8 - 25.3</td>
<td>11.5 - 17.2 - 23</td>
</tr>
<tr>
<td>Lower epidermis</td>
<td>15.3 - 16.1</td>
<td>9.8 - 10.3 - 10.6</td>
</tr>
<tr>
<td>Stomata</td>
<td>11.5 - 12</td>
<td>9 - 9.8 - 10.3</td>
</tr>
<tr>
<td>Subsidiary cells</td>
<td>19.5 - 20.7</td>
<td>10.3 - 11.5 - 12.6</td>
</tr>
</tbody>
</table>

1. Numerous unicellular wooly large characteristic nonglandular trichomes, strongly thickened with smooth cuticle.
2. Numerous fragments of broken nonglandular trichomes.
3. Fragments of upper epidermis which consists of polygonal isodiametric cells with straight walls, covered with smooth cuticle showing cicatrix and devoid of stomata. Few cells bear the previously described trichomes.
4. Fragments of lower epidermis which consists of polygonal isodiametric cells with straight walls, covered with smooth cuticle showing paracytic stomata and bearing numerous trichomes.
5. Fragments of the leaf lamina in sectional view.

**Figure 8:** Typical ethyl acetate pattern of guava leaf.

A: Ethyl acetate extract of guava leaf, 1: Quercetin, 2: Quercetin-3-O-α-L-arabinofuranoside, 3: Quercetin-3-O-β-D-arabinopyranoside, 4: Quercetin-3-glucoside and 5: Quercetin-3-galactoside.

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6. Fragments of cortical parenchyma showing shizogenous and shizolysigenous oil glands, numerous clusters and few prisms of calcium oxalate.

7. Numerous clusters and few prisms of calcium oxalate.

8. Fragments of lignified pericyclic fibres with wavy walls and acute apices.


### Identification by TLC

Extract 2 g powdered Guava leaves with 20.0 ml of boiling water for 10 minutes, cool and filter. Concentrate 10 ml of this filtrate to about 5 ml then shake with 10 ml of ethyl acetate. Separate the ethyl acetate layer, dry and redissolve in 2 ml of methanol. Examine by TLC using the references mentioned below. For TLC examination use silica gel G layers, 0.25 mm thick and ethyl acetate - methanol - water -acetic acid (100:2:1:4 drops) as developing solvent and ammonia as revealing reagent. Detect the resolved bands by UV. The Following figure is the typical ethyl acetate pattern.

### CONSTITUENTS

#### 1. Flavonoids

i. Quercetin and its glycosides:
A summary of the isolated quercetin and its glycosides are given in table 2.

ii. Other flavonoids include morin-3-O-α-L-lyxopyranoside,[10] morin-3-O-α-L-arabinopyranoside,[10] kæmpferol,[7] luteolin-7-O-glucoside[7] and apigenin-7-O-glucoside.[7]

#### 2. Tannins

i. Amritoside (ellagic acid 4-gentiobioside).[11]

ii. Guavin B(structure I)[14] and Guavins A, C and D (structuresII, III, IV respectively).[15]

iii. Antidiabetic agents: Isostrictinin (V),[14] Strictinin (VI),[16] Pedunculagin (VII).[16]

iv. Antimutagenic agents: (+)-gallocatechin (a bio-

#### 3. Isoprenoids

i. Monoterpenes:

ii. Terpenoids:
A summary of the isolated triterpenoidal compounds are given in table 4.

### Table 2: Quercetin and its glycosides of guava leaves.

<table>
<thead>
<tr>
<th>Flavonoid</th>
<th>R₁</th>
<th>R₂</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quercetin</td>
<td>H</td>
<td>H</td>
<td>7, 8, 9, 10, 11</td>
</tr>
<tr>
<td>Avicularin: Quercetin 3-O-L-arabinofuranoside</td>
<td>L-arabinofuranose</td>
<td>H</td>
<td>9</td>
</tr>
<tr>
<td>Guaiavener: Quercetin 3-O-α-L-arabinopyranoside</td>
<td>α-L-arabinopyranose</td>
<td>H</td>
<td>8, 9, 10, 11</td>
</tr>
<tr>
<td>Isoquercetin: Quercetin 3-O-β-D-glucoside.</td>
<td>β-D-glucose</td>
<td>H</td>
<td>8, 12</td>
</tr>
<tr>
<td>Hyperin: Quercetin 3-O-β-D-galactoside.</td>
<td>β-D-galactose</td>
<td>H</td>
<td>8</td>
</tr>
<tr>
<td>Quercitin: Quercetin 3-O-β-L-rhamnoside.</td>
<td>β-L-rhamnose</td>
<td>H</td>
<td>7, 8, 12</td>
</tr>
<tr>
<td>Quercetin 3-O-β-D arabinopyranoside</td>
<td>β-D-arabinopyranose</td>
<td>H</td>
<td>13</td>
</tr>
<tr>
<td>Quercetin 3-O-gentiobioside</td>
<td>Gentiobiase</td>
<td>H</td>
<td>8</td>
</tr>
<tr>
<td>Quercetin 4'-glucuronide</td>
<td>H</td>
<td>Glucuronic acid</td>
<td>12</td>
</tr>
</tbody>
</table>
intraperitoneal injection of the extract. The LD₅₀ of guava leaf extract was more than 5 g/kg. These results suggested that guava leaf extract is recommended as a cough remedy. [30] Meanwhile a recent study conducted on the Egyptian plant showed that the alcoholic extract (in a dose starting from 4 µg/ml), the aqueous extract (from 8 µg/ml), the ethyl acetate extract (from 6 µg/ml), the essential oil (16 µg/ml) as well as quercetin (30 µg/ml) produces a significant drop in contractile

PHARMACOLOGICAL ACTIONS OF P. GUAJAVA L. LEAF

1. Anticough action
Guava leaf has been used in Bolivia and Egypt for a long time to treat ailments including cough and pulmonary diseases. [29] The aqueous extract decreased the frequency of cough induced by capsaicin aerosol within 10 minutes after

Table 3: Tannins isolated from guava leaves.

<table>
<thead>
<tr>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>COCH₃</td>
<td></td>
</tr>
<tr>
<td>R'</td>
<td>H</td>
<td>OH</td>
</tr>
</tbody>
</table>

(V): R¹ = (β)-OG, R² = R³ = H
(VII): R¹ = OH, R² – R³ = (S)-HHDP (hexahydroxydiphenoyl)
### Table 4: Terpenoids previously isolated from guava leaves.

<table>
<thead>
<tr>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R5</th>
<th>R6</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OH</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>-OAc</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>Guavanoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
<td>R</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>Guavacoumaric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OMe</td>
<td>R</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>26</td>
</tr>
<tr>
<td>Guajanoic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>26</td>
</tr>
<tr>
<td>Ursolic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>2α-hydroxyursolic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>-OH</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>Asiatic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OH</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>Jacoumaric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>OH</td>
<td>CH₃-</td>
<td>CH₃-</td>
<td>H</td>
<td>CH₃-</td>
<td>25</td>
</tr>
<tr>
<td>Isoneriucoumaric acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Guajavanoic acid**

**Guajavolide**

**Guavenoic acid**
response of isolated guinea pig trachea treated with histamine (2 µg/ml), acetyl choline (1 µg/ml) or serotonin (1 µg/ml). This study concluded that the extracts as well as the essential oil are safe for use as anti-cough concerning their effect on isolated trachea, their smooth muscle relaxant and their anti-inflammatory effects. Large doses may inhibit the ventricular contraction of the heart as tested on isolated rabbit heart.[7]

Moreover the high percentage of essential oil (0.46%) and its broad antimicrobial activity,[28] may be beneficial in cough treatment.[7]

2. Spasmolytic activity
Several researches assure that guava leaf extracts possess a spasmolytic activity[7,9,31-36] which is mainly attributed to the polyphenolic fraction[36] and is due to the aglycone quercetin (guava leaf is a rich source of quercetin glycosides which are hydrolyzed by the gastrointestinal fluid to give the aglycone quercetin).[8] Quercetin produces smooth muscle relaxation on isolated guinea pig ileum previously contracted by a depolarizing KCl solution and also inhibits intestinal contraction induced by different concentrations of Ca2+.[31]

Furthermore, the study conducted on the Egyptian plant showed that the alcoholic extract (4 µg/ml), the aqueous extract (8 µg/ml), the ethyl acetate extract (6 µg/ml), the essential oil (16 µg/ml) as well as quercetin (30 µg/ml) produced a significant muscle relaxant effect on isolated guinea pig ileum and rabbit intestine previously treated with histamine (2 µg/ml).[7]

Asiatic acid isolated from guava leaf also showed dose-dependant (10-500µg/ml) spasmolytic activity in spontaneously contracting isolated rabbit jejunum preparations.[25]

3. Antibacterial activity
A summary of the antibacterial studies previously done for the leaves and its fractions are given in table 5. Flavonoids morin-3-O-alpha-L-lyxopyranoside and morin-3-O-alpha-L-arabopyranoside were recently isolated from the leaves and have MIC of 200 µg/ml for Salmonella enteritidis, and 250 µg/ml and 300 µg/ml against Bacillus cereus, respectively.[10]

4. Antiamoebic activity
The leaf possesses antiamoebic activity which is concentrated in the polyphenolic fraction.[36]

5. Antifungal activity
P. guajava L. leaf extracts possess high antifungal activity[13,43,44] (the hot water extract and the methanol extract were measured for their antifungal activity against Arthrinium sacchari M001 and Chaetomium funicola M002 strains).[44]

6. Antidiarrheal activity
P. guajava L. leaf has a long history of use as an antidiarrheal agent.[6,8] This activity could be explained through understanding the spasmolytic, antibacterial and antiamoebic activities, together with the following findings:

a. Quercetin was found to reduce the capillary permeability in the abdominal cavity.[34]

b. The alcoholic extract of the leaves possesses a morphine-like inhibition of acetyl choline release in the coxially stimulated ileum, this morphine-like inhibition was found to be due to quercetin.[32]

c. The aqueous extracts produce a dose-related antidiarrheal effect. A dose of 0.2 ml/kg fresh leaf extract produced 65% inhibition of propulsion (inhibition of microlax-

### Table 5: Antibacterial studies of Psidium guajava L. leaf.

<table>
<thead>
<tr>
<th>Leaf extract</th>
<th>Activity</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous</td>
<td>In vitro</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ß-streptococcus group A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 enterobacteria pathogenic to man (benefit in treatment of gastrointestinal disorders)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella paratyphi (causative agent for enteric fever)</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>in mice infected by H. strep &quot;Richards&quot;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella typhi infection in wistar rats (causative agent for enteric fever)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Causative agent for desentry (Shigella dysenteriae, Shigella flexneri and Shigella sonnei)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and cholera (Vibrio cholerae).</td>
</tr>
<tr>
<td>Alcoholic</td>
<td>In vitro</td>
<td>Neurospora crassa and 20 other clinically important bacteria</td>
</tr>
<tr>
<td></td>
<td>In vivo</td>
<td>in mice infected by H. strep &quot;Richards&quot;</td>
</tr>
<tr>
<td>50% ethanolic</td>
<td>In vitro</td>
<td>E. coli (constitute a feasible treatment option for diarrhea caused by E. coli or by S. aureus-produced toxins)</td>
</tr>
<tr>
<td>Methanolic</td>
<td>In vitro</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ß-streptococcus group A</td>
</tr>
<tr>
<td>Chloroformic</td>
<td>In vitro</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ß-streptococcus group A</td>
</tr>
<tr>
<td>Acetonic</td>
<td>In vitro</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>E. coli</td>
</tr>
<tr>
<td>Essential oil</td>
<td>In vitro</td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella sp.</td>
</tr>
</tbody>
</table>
induced experimental diarrhea with narcotic like extracts of *Psidium guajava* leaf in rats.\[^{45}\]

d. Oral administration of the methanol extract of the leaves reduces intestinal transit time and prevents castor oil-induced diarrhea in mice.\[^{60}\]

e. Another study showed that the aqueous extract of the leaves significantly retarded the propulsion of charcoal meal and significantly inhibited the PGE\(_2\)-induced enteropooling.\[^{47}\]

f. A recent study suggested that the antidiarrhoeal activity is through the inhibition of intracellular calcium release.\[^{48}\]

### 7. Anticestodal activity

The leaf extract of *P. guajava* possesses anticestodal efficacy. Study supports its folk medicinal use in the treatment of intestinal-worm infections in northeastern part of India.\[^{49}\]

### 8. Antidiabetic, hypoglycemic and anti-hyperlipidemic activities

Guava leaf has been used for the purpose of medical treatment of diabetes mellitus among people for many years.\[^{50}\]

a. Reduction of postprandial blood glucose elevation: Oral administration of a 50% ethanolic extract of the leaves and/or n-butanol soluble fraction from the ethanolic extract was found to inhibit the hyperglycemia in alloxan-induced diabetic rats. The active principles in the ethanolic and n-butanol extracts were identified as the tannins isostictinin, strictinin, and pedunculagin,\[^{10}\] while effective oral dose of the water extract which showed statistically significant hypoglycemic activity on alloxan-induced diabetic rats; in both acute and sub-acute tests was 250 mg/kg.\[^{61}\]

b. Inhibition of alpha – glucosidase enzymes: Guava tea manufactured from hot water extract of the leaves, have been confirmed to inhibit the activities of carbohydrate-degrading enzymes and to suppress the postprandial blood glucose level of human subjects.\[^{53}\]

Single ingestion of guava extract can reduce postprandial glucose elevation via the inhibition of alpha-glucosidase in mice and human subjects with or without diabetes. Furthermore, it was found to have milder activity than voglibose.\[^{53}\]

c. Improvement of diabetes symptoms, hyperlipidemia, hypercholesterolemia and hypoadeponectinemia. It was shown that the consecutive ingestion of Guava Leaf Tea together with every meal improves not only hyperglycemia but also hyperlipidemia and hypercholesterolemia in pre-diabetic and diabetic patients with or without hyperlipidemia. The consecutive ingestion also ameliorates high blood cholesterol level in subjects with hypercholesterolemia or borderline hypercholesterolemia.\[^{55}\]

The leaf extract is also claimed as lipase inhibitors inhibiting carbohydrate absorption and preventing obesity, heart disease and atherosclerosis.\[^{54}\]

Guava leaf extracts are potent antiglycation agents, which can be of great value in the preventive glycation-associated complications in diabetes.\[^{55}\]

Thus, it is suggested that guava may be employed to improve and/or prevent the disease of diabetes mellitus.\[^{53,56}\]

### 9. Antioxidant activity

Guava fruit is a suitable source of natural antioxidants. Peel and pulp could also be used to obtain antioxidant dietary fiber (AODF), a new item which combines in a single natural product the properties of dietary fiber and antioxidant compounds.\[^{57}\] The fruit is preferable as a dietary supplement for the prevention of atherosclerosis due to its content of polyphenols.\[^{58}\]

Meanwhile the leaf was proven to have strong antioxidant activity,\[^{59,60}\] since it possesses strong DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity, potent inhibitory activity of lipid peroxidation and strong inhibition against oxidative cell death (H\(_2\)O\(_2\)-induced oxidative cell death)\[^{59}\] and therefore guava could be used to extend the shelf life of foodstuffs, to reduce wastage and nutritional losses by inhibiting and delaying oxidation. As a conclusion, supplementing a balanced diet with guava leaf extracts may provide health-promoting effects.\[^{60}\]

### 10. Cardioprotective effects of *Psidium guajava* L. leaves extract against ischemia-reperfusion injury in perfused rat hearts

*P. Guajava* L. possesses cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts, primarily through their radical-scavenging actions. The extract significantly attenuates ischemic contracture during ischemia and improves myocardial dysfunction after reperfusion. Quercetin and gallic acid also exerted similar beneficial effects.\[^{62}\]

The study performed lately on the Egyptian plant showed that the alcoholic extract (400 µg/ml), aqueous extract (550 µg/ml), ethyl acetate (500 µg/ml) and essential oil (700 µg/ml) of the leaves inhibit the ventricular contraction of isolated rabbit heart. Isolated quercetin exhibits a dose related inhibition of ventricular contraction from
100 µg/ml. Extracts of the leaf depress myocardial inotropism.\cite{63}

Aqueous leaves extract of *Psidium guajava* significantly and dose-dependently (0.25-2 mg/ml) contracted aorta rings.\cite{64}

11. **Antimutagenic activity**

*P. guajava* L. leaves possesses antimutagenic activity,\cite{65,66} the leaves contain flavonoids which may be responsible for this activity and probably the anticarcinogenic properties of the leaves.\cite{65}

(+)-gallocatechin isolated from the methanol extract of guava leaves was found to be a bio-antimutagenic compound against UV-induced mutation in *Escherichia coli*.\cite{67}

Lately cytotoxic phenylethanoid glycosides were isolated from the seeds.\cite{68}

12. **Relative retroviral reverse transcriptase inhibitory activity**

At a concentration of 125 µg/ml, hot water extract showed 61% a relative retroviral reverse transcriptase inhibitory activity.\cite{69}

13. **Depressant activity on central nervous system**

The methanol, hexane, ethyl acetate extracts of the leaves and the isolated sesquiterpenes, especially caryophyllene oxide and α-selinene, which were by far the largest single components, exhibit a CNS depressant activity by potentiating the phenobarbitone sleeping time in mice.\cite{18,46,70}

The essential oil and the aqueous extracts produce a moderate tranquilizing effect while ethyl acetate and alcoholic extracts are more active.\cite{71}

14. **The antinociceptive/analgic activity**

The hexane, ethyl acetate, methanol extracts of the leaves as well as the leaf essential oil were demonstrated dose-dependent antinociceptive effects.\cite{46,70,71} The methanol extract also exhibits antipyretic effect,\cite{46} meanwhile the aqueous extract was found to possess analgesic activity.\cite{72}

15. **Anti-inflammatory activity**

The essential oil, the aqueous, the alcoholic, the methanolic and the ethyl acetate extracts, all produce a significant anti-inflammatory activity.\cite{7,46,72}

Meanwhile benzophenone glycosides, sesquiterpenes, and flavonoids purified from the leaves are claimed as allergy inhibitors.\cite{12}

16. **Effect of guava extract on the arterial blood pressure**

Guava extract following oral and intraperitoneal administration produces a significant reduction in arterial blood pressure without affecting the heart rate or respiratory rate.\cite{73} A recent study suggested that the antihypertensive use in traditional medicine is through the inhibition of intracellular calcium release.\cite{60}

17. **Antiulcer activity**

*P. guajava* possesses antiulcer activity acid secretion inhibitory effect in aspirin induced gastric ulcer model mediated through prostaglandins.\cite{74}

18. **Hepatoprotective activity**

The aqueous extract of leaves of guava plant possesses good hepatoprotective activity.\cite{75}

19. **Other activities**

a) The aqueous extract of the leaves, barks, and flowers were stated to have nicotinic receptor antagonist properties.\cite{23}

b) The alcoholic extract (0.2 mg/100ml), aqueous extract (0.3 mg/100ml), ethyl acetate extract (0.4 mg%), essential oil (0.5 mg%) of the leaves and quercetin (0.5 mg%) produce a marked inhibition of isolated rat uterus.\cite{7}

c) The leaf extract inhibits alpha-amylase activity and can be used as health beverage\cite{79} and was found to inhibit the adherence of the early plaque settlers which include *Streptococcus mitis*, *Streptococcus sanguinis* and *Actinomyces* species. The mechanism of which may involve a modification of the hydrophobic bonding between the bacteria and the salivary components covering the tooth surfaces.\cite{77}

**TOXICITY OF GUAVA LEAF EXTRACT**

The water extract of *P. guajava* leaves has no short term harmful effect,\cite{79} and was found to be non toxic to rats and mice at a dose of 5g/Kg i.e. LD$_{50}$ was more than 5 g/kg.\cite{70}

The study performed on the Egyptian plant lately stated that the ethyl acetate extract is not toxic in doses up to 1.40 g/kg body weight, the alcoholic extract up to 2.05 g/kg, the aqueous extract up to 2.35g/kg and the essential oil up to 0.62 g/kg.\cite{7}

Guava tea intake raises no changes in parameters of iron metabolism, liver and kidney functions and of blood chemistry data. In addition hypoglycemia is not caused by excess ingestion of guava tea.\cite{54}
In single-dose and 1-month repeated dose toxicity studies, the oral administration of GvEx (200 and 2000 mg/kg/day) caused no abnormal effects in rats, indicating that there is neither acute nor chronic toxicity. Guava Leaf Tea had a lower mutagenic activity than commercial green tea and black tea in a DNA repair test (Rec-assay); however, these teas showed no mutagenic activity in a bacterial reverse mutation test (Ames test). Moreover, GvEx did not induce chromosomal aberrations in a micronuclear test using peripheral blood erythrocytes, which were prepared from mice by a single oral administration of GvEx (2000 mg/kg). From these findings, it is suggested that Guava Leaf Tea and these commercial teas have no genotoxicity.\[53\]

**ASSAY**

**Quercetin content**

*Quercetin content of the powdered leaves (using the previously published method)[75]*

1. Extract 2 g powdered Guava leaves with 20.0 ml of boiling water for 10 minutes, cool and filter.
2. To 10.0 ml of the previously prepared filtrate add 2 ml 25% HCl and heat on a water-bath for 25 minutes then cool and filter.
3. Extract this filtrate with 4 successive quantities, 25 ml each, of butanol.
4. Concentrate the combined butanol extracts under reduced pressure and redissolve in methanol adjusting the volume to 10.0 ml (volumetric flask). The resulting 10.0 ml is equivalent to 1 g powdered guava leaf.
5. Apply bands of this sample alongside with bands of standard quercetin (0.5 μg/μl) on 20 × 10 cm aluminium silica gel 60 F254 HPTLC plates by means of Linomat IV automated spray-on band applicator operated with the following settings: band length 6 mm, application rate 15 s/μl, distance between bands 4 mm, distance from the plate side edge 1.2 cm, and distance from the bottom of the plate 1.5 cm.
6. For construction of the calibration curve apply 1.00 μl, 2.00 μl, 3.00 μl, 4.00 μl, 5.00 μl and 6.00 μl of the quercetin standard (0.5 μg/μl). Apply each concentration trice and calculate the average.
7. Apply each sample as triplicate of 5.00 μl and then average is calculated.
8. Develop the plate to a distance of 6 cm beyond the origin with toluene-acetone-methanol-formic acid (46:8:5:1) in a vapour equilibrated Camag HPTLC twin trough chamber. After development, air dry the plates for 15 minutes.
9. Densitometrically quantify the bands using Camag TLC scanner 3 and WINCATS software. Operate the scanner in the Absorption/Reflection mode with 5 × 0.1 mm slit dimension, 20 mm/sec scanning rate and 20 nm monochromator bandwidth at an optimized wavelength 254 nm.
10. Calculate the concentration of quercetin in the sample with respect to the calibration curve constructed.
11. Quercetin concentration should not be less than 200 mg/100 g powdered leaf.

**Quercetin content of the prepared extract**

Procede from step two from the assay of the powdered leaves.

Calculate the quercetin content of 1 ml of the extract.

Calculate the quercetin content of the powdered leaves from which the extract was prepared from.

**Quercetin content of the final product**

Procede from step two from the assay of the powdered leaves.

Calculate the quercetin content of 1 ml of the final product.

**APPENDIX I**

**Selection of the best solvent and method for the extraction of Psidium guajava L. leaves using quercetin as a marker compound**

Samples of the same powdered guava leaves (2 g each) were extracted separately by 20.00 ml of water-ethanol in different proportions using maceration in order to determine the best water-ethanol ratio for extraction of the quercetin content.

Samples of the same powdered guava leaves (2 g each) were extracted separately by 20.00 ml of water using infusion and decoction (boiling under reflux for certain time) once for 10 minutes and once for 20 minutes.

Each extract was filtered and 10.00 ml of the extract was quantitatively estimated by the previously proposed method. Accordingly, the extract was hydrolysed using 2 ml of HCl, extracted with butanol, dried, redissolved in methanol adjusting the volume to 10.00 ml and this solution was finally quantified for its quercetin content using HPTLC with the same parameters in order to determine the best solvent that gives the highest yield of quercetin and its glycosides. The trials were conducted in triplicate. The details of the extraction procedures are given in table 6.

**RESULTS**

Results of triplicate trials are presented in table 7.
five different stages of growth, namely the flowering stage, the premature fruiting stage i.e. while fruits were small and green, the mature fruiting i.e. while the fruits were mature and yellow, post harvesting the fruits and post harvesting and preflowering. The collected samples are analysed for their quercetin content according to the proposed method in order to reveal the best stage of growth during which guava trees yield the highest quercetin content. The results are presented in table 8.

**DISCUSSION**

From table 6 it is obvious that the best solvent for extracting quercetin and its glycosides is the 16% ethanol and maceration for 24 hours, this is indicated by the highest quercetin percent (255.98%). On increasing the strength of ethanol, the yield of quercetin was decreased, reaching a minimum yield with absolute ethanol (89.65%). For the aqueous extraction the decoction is much better than the infusion. It is also obvious that 10 minutes decoction is very enough for the extraction with water and increasing the time does not increase significantly the amount extracted. From table 7 it is obvious that the highest quercetin content is during the premature fruiting stage. Nevertheless, and from the economic point of view, samples taken post harvesting of the fruits contain good amount of quercetin glycosides, as well. From table 8 it is obvious that samples collected from Maamora showed the best results. Samples collected from Anshas, Northern coast showed also good results.

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