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EXPLORATION OF GUAVA (*PSIDIUM GUAJAVA LINN.*) LEAVES EXTRACT IN PHARMACEUTICAL DOSAGE FORM AND ITS EVALUATION

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ABSTRACT
Guava (*Psidium guajava L.*) family Myrtaceae is a small tree or shrub. This plant is native to South America and Mexico. This plant shows many biological activities such as antimicrobial, anti-inflammatory, anti-diarrheal, hepato-protective. Alcohol and ethyl acetate extract contains many phyto-constituents that was alkaloids, flavanoids, saponins, tannins, steroids, glycosides, carbohydrates, phenolic compounds. Nanogel formulation was prepared by using ethyl acetate extract of leaves, sodium alginate, sodium benzoate, propylene glycol, distilled water, triethanolamine for pH adjustment. The pH was found to be 6-7. The formulated gel was greenish in color, homogeneous, non-irritant to skin, translucent, easily spread. The concentration in formulated gel was found to be 0.79%.

KEYWORDS: *Psidium guajava L.*, Quercetin, Linearity

1. INTRODUCTION

1.1History of guava

The Guava is generally native to central America or Mexico and it is later spread across the world by man and nature. In India it was introduced by the Portuguese in the early 17th century.
Its botanical name is Psidium guajava and it belonging to the large family of Myrtaceae that also includes the Eucalyptus tree and several tropical species like Cloves, Nutmeg, Allspice, Cinnamon and also Jamun. The Guava is known for the "apple of tropics" because it contains vitamin C, pectin and other nutrients in higher amount.
The Guava is very popular fruit and available almost all throughout the year, but it mainly bears fruit twice a year, ripening during the rainy and winter season. In rainy season the production of fruit is high and the quality of fruit is inferior which grows in winter. Guavas are evergreen fast growing trees relatively easy to grow and capable of high fruit yield with very little care. Its production cost is low because it does not require much fertilizer, irrigation and plant protection.

Cultivation: Owing to its hardy nature, Guavas can grow in a wide range of soil in tropical and subtropical regions. The best quality guavas are grown where low night temperature is higher in winter season. Again it tolerates high temperature and draught conditions during summer in north India.
pH of soil is 4.5 to 8.2 to grow the plant and the roots can usually penetrate only up to 25cm under the ground. This is why the surface soil should provide enough nutrients to the plant. In orchards, pit is prepared of 60 cm diameter and depth and guava is planted at a distance of 5 to 6 meters. In each pit manure mixture of 10kg of cowdung or farmyard manure is applied, 300g of super phosphate and 150g of potash mixed with soil before planting.

1.2Quercetin:

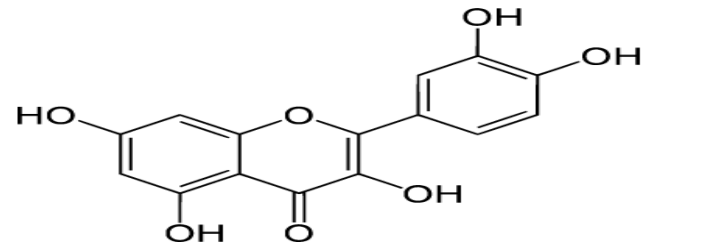


Fig. No. 1.1 Structure of Quercetin

Quercetin is a plant flavanol from which the flavanoid group of polyphenols. It is present in many fruits, vegetables, leaves, seeds, and grains; red onions and kale and these are common foods containing appreciable amounts of quercetin. Quercetin has a bitter flavor and is used as an ingredient in dietary supplements, beverages, and foods.
Quercetin is a flavanoid. It is widely distributed in nature. The name Quercetin is used since 1857, which is derived from quercetum (oak forest), after the oak genus Quercus. It is polar auxin transport inhibitor, occurring naturally. Daily consumption of Quercetin is 25–50 milligrams. It is most abundant dietary Flavonoids.
Mol. Formula: C15H10O7
Mol. Weight: 302.23 g/mol
Appearance: Solid
Color: Yellow needles
Melting point: 316.5°C
Solubility: It is very soluble in ether, methanol and soluble in ethanol, acetone, pyridine, acetic acid
Physical Description: Quercetin appears as yellow needles or yellow powder. It converts to anhydrous form at 203-207°F. Alcoholic solutions taste is very bitter.

1.3Validation:
Validation is the process of establishing documentary evidence demonstrating that a procedure, process, or activity carried out in testing and then production maintains the desired level of compliance at all stages.
In the pharmaceutical industry, it is very important that in addition to final testing and compliance of products, it is also assured that the process will consistently produce the expected results. The desired results are established in terms of specifications for outcome of the process. Qualification of systems and equipment is therefore a part of the process of validation.
Validation is used in food, drug and pharmaceutical regulating agencies such as the USFDA and their GMP guidelines. Since a wide variety of procedures, processes, and activities need to be validated, the field of validation is divided into a number of subsections including the following:

- Equipment validation
- Facilities validation
- HVAC system validation
- Cleaning validation
- Process Validation
- Analytical method validation
- Computer system validation

Similarly, the activity of qualifying systems and equipment is divided into a number of subsections including the following:

- Design qualification (DQ)
- Component qualification (CQ)
- Installation qualification (IQ)
- Operational qualification (OQ)
- Performance qualification (PQ)

1.4Linearity Assay:
Linearity is the ability of a quantitative analytical method to provide results that are directly proportional to the concentration or activity of an analyte in test samples, within a given measuring interval. A measurement procedure is linear when there is a mathematically verified straight-line relationship between the measured values and the true values.

2. MATERIAL AND METHODS
Guava (Psidium guajava L.) leaves were selected for the work
Fresh leaves of guava were collected from the farm in Yeola, Dist. Nashik, Maharashtra,
India. Leaves were dried in shade for 14 days. Sizing of the leaves to coarse powder & stored.

2.1Physicochemical parameters
2.1.1Determination of Ash Value of Leaves of P. guajava L.
The ash remaining following ignition of medicinal plant materials is determined by three different methods which measure total ash, acid-insoluble ash, and water soluble ash.
2.1.1.1Determination of Total Ash of Leaves of P. guajava L. Procedure:
2 gm of powdered drug was accurately weighed and taken in previously weighed silica crucible. The drug was spread uniformly in crucible and ignited. The temperature of furnace was gradually increased up to 500-600°C until it was white which indicated absence of carbon. It was kept in desicator for 30 min to cooling and weighed. The percentage w/w of ash with reference to the air-dried plant material was calculated.
2.1.1.2Determination of Acid-insoluble Ash of Leaves of P. guajava L. Procedure:
The total ash was boiled with 25 ml of 2M HCl for 5 min. Then filtered with ash-less filter paper and collected the acid insoluble ash on the filter paper and washed with hot water. Transferred the filter paper in crucible and ignited at 450°C to constant weight. Then kept in desicator for 15 min for cooling and weighed. The percentage w/w of acid insoluble ash with reference to the air dried plant material was calculated.

2.1.1.3Determination of Water- soluble Ash of Leaves of P. guajava L. Procedure:
The total ash was boiled with 25 ml of distilled water for 5 min. Then filtered with ash-less filter paper and collected the water insoluble ash on the filter paper and washed with hot water. Transferred the filter paper in crucible and ignited at 450°C to constant weight. Then kept in desicator for 15 min for cooling and weighed. Then subtract this residue from total ash. The percentage w/w of water soluble ash with reference to the air-dried plant material was calculated.

2.1.2Determination of Extractive Matter of Leaves of P. guajava L.
This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. It is employed for materials for which as yet no suitable chemical or biological assay exists.
2.1.2.1Determination of Alcohol Extractive Matter of Leaves of P. guajava L. Procedure:
4 gm of powdered drug was weighed accurately and taken in glass-stoppered conical flask and macerated with 100 ml of ethanol specified for the plant material concerned for 6 hours, shaked frequently, then allowed to stand for 18 hours. Filtered and 25 ml solvent transferred to the previously weighed flat-bottomed dish and evaporated to dryness on water bath. Then the dish was kept in desicator for 30 min and weighed immediately. The percentage w/w of alcohol extractive matter with reference to the air dried plant material was calculated.
2.1.2.2 Determination of Water Extractive Matter of Leaves of P. guajava L. Procedure:
4 gm of powdered drug was weighed accurately and taken in glass-stoppered conical flask and macerated with 100 ml of water specified for the plant material concerned for 6 hours, shaked frequently, then allowed to stand for 18 hours. Filtered and 25 ml solvent transferred to the previously weighed flat-bottomed dish and evaporated to dryness on water bath. Then the dish was kept in desicator for 30 min and weighed immediately. The percentage w/w of water extractive matter with reference to the air dried plant material was calculated.

2.1.3Determination of Foreign Matter of Leaves of P. guajava L.
Foreign matter is material consisting of any or all of the following:
-parts of the medicinal plant material or materials other than those named with the limits specified for the plant material concerned;
-any organism, part or product of an organism, other than that named in the specification and description of the plant material concerned;
-mineral admixtures not adhering to the medicinal plant materials, such as soil, stones, sand and dust.
Procedure:
50 gm of medicinal plant material was accurately weighed. It was spread in a thin layer. Plant material was sorted the foreign matter by visually with the help of sieve no. 100. Remained sample was sifted through sieve no. 250. The portions of sorted foreign matter was weighed. The percentage w/w of foreign matter with reference to the air dried plant material was calculated.

2.1.4Determination of Moisture Content (Loss on Drying) of Leaves of P. guajava L.
Loss on drying is the loss of weight expressed as percentage w/w resulting from water and volatile matter of any kind that can be driven off under specified condition.
Procedure:
1gm of powdered drug was weighed accurately and taken previously weighed glass-stoppered shallow plate. The shallow plate without lid was kept in hot air oven at 105°C for 1 hour. The shallow plate was then kept in desicator for 30 min for cooling and weighed immediately. The percentage w/w of moisture content with reference to the air dried plant material was calculated.

2.1.5Determination of Crude Fiber Content of Leaves of P. guajava L.
Crude fiber consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60-80% of the cellulose and 4-6% of the lignin.
Procedure:
0.255 ± 0.005 N sulphuric acid solution and 0.313 ± 0.005 N sodium hydroxide solution was prepared.
2gm of plant material was weighed and extracted with pet ether. 2 gm of dried material was boiled with 200 ml of sulphuric acid for 30 min with bumping chips. The material was filtered and washed with boiling water. Again the material was boiled with 200 ml of sodium hydroxide solution for 30 min and filtered again, washed with 25 ml of 1.25% H_2SO_4, three 50 ml portions of water and lastly 25 ml alcohol. The residue was removed and transferred to pre-weighed ashing dish (W1). The residue was dried for 2 hours at 130°C and cooled the dish in desicator and weighed (W2). It was ignited for 30 min at 600°C, cooled in desiccator and reweighed (W3). % crude fiber content was calculated by –
Formula:

$$\frac{\text{Loss in weight on ignition (W2-W1)} - \text{(W3-W1) X 100}}{\text{Weight of the sample}}$$

2.2Successive extraction of P. guajava leaf powder
The Psidium guajava L. leaves were shade dried and 300 g of coarse powder each batch was successively extracted with petroleum ether, chloroform and ethanol respectively in increasing order of polarity. The extracts were concentrated. After drying, the respective extracts were weighed and percentage yield was calculated.

2.3Determination of Phytochemical Screening of P. guajava L. Leaves Extract

2.3.1Preliminary phytochemical screening

1.Test for carbohydrates

Molisch's test: To the T.S., few drops of α-naphthol solution was added in alcohol, shake and added conc. H_2SO_4 from side of the test tube. Violet ring is formed at the junction of two liquids which shows presence of carbohydrates.

2.Test for Proteins

Biuret test: To 3 ml of T.S. 4% of NaOH was added and few drops of 1% $CuSO_4$ solution. Pink or violet color appears which shows presence of proteins.

3.Test for Amino Acid

Ninhydrin test: 3 ml T.S. + 3 drops 5% Ninhydrin solution was heated in boiling water bath for 10 min. Purple or bluish color appears.

4.Test for Steroids

Liberamann –Burchard reaction: 2 ml extract + chloroform + 1-2 ml acetic anhydride and 2 drops conc. H_2SO_4 from the side of the test tube. First red, then blue and finally green color appears.

5.Test for Glycosides

Keller-killani test: 2 ml T.S. + glacial acetic acid + 1 drop 5% ferric chloride and conc. H_2SO_4. Reddish brown color appears at junction of the two liquid layers and upper layer appears bluish green.

6.Test for Saponin

Foam test: Shake the drug extract or dry powder vigorously with water. Persistent stable foam observed.

7.Test for Flavonoids

Shinoda test: 2 ml T.S. was taken in test tube and to it magnesium powder and few drops of conc. HCl were added which gives pink color, indicates presence of flavonoids.

8.Test for Tannins and Phenolic compounds

Ferric chloride test: 2-3 ml T.S. + 5% $FeCl_3$ solution gives deep blue- black color indicates presence of tannins and phenolic compounds.

Lead acetate test: 2-3 ml T.S. + lead acetate solution gives white ppt indicates presence of tannins and phenolic compounds.

9.Test for Alkaloids

Dragendorff's test: 2-3 ml T.S. + few drops of Dragendorff's reagent. Orange brown ppt formed indicates presence of alkaloids.

Mayer's test: 2-3 ml T.S. + few drops of Mayer's reagent gives ppt indicates presence of alkaloids.

10.Anthraquinone glycosides

Brontrager's test: 3 ml of extract + dil. H_2SO_4, boiled and fltered. To cold filtrate add equal volume benzene or chloroform. Shake well. Separate the organic solvent. Add ammonia. Ammonical layer layer turns red or pink indicates anthraquinone glycosides.

2.3.2 Fingerprinting of Alcohol and Ethyl acetate extract by TLC

Thin layer chromatography is the best known techniques of plant biochemistry. TLC is used for preliminary separation and determination of plant constituents. It is helpful for proper identification, authentication of phytoconstituents.

Required material (Reagents/chemicals and apparatus):

F254 silica plate, chromatogram development chamber, glass capillaries (applicator), petri plate, spraying bottle, toluene, ethyl acetate and formic acid, alcoholic extract, ethyl acetate extract, 1% ferric chloride solution standard quercetin.

•Solvent system: Toluene: Ethyl acetate: Formic acid (5:4:1)

Formula:

$$Rf = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

2.3.3Fingerprinting of Alcohol and Ethyl acetate extract by HPTLC (Pre-validation) Required material (Reagents/chemicals and apparatus):

F254 silica plate (10 x 10), spraying bottle, toluene, ethyl acetate and formic acid, alcoholic extract, ethyl acetate extract, NP reagent (Natural products-polyethylene glycol), standard quercetin.

HPTLC parameters:

1)Equipment use: HPTLC instrument: CAMAG Linomet syringe V, CAMAG TLC scanner V, CAMAG Digistore-Reprostar 3, CAMAGTwin Trough Chamber, Win CATS version 1.4.2. software (CAMAG Switzerland), UV chamber (CAMAG Switzerland), Pre-coated silica gel 60 F254 alumiFtablenium plates (0.2mm thick, Merck, Germany).

2)Chromatographic condition:

- Stationary phase: Pre-coated silica gel G F60 (HPTLC plate) (0.2 mm thick size: 10 x 10 cm)
- Mobile phase: Toluene : Ethyl acetate : Water (5:4:1)
- Saturation time: 20 min
- Wavelength: 370 nm
- Spray reagent: NP reagent

Sample Preparation

A)Standard preparation:

2 mg Quercetin / 2 ml methanol 1000 ppm
B)Sample preparation:
1)PG-A 10 mg / 10 ml methanol 1000 ppm
2)PG-EA 10 mg / 10 ml methanol 1000 ppm

2.4Formulation dosage form:
Formulation oral nanogel of *P. guajava L.* ethyl acetate extract Oral nanogel formulation
Required material
Chemicals: Sodium alginate, Leaves extract, Propylene glycol, Sodium benzoate, Triethanolamine, Distilled water.
Apparatus: Weighing balance, beakers, stirrers
Equipment: Probe sonicator
Parameters: Temperature: 35°C, Time: 10 min, Instrument details: Ultrasonic homogenizer, Model: Athena Technology
Preparation of the gel:
Sodium alginate was soaked in water for 30 min. Sodium benzoate was mixed with propylene glycol. Mixed the above two mixtures. Make up the volume up to 50 ml with water. Sonicate the gel base with the addition of extract by Probe sonicator. pH was adjusted by the drop wise addition of triethanolamine

Table No. 2.1 Observation table for composition and amount of ingredients

Ingredients	F1	F2	F3
Sodium alginate	1.75 g	2 g	2.5 g
Leaves extract	1%	1%	1%
Propylene glycol	2.5 ml	2.5 ml	2.5 ml
Sodium benzoate	0.1 g	0.1 g	0.1 g
Triethanolamine	q. s.	q. s.	q. s.
Distilled water	Up to 50 ml	Up to 50 ml	Up to 50 ml

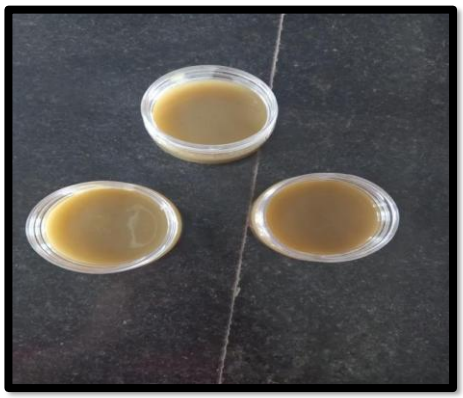


Fig. No. 2.1 Gel

3.RESULT AND DISCUSSION
3.1 Physicochemical parameters

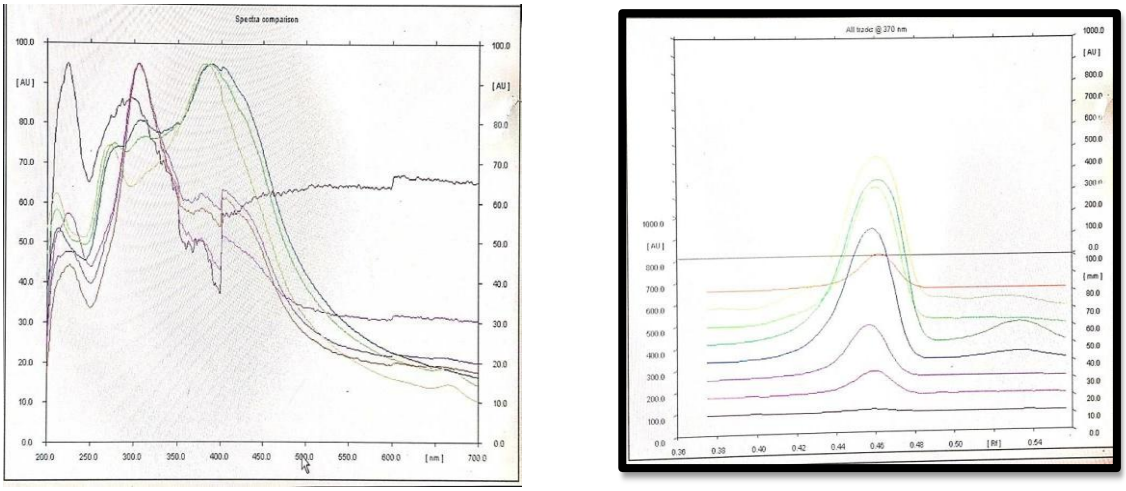
Table No. 3.1 Physicochemical parameters

Parameter	Percentage
Total ash	8.25 % w/w
Water soluble ash	1.5 % w/w
Acid insoluble ash	1.3 % w/w
Water extractive matter	7% w/w
Alcohol extractive matter	15% w/w
Foreign Matter	0.052 %w/w
Moisture content (Loss on drying)	7.6 %w/w
Crude fiber content	%w/w

3.2 Preliminary phytochemical screening

Table No. 3.2 Preliminary phytochemical screening

Sr. No.	Phytochemical test	Extract
1.	Carbohydrates	+
2.	Protein	-
3.	Amino acid	-
4.	Fats & oil	+
5.	Steroids	+
6.	Glycosides	+
7.	Saponin	+
8.	Flavanoids	+
9.	Tannins and Phenolic compounds	+
10.	Alkaloids	+
11.	Anthraquinine glycosides	-



(A) (B)

Fig. No. 3.1 (a) Wavelength Overlay (b) 3D Overlay

Table No. 3.3 Fingerprinting of Alcohol and Ethyl acetate extract by HPTLC (Pre-validation)

Track No.	Track	Volume	Rf	AUC
1	PGA	1.0 µl	0.46	298.6
2	PGA	10.0 µl	0.46	2027.7
3	PGA	20.0 µl	0.46	4693.4
4	STD-Q	2.0 µl	0.46	13660.8
5	STD-Q	5.0 µl	0.46	20180.6
6	PGEA	10.0 µl	0.46	15195.9
7	PGEA	20.0 µl	0.46	18630.9
8	PGEA	1.0 µl	0.46	3069.6

3.3 Evaluation of oral gel

Table No. 3.4 Evaluation of oral gel

Sr. No.	Parameter	F1	F2	F3
1.	Organoleptic characters			
	1. Color	Greenish	Greenish	Greenish
	2. Odor	Characteristics	Characteristics	Characteristics
	3. Texture	Smooth	Smooth	Smooth
	4. Nature	Semisolid	Semisolid	Semisolid
2.	pH	6-7	6-7	6-7
3.	Spreadability	17.14	14.28	11.76

4.	Homogeneity	Homogeneous	Homogeneous	Homogeneous
5.	Skin irritation test	No irritation	No irritation	No irritation

3.4 Evaluation by analytical method: HPTLC (Validation of formulation): Linearity Assay



(a)



(b)

Fig. No. 3.2 (a) @366 nm Linearity Assay (b) After Deerivatization Linearity Assay

Linear Calibration 1

Table No. 3.5 AUC of Linearity calibration 1

Track No.	**Track**	**Volume**	**Rf**	**AUC**
1	S-Q	4.0µl	0.38	2774.9
2	S-Q	4.0µl	0.37	2333.1
3	S-Q	6.0µl	0.37	2977.4
4	S-Q	8.0µl	0.36	3701.2
5	S-Q	10.0µl	0.36	4653.9
6	S-Q	12.0µl	0.36	4839.9
7	PGEA	5.0µl	0.36	898.1
8	PGEA	10.0µl	0.35	1610.5
9	PGF	5.0µl	0.35	1772.7
10	PGF	10.0µl	0.36	2903.9

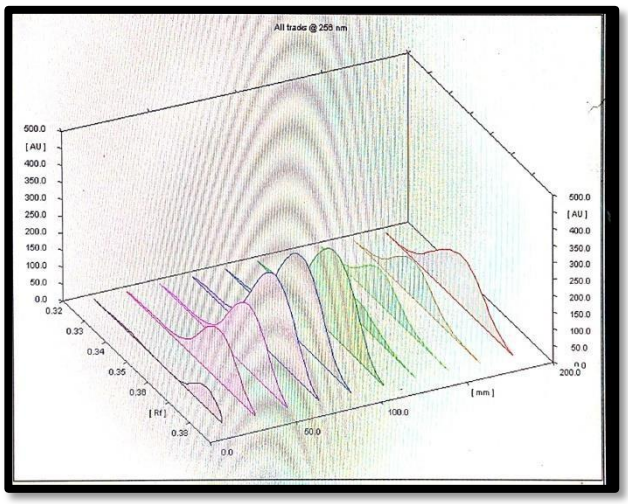


Fig. No, 3.3 3D Overlap

Linear Calibration 2

Table No. 3.6 AUC of Linearity calibration 2

Track No.	Track	Volume	Rf	AUC
1	S-Q	4.0µl	0.38	2828.0
2	S-Q	4.0µl	0.37	2385.6
3	S-Q	6.0µl	0.37	3099.1
4	S-Q	8.0µl	0.36	3965.9
5	S-Q	10.0µl	0.36	4849.1
6	S-Q	12.0µl	0.36	5110.4
7	PGEA	5.0µl	0.36	928.5
8	PGEA	10.0µl	0.35	1650.5
9	PGF	5.0µl	0.35	1772.8
10	PGF	10.0µl	0.36	2983.8

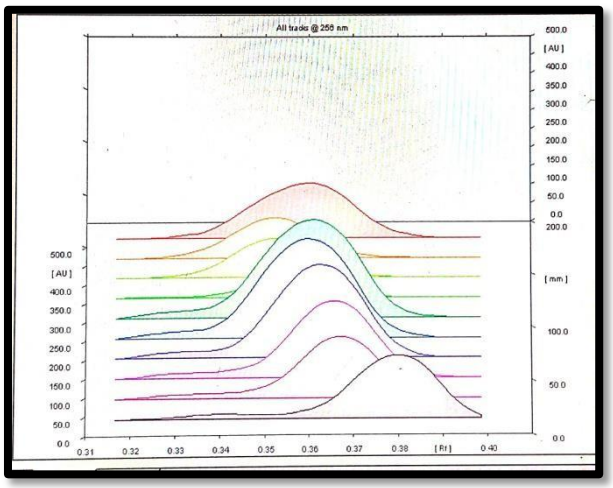


Fig. No, 3.4 3D Overlap

Linear Calibration 3

Table No. 3.7 AUC of Linearity calibration 3

Track No.	Track	Volume	Rf	AUC
1	S-Q	4.0µl	0.38	2847.0
2	S-Q	4.0µl	0.37	2392.2
3	S-Q	6.0µl	0.37	3401.8
4	S-Q	8.0µl	0.36	4742.9

5	S-Q	10.0µl	0.36	5335.2
6	S-Q	12.0µl	0.36	5777.9
7	PGEA	5.0µl	0.36	904.7
8	PGEA	10.0µl	0.35	1878.9
9	PGF	5.0µl	0.35	1832.8
10	PGF	10.0µl	0.36	2952.1

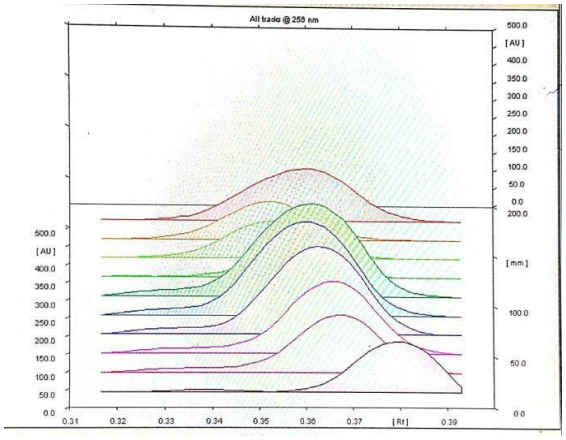


Fig. No, 3.5 3D Overlap

Table No. 3.8 SD and RSD

µL	Ng	Lin 1	Lin 2	Lin 3	Average	SD	RSD
4	400	2333.1	2385.6	2392.2	2370.3	32.38472	1.366271
6	600	2977.4	3099.1	3401.8	3159.433	218.5382	6.917005
8	800	3701.2	3965.9	4742.9	4136.667	541.4385	13.08876
10	1000	4653.9	4849.1	5335.2	4946.067	350.848	7.093475
12	1200	4839.9	5110.4	5777.9	5242.733	482.7992	9.208922
10	1000	1610.5	1650.5	1878.9	1713.3	144.8017	8.451623
10	1000	2903.9	2983.8	2982.1	2956.6	45.64745	1.543917

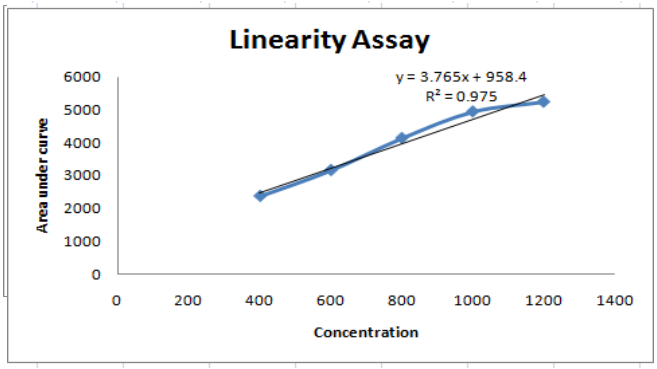


Fig. No 3. 6 Linearity curve

Evaluation of physicochemical properties of formulated P. guajava gel

From this study physicochemical properties of the P. guajava gel formulation from leaves ethyl acetate extract within acceptable limit. It showed good homogeneity, spreadability, non irritant to skin, smooth in texture, greenish in color, pH was ranged from 6-7.

Unknown concentration of standard in formulation and extract was found to be 0.79% and 2% by linearity assay by HPTLC.

4.CONCLUSION

Guava is a Indian folk medicine. Guava leaves extract contains carbohydrates, saponins, alkaloids, flavanoids, steroids, glycosides, tannins and phenolic compounds.

Guava leaves shows antibacterial, anti-inflammatory, antifungal, hepato-protective, antioxidant activities. It can be used to treat many diseases such as diabetes, diarrhea, mouth ulcer.
Fingerprinting of ethyl acetate and alcohol extract shows the presence of quercetin present in extract and formulation as a standard.
The calibration curve of querectin was found to be linear and the regression coefficient was
1.97as good.

REFERENCE

1. Mital Kaneria, Sumitra Chanda. Phytochemical and pharmacognostic evaluation of leaves of Psidium guajava L. (Myrtaceae). Pharmacognosy Journal. 2011; Vol 3 Issue 23 41-45.
2. Vikrant Arya, Narender Thakur, C. P. Kashyap. Preliminary phytochemical analysis of the extracts of Psidium leaves. Journal of Pharamacognosy and Phytochemistry. 2012; 1(1): 1-5.
3. Gayatri, V., Kiruba, D. Preliminary phytochemical analysis of leaf powder extract of Psidium guajava L. International Journal of Pharmacognosy and Phytochemical Research. 2014; 6(2): 332-334.
4. S. Anbuselvi, Jeyanthi Rebecca. Phytochemical biochemical and antimicrobial activity of Psidium guajava leaf extract. Journal of Pharmaceutical Sciences and Research. 2017; 9(12): 2431-2433.
5. Shruti Shirur Dakappa, Pharmacognostic evaluation of Psidium guajava Linn. leaves (Myrtaceae). Medicinal ans Aromatic Plant Science and Biotechnology2011; 5(2): 156-159.
6. Arain et al. Essential oil from Psidium guajava leaves: An excellent source of β-caryophyllene. Natural Product Communication. 2019: 1-5.
7. Adolf Nahrstedt. Polymeric proanthocyanidins from Psidium guajava. Scientia Pharmaceutica. 2005; 112-125.
8. SM EI Sohafy, AM Metawalli, FM Harraz, AA Omar. Quantification of flavanoids of Psidium guajava L. Preparation by Planer Chromatography (HPTLC). Phcog Mag 2009; 5: 61-6.
9. Arima H, Danno G. Isolation of antimicrobial compounds from guava (Psidium guajava L.) and their structural elucidation. Bioscience, Biotechnology and Biochemistry. 2002; 66(8): 172701730.
10. Elixabet Diaz-de-Cedrio, Vito Verado, Ana Maria Gomez-Caravaca, Alberto, Fernandez-Gutierrez, and Antanio Segura-Carretero. Determination of Polar Compounds in Guava Leaves Infusion and Ultrasound Extract by HPLC-ESI-MS. Hindawi Publishing Corporation Journal of Chemistry. 2015; Article ID 250919.
11. Batubara I, Suparto I H and Walundari N S. The best extraction technique for Kaemferol and Quercetin isolation from guava leaves (Psidium guajava). IOP Conf. Series: Earth and Environmental Science 58 (2017) 012060.
12. Ekeleme Kenneth, Tsaku Paul, Nkene Istifanus, Ufomadu Uba, Abimiku Rejoice, Oti Victor and Sidi Mohammed. Phytochemcal analysis and antibacterial activity of Psidium guajava L. leaf extracts.GSC Biological and Pharmaceutical Sciences. 2017; 01(02): 013-019.
13. Deepa Philip C, Indira Kumari R, Lavanya B. Phytochemical analysis, antioxidant and anti microbial activity of white & pink Psidium guajava Linnaeus. International Journal of Current Pharmaceutical Research.2015; Vol. 7, Issue 2, 29-31.
14. Gurnani P, Krishnan CGA, Gurnani R, Ghosh A, Shah A. Antibacterial activity of guava leaves extract against Lactobacillus acidophilus: An in-vitro study. International Journal of Oral Health and Medical Research. 2016; 2(6): 37-40.
15. Das M, Goswami S, Antifungal and antibacterial property of guava (Psidium guajava) leaf extract: role of phytochemicals. Int J Health Sci Res. 2019; 9(2): 39-45.
16. Biswas B, Rogers K, McLaughlin F, Daniels D, Yadav A. Antimicrobial activities of leaf extract of guava (Psidium guajava L.) on two gram-negative and gram positive bacteria. International Journal of Microbiology. 2013; Art. ID: 746165, 7 pages.
17. Manisha Pandey, Afifa Qidwai, Rajesh Kumar, Anand Pandey, Shashi Kant Shukla, Ashutosh Pathak and Anupam Dikshit. Pharmacological and antibacterial aspect of Psidium guajava L. against Acne vulgaris. International Journal of Pharmaceutical Sciences and Research. 2017; 8(1): 145-150.
18. Mushtaq M, Daud M, Khattak S, Samrana S. In vitro antimicrobial activity of guava leaves extract against important bacterial and fungal strain. International Journal of Bioscience. 2014; 10(4): 188-192.
19. Goncalves F. A., Andrade Nedo M., Bezerra J. N. S., Macrade A., Sousa O. V., Fonteles-Filho A. A. & Vieira R. H. S. F. Antibacterial activity of guava , Psidium guajava Linnaeus, leaf extract on diarrhea- causing enteric bacteria isolated from Seabob shrimp. Xiphopenaceus kroyeri (Heller). Rev. Inst. Med. Trop. S. Paulo. 2008; 50(1): 11-15.
20. Shetty YS, Shankarapillai R, Vivekanandan G, Shetty RM, Reddy CS, Reddy H, Mangalekar SB. Evaluation of the efficacy of guava extract as an antimicrobial agent on periodontal pathogens. Journal of Contemporary Dental Practice. 2018; 19(6): 690-697.
21. Fumi Yamanaka, Tsutomu Hatano. Antibacterial effects guava tannins and related polyphenols on Vibrio and Aeromonas species. Natural Product Communications. 2008; 3(5): 711-720.
22. Jongkwon Seo, Soojung Lee, Marcus L. Elam, Sarah A. Johnson, Jonghoon Kang, Bahram H. Arjmandi. Study to find the best extraction solvent for use with guava leaves (Psidium guajava L.) for high antioxidant efficacy. Food Science & Nutrition. 2014; 2(2): 174-180.
23. Julio Cesar Camarena-Tello, Hector Eduardo Martinez-Flores, Ma.Guadalupe Garnica-Romo,Jose Saul Padilla-Ramiewz, Alfredo Saavedra-Molina,Osvaldo Alvaerez-Cortes, Maria Carmen Bartolomo-Camacho and Jose Octavio Rodiles-Lopez. Quantification of Phenolic Compounds and In Vitro Radical Scavenging Abilities with Leaf Extracts from Two Varieties of Psidium guajava L. MDPI. 2018; 7:34.

24. Subba Rao Ch, Arun Kumar S, Javed Ali, Priyadarshini P, Keerthi R, Nivedita L, Priyanka D. In vitro antioxidant activity of Psidium guajava Linn. by using ethanolic extract fraction of leaves and bark. Adv Cell Sci Tissue Cult. 2018; volume 2 Issue 1 19-22.
25. K. Iamjud, N. Banyen, U. Boonprakob and K. Thaipong. Ascorbic Acid, Total Phenolics and Antioxidant Activity of Guava Leaf Extracts. Acta Horticulture. 2014; 366-372.
26. Salgado, H. R. N. Roncari, A. F. F. , Michelin, D. C., Moreira , R. R. D. Evaluation of antidiarrheal effects of Psidium guajava L. (Myrtaceae) aqueous leaf extract in mice. Journal of Basic and Applied Pharmaceutical Science. 2006; vol. 27: 89-92.
27. Noer Laily, Retno Windya Kusumaningtyas, Iim Sukarti, Maria Rosari Devi Kartika Rini. The Potency of Guava Psidium guajava (L.) Leaves as a Functional Immunostimulatory Ingredient. Procedia Chemistry. 2015; (14): 301-307.
28. . Baby Joseph & R Mini Priya. Preliminary Phytochemicals of Psidium guajava L. Leaf of Methanol Extract and its Cytotoxic Study on HeLa Cell Lines. Inverti Rapid: Ethnopharmacology. 2010; vol. 1 [ISSN 0976-3805].
29. Steve Endeguele Ekon, Jean-De-DieuTamokou. Methanol leaves extract of Psidium guajava Linn. exhibited antibacterial and wound healing activities. International Journal Current Microbiology and Applied Science. 2018; 7(7): 4008-4023.
30. Richa Singh, Sagar Bansal and Manoj Kumar Mishra. Formulation and evaluation of herbal oral gel containing extracts of powdered Psidium guajava Linn leaves with Curcuma longa Linn rhizomes to test mouth ulcer. International Journal of Drug Development and Research. 2020; 12(2): 150.
31. Shaikh Sabir, Shete Amol, Doijad Rajendra. Formulation and evaluation pharmaceutical aqueous gel of powdered guava leaves for mouth ulcer treatment. PharmaTutor. 2018; 6(4): 32-38.
32. Prashant P. Bhalchim, Manjusha N. Dole. Formulation and Evaluation of Herbal Gel Containing Psidium guajava Linn Leaves Extract. Asian Journal of Pharmaceutical Technology and Innovation. 2013; 03(15): 24-28.
33. Akila B, Vijaylakshmi R, Hemlatha G & Arunkumar R. Development and evaluation of functional property of guava leaf based herbal tea. Journal of Pharmacognosy & Phytochemistry. 2018; 7(3): 3036-3039.
34. Meghraj Ashok Patil. Phytochemical and in-vitro evaluation of formulated polyherbal hair conditioner. Journal of Pharmacognosy and Phytochemistry. 2019; 8(6): 35-39.
35. Firdous Shaikh, Priyanka Bhosale, Snehal More. Formulation and evaluation of herbal hand sanitizer using Psidium guajava leaves extract. International Journal of Pharmaceutical Sciences Review and Research. 2020; 62(2): 37-39.
36. Nur kamila Ramli, Zahid Majeed, Anis Suhaila Shuib, Nurlidia Mansor, Zakaria Man.
37. The potential of quercetin in Psidium guajava L. leaves extract as bioinhibitor for controlled released fertilizer. Advanced Materials Research. 2014; Vol. 970; 16-18.
38. N.S. Sampath Kumar, Norizah Mhd Sarbon, Sandeep Singh Rana, Anjani Devi Chintagunta, S. Pratibha, Satheesh Kumar Ingilala, S. P. Jeevan Kumar, B. Sai Anvesh and Vijaya Ramu Dirisala. Extraction of bioactive compounds from Psidium guajava and its utilization in preparation of jellies. AMB Express. 2021; 11:36 https://doi.org/10.1186/s13568-021-01194-9.
39. Shaik Shaheena, Anjani Devi Chintagunta, Vijaya Ramu Dirisala and N. S. Sampath Kumar. Extraction of bioactive compounds from Psidium guajava and their application in dentistry. AMB Express. 2019; 9:208 https://doi.org/10.1186/s13568-019-0935-x.
40. K. R. Kirtikar, B. D. Basu & I.C.S., Indian Medicinal Plants, edited, revised, & mostly rewritten by E. Blatter, J. F. Caius & K. S. Mhaskar, Volume II, Published by Lalit Mohan Basu, M.B. 49, Leader Road, Allahabad, India. Page no. 1045-1048.
41. Dr. K. M. Nadkarni's Indian Materia Medica, Originally edited by The Late Dr. K. M. Nadkarni, revised and enlarged by A. K. Nadkarni, Forwarded to Coloneal Sir R. N. Chopra, Volume One, Published by Popular Prakashan, Page no. 1017-1019.
42. Major B. D. Basu, I. M. S. (Retired), Indian Medicinal Plants, Plates Part II, from 268-514, Published by Periodical Experts Book Agency, D-42, Vivek Vihar, Delhi-110 095, Plate No. 421.
43. Indian Pharmacopoea 2014, volume I, Government of India Ministry of Health and Family Welfare, Published by The Indian Pharmacopoea Comission, Ghaziabad. Page no. 162-163.
44. 32. Quality Control Methods for medicinal plant materials, World Health Organization Geneva.
45. 33. Indian Pharmacopoea 1985, volume I, Government of India Ministry of Health and Family Welfare, Published by The Indian Pharmacopoea Comission, Ghaziabad, Page no. Appendix 5, A-124.
46. 34. Dr. S. S. Khadbadi, Dr. L. L. Deore, Dr. B. A. Baviskar, Experimental Phytopharmacognosy, Nirali Prakashan, Page no. 4.9
47. 35. Dr. K. R. Khandelwal, Dr. Vrunda Sethi, Practical Pharmacognosy Techniques and Experiments, Nirali Prakashan, Page no. 10.1- 10.4, 25.1-25.8.
48. Miss. Shinde P. R., Dr. Patil P. S, Dr. Bhambar R. S., A Textbook of natural Drug Technology, Unique Publications Pvt. Ltd., Page no. 165-171.
49. Lachman/ Lieberman's The theory and practice of Industrial Pharmacy, Fourth edition, CBS publisher and distributor, Page no. 741.
50. Mrudula H. Bele, Pharmaceutics, formulation and practicing of conventional dosage form, Career publication, Page no. 253.
51. The Merck Index, An encyclopedia of chemicals, drugs and biological, thirteenth edition, published by Merck research laboratories division of Merck and co. inc. Page no. 1438.