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EXPLORING AND INVESTIGATING THE POTENTIAL PHYTOCHEMICALS OF *PSIDIUM GUAJAVA* L. STEM EXTRACTS

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ABSTRACT: Objective: This study aimed to carry out a phytochemical, pharmacognostic, qualitative, and quantitative evaluation of the medicinal plant *Psidium guajava* L., also known as guava, a member of the Myrtaceae family. **Methods:** The present study provides pharmacognostic, phytochemical, and quantitative details of *Psidium guajava* L. **Results:** Plant-dried stem powder material was subjected to continuous hot extraction Soxhlet using solvents pet-ether, acetone, and ethanol. Phytochemical standardization was undertaken to detect the presence of bioactive agents along with TLC. Different physical parameters, like ash values, extractive value, loss on drying, etc., were evaluated for the powdered drug. The extracts were obtained from the Soxhlet method and subjected to preliminary phytochemical evaluation. **Conclusions:** The results of this study can serve as a valuable source of information for identifying this plant for future investigation and applications.

INTRODUCTION: The medicinal plant *Psidium guajava* L. also known as guava, is a member of the myrtaceae family. It is abundantly available throughout India. The *P. guajava* tree's leaves and bark have a long history in medicine and are being used today. Although it is originally from Central America, it is now farmed and disseminated, and its fruits have improved the diets of millions of people worldwide in tropical regions. It grows to about 10 m, has spreading branches, and is tolerant of various soil types. It's also referred to as "poor man's apple" ¹.

Psidium guajava L. is now grown in South Florida, Bermuda, the Bahamas, Cuba, Trinidad, and down to Brazil in the West Indies.

Morphology: The guava is a fast growing tree that can grow to a height of three -10 m. It has a shallow root system. Guava produces low drooping branches from the base and suckers from the root. The trunk is slender, 20cm in diameter, covered with the bark. The bark is reddish brown, thin, smooth, and flaky.

The roots are extensive but only superficial. The fruit has a strong, sweet, musky odor and can be round, ovoid, or pear-shaped. The leaves are grown in pairs, opposite to each other. The flower is white in color, about 3cm in diameter, solitary or in 2-3 flower clusters borne at the axils of the newly emerging lateral shoots ¹³.

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Flower

Branches with Leaves and Fruits

Fruit

FIG. 1: *PSIDIUM GUAJAVA*TABLE 1: COMMON NAME FOR *PSIDIUM GUAJAVA* L.:

Amba	Nepal
Amrood	India
Guava	Indonesia, Shri Lanka, USA, Tanzania, Mexico
Amrud	Fiji
Amrut	Fiji

TABLE 2: TAXONOMICAL CLASSIFICATION OF *P. GUAJAVA*

Kingdom	Plantae
Division	Magnoliophyta
Subclass	Rosidae
Order	Myrtales
Family	Myrtaceae
Subfamily	Myrtoideae
Genus	<i>Psidium</i>
Species	<i>Guajava</i>
Binomial name	<i>Psidium guajava</i>

Photochemistry of *P. guajava*: (Rohit Kumar Bijauliya, 2018): *Psidium guajava* is an important food crop and medicinal plant worldwide. Different pharmacological experiments in several *In-vitro* and *in-vivo* models have been carried out and identified the medicinally important Phyto-constituents.

Fruit: Vitamin C, Vitamin A, Iron, Calcium, Manganese, Phosphoric, Oxalic and Malic Acids, Saponin Combined with Oleanolic Acid. Morin-3-O- α -L-Lyxopyranoside and Morin-3-O- α -L-Arabopyranoside, (Z)-3-Hexen-One-Ol, 6-Methyl-5-Hepten-2-One Limonene, Octanol, Ethyl Octanoate (Pink Guava Fruit), Fiber, Fatty Acid.

Leaves: α -Pinene, β -Pinene, Limonene, Menthol, Terphenyl Acetate, Isopropyl Alcohol, Longicyclene, Caryophyllene, β -Bisabolene, Caryophyllene Oxide, β -Copanene, Farnesene, Humulene, Selinene, Cadinenes And Curcumene, Mallic Acids, Nerolidiol, β - Sitosterol, Ursolic,

Crategolic, and Guayavolic acids, Cineol, Quercetin, 3-L4-4- Arabino furanoside (Avicularin) And Its 3-L-4-Pyranoside (Essential Oil), Resin, Tannin Eugenol, Caryophyllene, Guajavolide (2 α ,3 β -,6 β -,23-Tetrahydroyurs-12-en-28,20 β - Olide; 1) And Guavenoic Acid (2 α -,3 β -,6 β -,23-Tetrahydroyurs-12,20(30)-dien-28-oic Acid, Triterpene oleanolic Acid, Triterpenoids, Flavinone-2,2'-ene, Prenol, Dihydrobenzo Phenanthridine And Crypto Nine.

Bark: Polyphenols, Resin, and Crystals of Calcium Oxalate

Stem: Flavonoids, Guaijavarin, Quercetin, Myricetin and Epigenin. Essential Oil Contains Hexanal, 2,4-Hexadienal, 3-Hexenal, 2-Hexenal, 3-Hexenyl Acetate and Phenol, β -Caryophyllene, Nerolidiol, 3-Phenylpropyl Acetate, Caryophyllene Oxide, Pentane-2-Thiol, 3-Penten-2-ol and 2-Butenylacetate, 3-Hydroxy-2-Butano3-Methyl-1-Butanol, 2, 3- Butanediol, 3-Methylbutanoic Acid.

Root: Tannin, Leukocyanidins, Sterols, Gallic Acid, Carbohydrates, Salts, Tannic Acid.

Seed: Proteins, Starch, Oils, Phenolic, and Flavonoid Compounds, Flavone Glycoside, Quercetin-3-O-B-D-(2"-Ogalloyglucoside)-4'-O-Vinylpropionate.

Twigs: Calcium, Magnesium, Phosphorous, Potassium, Sodium, Fluoride, Copper, Iron, Zinc, Manganese, and Lead. Flavonoids, Sesquiterpenes Alcohols, and Acid Triterpene.

Traditional uses of *Psidium guajava*: More recent pharmacological studies show that *Psidium guajava* L. is used in many parts of the world to

treat several diseases, e.g., diabetes, hypertension, caries, wounds, pain relief, and fever. Roots, barks, leaves, and immature fruits are commonly used to treat gastroenteritis, diarrhea, and dysentery. Leaves are applied to wounds, ulcers, and rheumatic pain while chewed to relieve toothache.

A decoction of new shoots is taken as a febrifuge. After childbirth, a combined decoction of leaves and bark is given to expel the placenta. A water leaf extract is used to reduce blood glucose levels in people with diabetes. Guava jelly is a tonic for heart and constipation. The astringent unripe fruit, the leaves, the cortex of bark, and roots are used for washing ulcers and wounds as an astringent¹³.

MATERIALS AND METHODS:

Collection, Identification and Authentication of Plant Material: Based on a literature survey, fresh stems were selected for the present project. Fresh stems of *Psidium guajava* L. were collected from the local region of Nanded City and identified based on its morphological features with the help of a taxonomist. The plant was authenticated by Dr. Shirang S. Bodke, Associate Professor and Head of Botany and Horticulture, Yeshwant Mahavidyalaya, Nanded. This has been submitted to Nanded Pharmacy College, Specimen No. NPC/M. Pharm/ Herbarium 2022-23/H-01 and authenticated as *Psidium guajava* L. (Family-Myrtaceae).



FIG. 2: HERBARIUM SHEET OF *PSIDIUM GUAJAVA*

Processing of Crude Drug: The fresh stems of plant *Psidium guajava* L. were subjected to shade drying and further crushed to coarse powder. Then,

the powder passed through mesh no. 14 and was stored in an airtight container for further use.



FIG. 3: STEMS OF *PSIDIUM GUAJAVA* L.



FIG. 4: STEM POWDER

Pharmacognostic Evaluation of Plant Material:

TABLE 3: MACROSCOPIC DESCRIPTION OF *PSIDIUM GUAJAVA* L.

Color	Yellowish cream
Odor	Characteristic
Taste	Slightly Bitter
Shape	Trunk is slender, 20 cm diameter
Size	3-10 m

Stem Microscopy: A section of the stem was stained with phloroglucinol and HCl (1:1). The microscopic study of the fresh stem section showed the presence of Epidermis, endodermis, vascular bundles, i.e., xylem and phloem, cuticle, trichomes, pith.

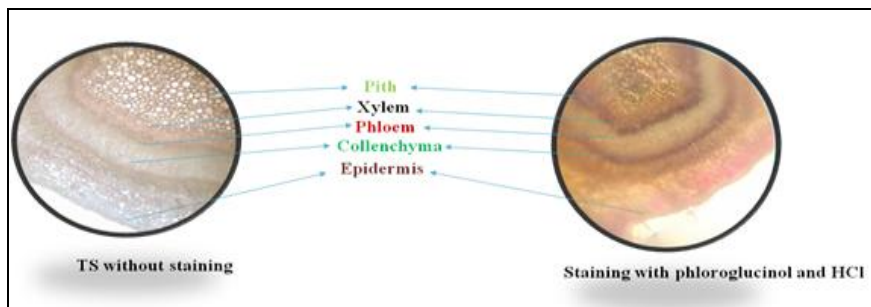


FIG. 5: TS OF *PSIDIUM GUAJAVA* L. STEM

Microscopic Evaluation of Powdered Drug:

Some powder drug was stained with Phloroglucinol and HCl (1:1). It gives pink colour to fibers and vascular bundles, which was observed under a

microscope with 40X lens. The powdered drug showed trichomes, fibers, Calcium oxalate crystals, and starch.

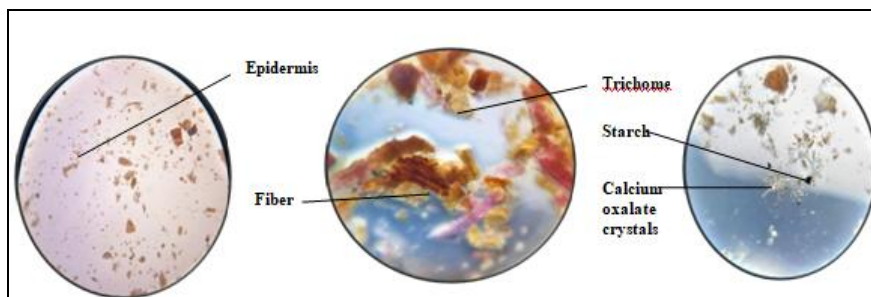


FIG. 6: POWDER CHARACTERISTICS OF *PSIDIUM GUAJAVA* L. STEM

Preparation of Plant Extract: Coarse powder (250 g) of *Psidium guajava* L. stem was exhaustively defatted using petroleum ether (60-80 °C) (PP-PE) and extracted successively with acetone and ethanol using Soxhlet apparatus.

All the extracts were collected, filtered through Whatman filter paper, concentrated, and stored in a tight desiccator, and the percentage yield was calculated.



FIG. 7: SOXHLET APPARATUS

Physical Evaluation: The dried stems of the plant were used for the determination of physicochemical parameters such as total ash value, acid insoluble

ash value, water soluble ash value, and moisture content (LOD).



FIG. 8: ASH VALUE DETERMINATION

RESULTS:

Pharmacognostic Evaluation of *Psidium guajava* L. Stems:

Determination of Loss on Drying (LOD):

TABLE 4: DETERMINATION OF LOSS ON DRYING (LOD)

Weight of empty crucible (g)	23.45 g
Weight of crude drug (sample) (g)	1.5 g
Weight of crucible (g) + Weight of crude drug (sample) (g)	24.95 g
Weight of crucible (g) + Weight of sample after drying (g)	24.72 g
Loss on drying (% w/w)	15.3%

The percentage of loss on drying was found to be 15.3 %.

Determination of Ash Value:

TABLE 5: DETERMINATION OF ASH VALUE

Sr. no.	Standardization parameters	Wt. of crucible + ash or powder (gm) (z)	Wt. of empty crucible (gm) (x)	Wt. of drug (gm) (y)	% of ash = $\frac{z}{y} \times 100$
1	Total Ash value	17.35	17.21	2	7
2	Acid insoluble ash	18.54	18.41	2	6.5
3	Water soluble ash	17.22	17.21	2	0.5

Ash values were found as, total ash 7 % w/w, acid-insoluble ash 6.5 % w/w and water-soluble ash 0.5 % w/w.

Phytochemical Test of *Psidium guajava* L. Stems Extract:

TABLE 6: QUALITATIVE ANALYSIS OBSERVATION

Chemical test	Pet ether	Acetone	Ethanol
(A) Test for carbohydrates			
Molisch test	+	+	+
Fehling's test	-	-	+
Benedict test	-	-	+
Barford's test	-	-	+
(B) Test for proteins			
Biuret test	-	-	-
(C) Test for amino acids			
Ninhydrin test	-	-	-
Cysteine test	-	+	-

(D) Test for steroids			
Salkowski test	-	+	+
(E) Test for Glycosides			
Legal Test	-	-	-
Foam Test	+	+	+
(F) Test for Flavonoids			
Shinoda Test	-	+	-
Sulphuric Acid Test	-	+	+
Lead Acetate Test	-	+	+
NaOH Test	-	+	+
Zinc Dust + HCl Test	-	+	+
(G) Test for Tannins and Phenolic Compounds			
5% FeCl ₃ Solution Test	-	+	+
Lead Acetate Test	-	+	+
Bromine Water Test	-	+	+
K ₂ Cr ₂ O ₇ Test	-	+	+
Dil. KMnO ₄ Solution	-	+	+
(H) Test for Alkaloids			
Mayer's Test	-	+	+
Wagner's Test	-	+	+
Hager's Test	-	+	+

Present (+) & absent (-): The above observation table shows the presence of phytoconstituents in the extracts. It reveals all two (i.e., Acetone and

Ethanolic) extracts contain carbohydrates, glycosides, steroids, proteins, tannins and phenols, alkaloids, and flavonoids.

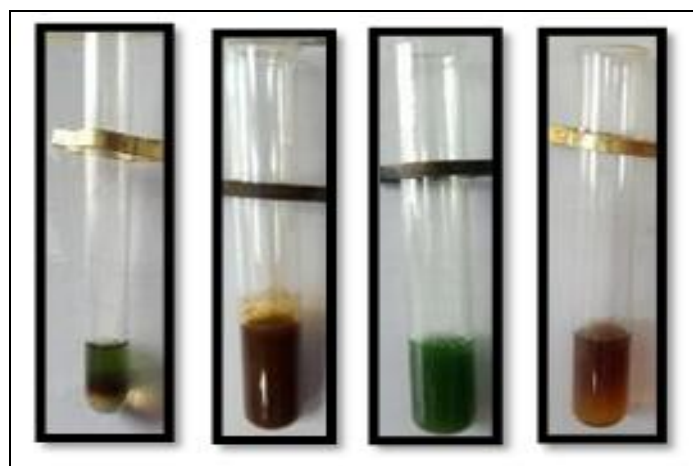


FIG. 9: TESTS FOR CARBOHYDRATES



FIG. 10: TESTS FOR FLAVONOIDS

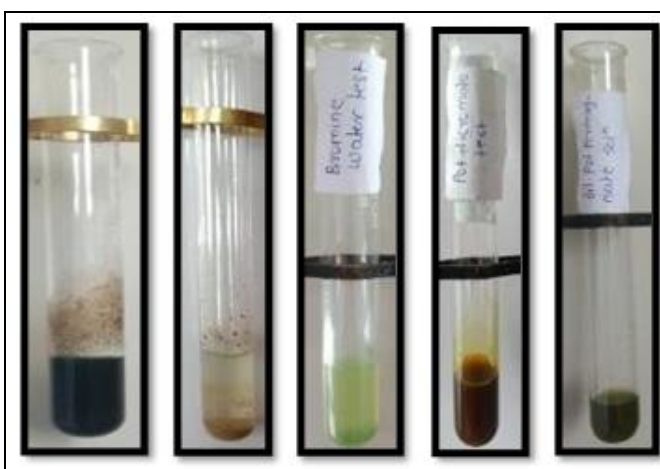


FIG. 11: TESTS FOR TANNINS AND PHENOLS

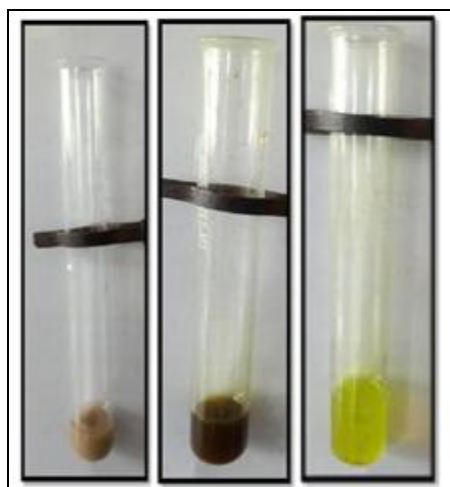


FIG. 12: TESTS FOR ALKALOIDS

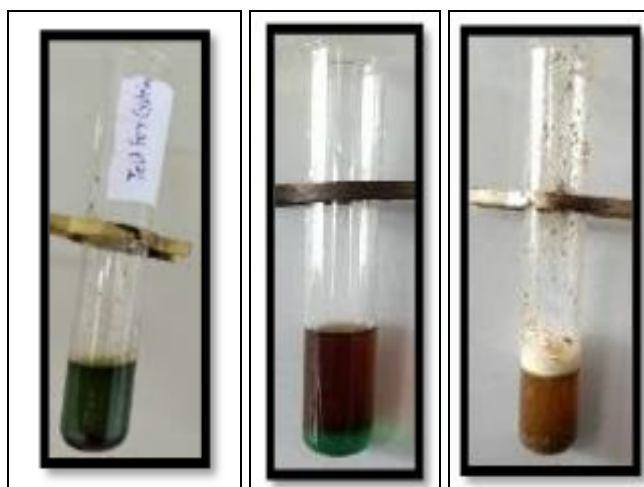


FIG. 13: TEST FOR AMINO ACID FIG. 14: TEST FOR STEROID FIG. 15: FOAM TEST

Confirmation of Phytoconstituents with Thin Layer Chromatography:

TABLE 7: R_f VALUES OF *PSIDIUM GUAJAVA L.* (PG) STEM EXTRACTS

Sr. no.	Extract	Solvent System with Proportion & Spraying agent	R _f Value	Colour of Spot	Chemical constituent
1	PG- Pet-ether	Benzene: acetone: Methanol (4:4:2) 10% H ₂ SO ₄	0.41	Yellowish green	Chlorophyll b
			0.50	Green	Chlorophyll a
2	PG- Acetone	Chloroform: EA: Methanol (8:2:1) 10% H ₂ SO ₄	0.28	Grey	Mg-free chlorophyll a
			0.4	Yellow orange	Flavonoid
			0.55	Green	Flavone
			0.64	Dark purple	Terpenes
			0.81	brown	Xanthophyll 2
3	PG- Ethanol	Chloroform: Acetone: methanol (8:1:1) 10% H ₂ SO ₄	0.1	Light Orange	Lutein
			0.27	Yellow	Anthocyanin
			0.38	Light Red	Anthocyanin
			0.49	Reddish brown	Anthocyanin
			0.73	Light Red	Epicatechin

The above observation table shows the presence of phytoconstituents in the extracts. It reveals all two (i.e., Acetone and Ethanolic) extracts contain

carbohydrates, glycosides, steroids, proteins, tannins and phenols, alkaloids, and flavonoids. (A. M. Motwally).

TLC Finger Printing:

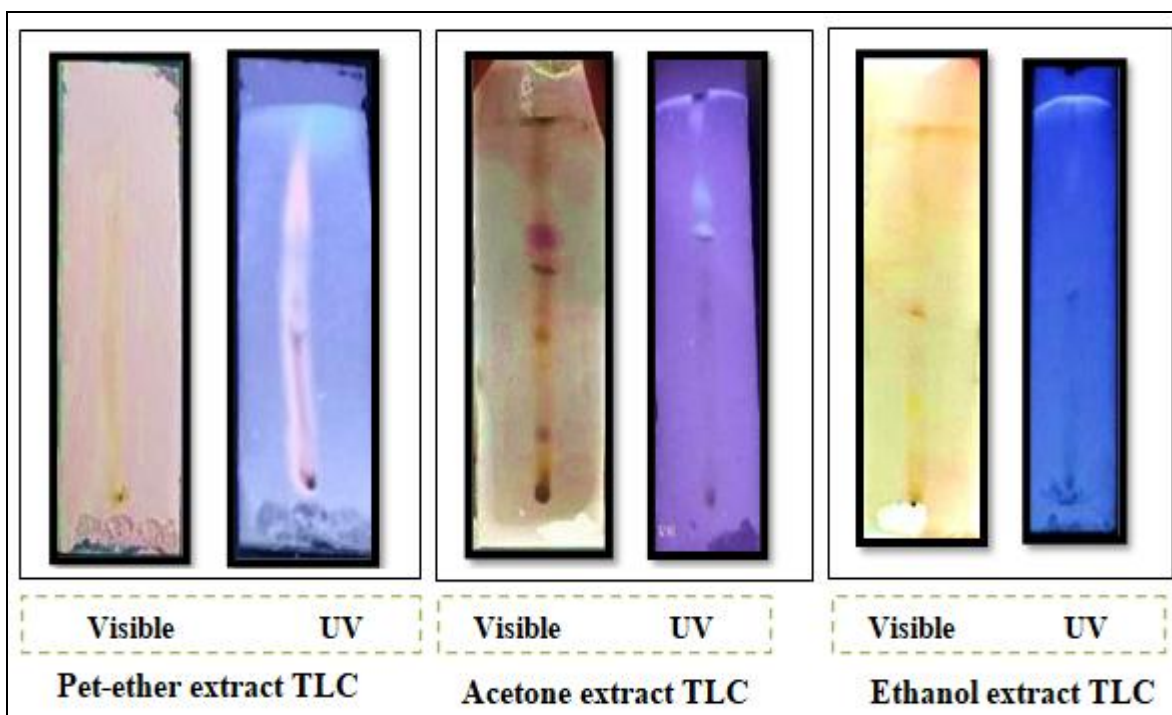


FIG. 16: TLC OF PETROLEUM ETHER, ACETONE, ETHANOL EXTRACT

Determination of Total Phenolic and Total Flavonoid Content of *Psidium guajava* L. Stem Extract:

Total Phenolic Content: Total phenolic content of standard gallic acid.

TABLE 8: TOTAL PHENOLIC CONTENT OF STANDARD GALLIC ACID

Sr. no.	Conc. (ug/ml)	Absorbance at 666 nm
1	2	0.101 ± 0.0008
2	4	0.141 ± 0.0008
3	6	0.218 ± 0.0008
4	8	0.307 ± 0.0008
5	10	0.372 ± 0.0008

Values represent mean ± SEM (n = 3).

The calibration curve for sequentially and independently prepared stock solution of standard gallic acid depicts the concentration of gallic acid against the absorbance. The absorbance values

increased proportionally upon increasing the concentration of gallic acid from 2µg/ml to 10 µg/ml.

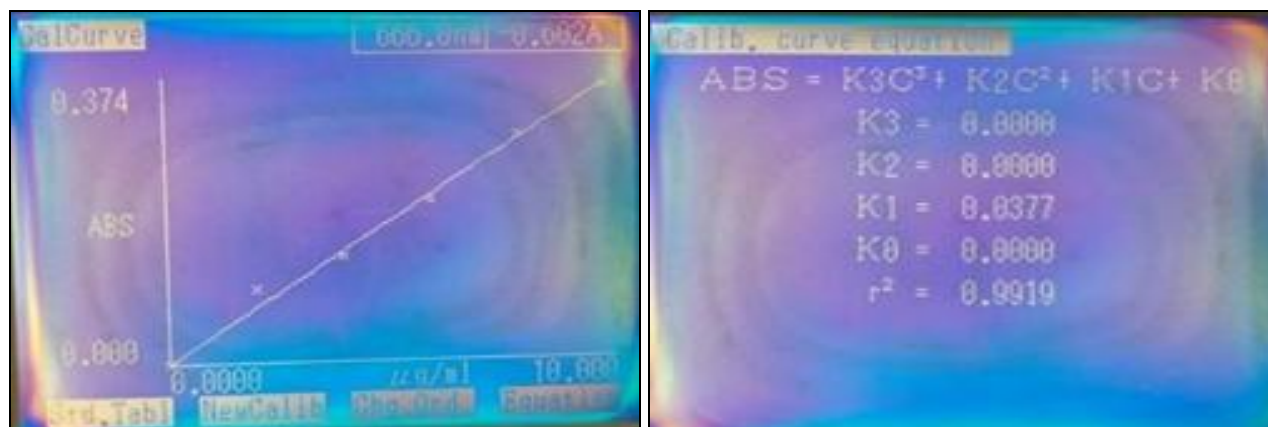


FIG. 17: CALIBRATION CURVE FOR STD. GALLIC ACID & CALIBRATION CURVE EQUATION

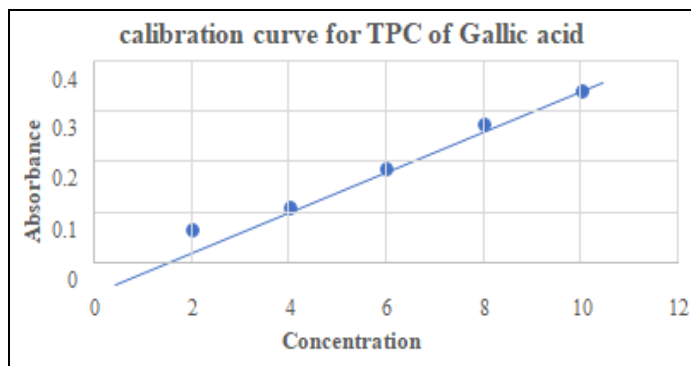


FIG. 18: CALIBRATION CURVE OF GALLIC ACID

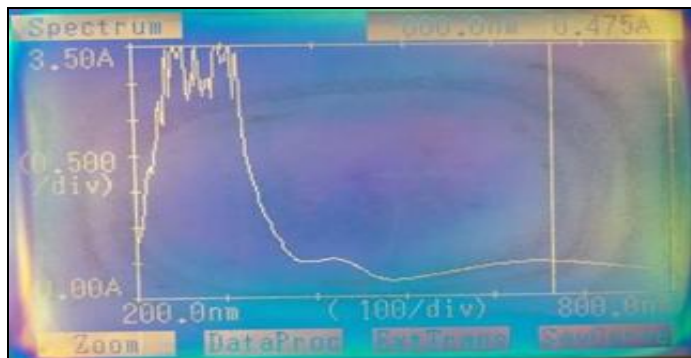


FIG. 19: λ MAX DETERMINATION OF STANDARD GALLIC ACID

Result of Total Phenolic Content of *Psidium guajava* L. Stem Extract:

TABLE 9: TOTAL PHENOLIC CONTENT OF *PSIDIUM GUAJAVA* L. STEM

Sr. no.	Extract	Conc. (ug/ml)	Absorbance	TPC (mg GAE/g DW)
1	Acetone	50	0.333 ± 0.0014	180.13 ± 0.81
2	Ethanol	50	0.312 ± 0.0017	168.73 ± 0.93

Values represent mean ± SEM (n = 3).

Note: GAE/g DW denotes Gallic Acid Equivalent per gram dry weight.

The above observation table reveals that Acetone and Ethanol have Phenolic content of 180.13 (mg GAE/g DW), and 168.73 (mg GAE/g DW), respectively.

Acetone extract shows more phenolic content than ethanol as per a comparative evaluation of the phenolic content of extracts.

Total Flavonoid Content:

Total Flavonoid Content of Standard Quercetin:

TABLE 10: TOTAL FLAVONOID CONTENT OF STANDARD QUERCETIN

Sr. no.	Conc. (ug/ml)	Abs at 510 nm
1	2	0.0213 ± 0.0008
2	4	0.033 ± 0.0008
3	6	0.048 ± 0.0008
4	8	0.0723 ± 0.0008
5	10	0.090 ± 0.0005

Values represents mean ± SEM (n = 3).

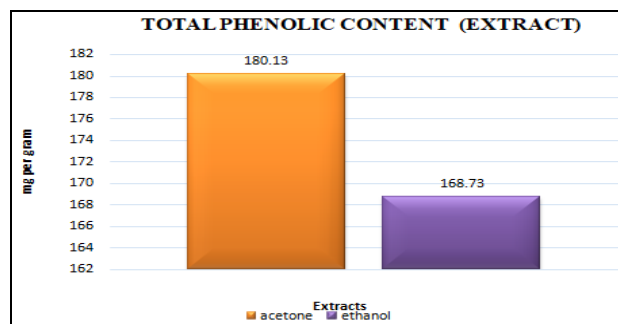


FIG. 20: TOTAL PHENOLIC CONTENT OF EXTRACTS

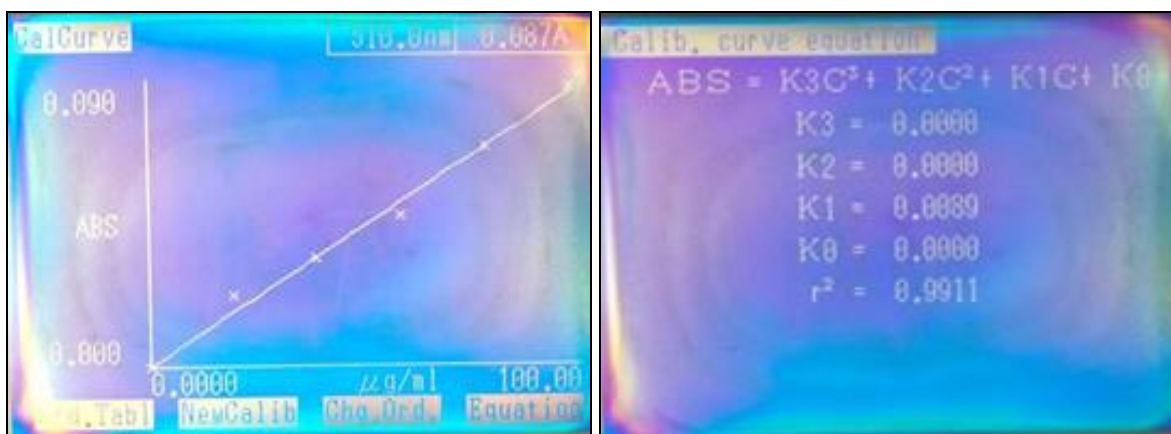


FIG. 21: CALIBRATION CURVE FOR STD. QUERCETIN & CALIBRATION EQUATION

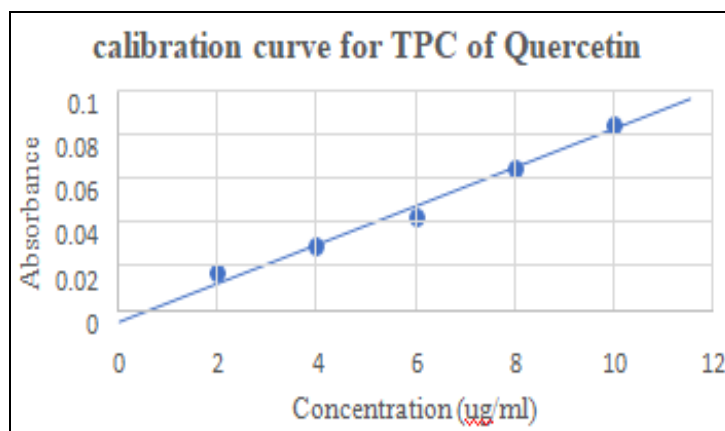


FIG. 22: CALIBRATION CURVE FOR TFC OF QUERCETIN

The calibration curve for quercetin's sequentially and independently prepared stock solution depicts the concentration of quercetin against the

absorbance. The absorbance values increased proportionally upon increasing the concentration of quercetin from 2µg/ml to 10 µg/ml.

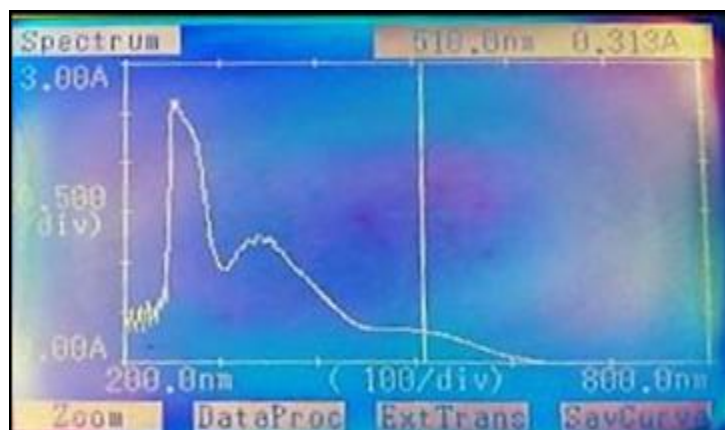


FIG. 23: DETERMINATION OF A MAX OF STANDARD QUERCETIN FOR TFC

Result of Total Flavonoid Content of *Psidium guajava* L. Stem Extract:

TABLE 11: TOTAL FLAVONOID CONTENT OF *PSIDIUM GUAJAVA* L. STEM EXTRACTS

Sr. no.	Extract	Abs	TFC In (Mg QE/g DW)
1	Acetone	0.052 ± 0.001	117.46 ± 3.94
2	Ethanol	0.039 ± 0.0008	88.26 ± 1.99

Values represents mean ± SEM (n = 3).

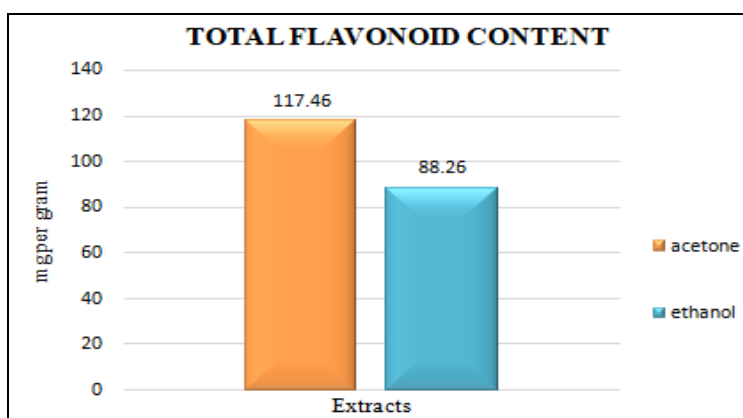


FIG. 24: EFFECT OF FLAVONOID CONTENT OF EXTRACT

Note: QE/g DW Denotes Quercetin Equivalent Per Gram Dry Weight: The above table indicates that the acetone and ethanol extracts contain flavonoids contain as like 117.46 (mg QE/g DW) and 88.26 (mg QE/g DW).

Acetone extract shows more flavonoid content than ethanol extract as per a comparative evaluation of the flavonoid content of extracts.

In-vitro Antioxidant Activity: The antioxidant activity of *Psidium guajava* L. was determined by using the *in-vitro* method DPPH free radical scavenging assay.

The result was compared with the reference standards of gallic acid, quercetin, and rutin.

TABLE 12: ABSORBANCE OF GALLIC ACID, RUTIN AND QUERCETIN

Sr. no.	Conc. (µg/ml)	Absorbance		
		Gallic acid	Rutin	Quercetin
1	20	0.423 ± 0.002	0.251 ± 0.002	0.484 ± 0.02
2	40	0.380 ± 0.001	0.218 ± 0.001	0.375 ± 0.001
3	60	0.316 ± 0.002	0.193 ± 0.001	0.282 ± 0.001
4	80	0.287 ± 0.001	0.163 ± 0.002	0.194 ± 0.001
5	100	0.195 ± 0.002	0.150 ± 0.001	0.155 ± 0.002

Values represents mean ± SEM (n = 3).

TABLE 13: DPPH RADICAL SCAVENGING ASSAY OF STANDARD GALLIC ACID, RUTIN AND QUERCETIN

Sr. no.	Conc. µg/ml	% inhibition		
		Gallic acid	Rutin	Quercetin
1	20	48.30 ± 0.36	69.26 ± 0.28	44.4 ± 0.10
2	40	53.59 ± 0.21	73.33 ± 0.17	54.20 ± 0.18
3	60	61.37 ± 0.28	76.43 ± 0.18	65.56 ± 0.18
4	80	64.95 ± 0.18	80.09 ± 0.25	76.22 ± 0.17
5	100	76.14 ± 0.24	81.68 ± 0.13	88.92 ± 0.10

Values represent mean ± SEM (n = 3).

The standard gallic acid, rutin, and quercetin show 48.30 %, 69.26%, and 44.4 % inhibition (percent scavenging activity) at 20µg/ml conc. At 100 µg/ml concentration shows 76.14%, 81.68 %, and 88.92% inhibition, respectively. The observation reveals

that quercetin shows better antioxidant activity from concentration 20µg/ml to 100µg/ml in a concentration-dependent manner, i.e., as conc. increases % inhibition of free radicals also increases.

TABLE 14: ABSORBANCE OF QUERCETIN, *PSIDIUM GUAJAVA* L. STEM EXTRACTS (ACETONE AND ETHANOL)

Sr. no.	Conc. (µg/ml)	Absorbance		
		Quercetin	Acetone extract	Ethanol extract
1	20	0.484 ± 0.02	0.482 ± 0.002	0.515 ± 0.002
2	40	0.375 ± 0.001	0.428 ± 0.005	0.475 ± 0.002
3	60	0.282 ± 0.001	0.387 ± 0.004	0.432 ± 0.001

4	80	0.194 ± 0.001	0.214 ± 0.003	0.375 ± 0.002
5	100	0.155 ± 0.002	0.155 ± 0.002	0.293 ± 0.001

Values represent mean ± SEM (n = 3).

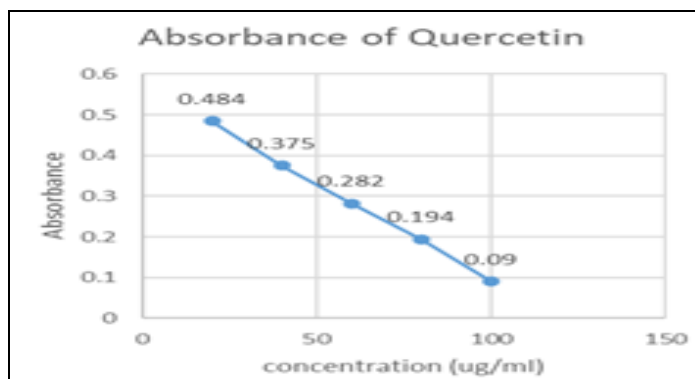


FIG. 25: ABSORBANCE CURVE OF QUERCETIN

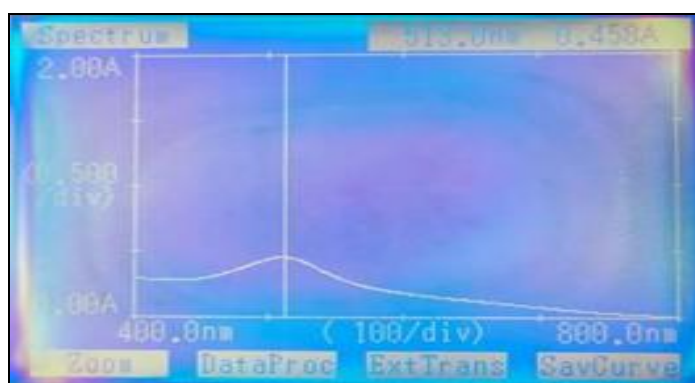


FIG. 26: DETERMINATION OF λ MAX OF STANDARD QUERCETIN FOR ANTIOXIDANT ACTIVITY

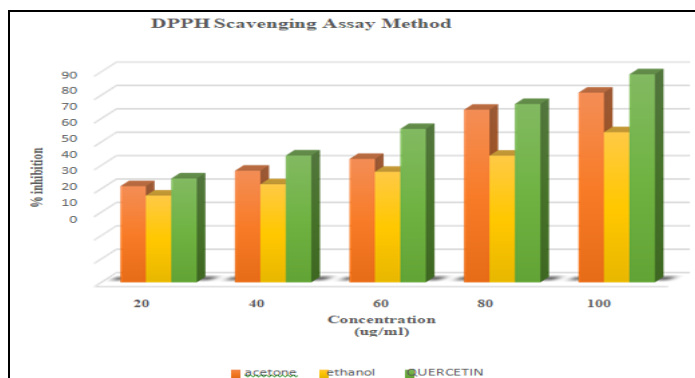


FIG. 27: DPPH RADICAL SCAVENGING ASSAY OF TEST EXTRACTS OF *PSIDIUM GUAJAVA* L. STEM EXTRACTS

TABLE 15: DPPH SCAVENGING ACTIVITY OF QUERCETIN, *PSIDIUM GUAJAVA* L. STEM EXTRACTS (ACETONE AND ETHANOL)

Sr. no.	Conc. µg/ml	% inhibition		
		Quercetin	Acetone extract	Ethanol extract
1	20	44.4 ± 0.10	41.1 ± 0.31	37.07 ± 0.24
2	40	54.20 ± 0.18	47.69 ± 0.70	41.91 ± 0.24
3	60	65.56 ± 0.18	52.74 ± 0.60	47.24 ± 0.21
4	80	76.22 ± 0.17	73.78 ± 0.39	54.12 ± 0.29
5	100	88.92 ± 0.10	81.07 ± 0.25	64.17 ± 0.17

Values represent mean ± SEM (n = 3).

The acetone extract of *Psidium guajava* L. shows more significant antioxidant properties than ethanolic extract.

CONCLUSION: In this modern world and hectic lifestyle, the graph of diseases is rising. Due to this, people are taking medicines that have a lot of side

effects and complications. So, the need for side effect-free medications is rising. There is a rising voice for side effects-free medications. From older generations, we have learned that they used plants or their parts as medicine for various diseases. In many developing countries, most people prefer traditional systems of medicine as their treatment.

India has a large geographical area, which allows us to explore different plants with different species. We have the support of knowledge of Ayurveda. The natural constituents in the plants with side effect-free treatment benefit us. Hence, we should work more towards natural products.

Psidium guajava L. Commonly known as the Poor man's apple plant, belongs to the Myrtaceae family. The leaves and fruits of plants have been used for many human ailments. However, a literature survey revealed no systematic and scientific study on the analgesic and anti-inflammatory activity of *Psidium guajava* L. stem extracts. Therefore, exploring this plant for its analgesic and anti-inflammatory activity was considered worthwhile.

A continuous hot extraction method is used for the extraction of a stem using the Soxhlet apparatus; three extracts of *Psidium guajava* L. were prepared with petroleum ether, acetone, and ethanol. The percentage yield of each extract was calculated after distillation and restoration of the solvent was done, and the extracts were stored in a container for further use.

A preliminary phytochemical investigation and TLC fingerprinting were carried out on all three extracts to determine the phytoconstituent present in the extract. The preliminary phytochemical estimation of plant extract reveals the presence of flavonoids, carbohydrates, proteins, amino acids, glycosides, tannin, alkaloids and saponin, etc. These phytochemicals were confirmed by qualitative analysis.

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