



Formulation And Evaluation Of Herbal Emulgel Of *Psidium Guajava* Leaves Extract For Antifungal Activity.

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1.ABSTRACT:

Herbal medicine has become an item of global importance both medicinal and economics. Although usage of these herbal medicines has increased but quality, safety and efficiency of these medicinal plants are serious concerns in industrialized and developing countries. Herbal remedies are getting increasing patient compliance as they are devoid of typical side effects of allopathic medicines. The present research has been undertaken with the aim to formulate and evaluate the herbal Emulgel containing *Psidium guajava* leaves extract. The Emulgel formulation was designed by using Carbopol 934, *Psidium guajava* leaves Hydro – alcoholic extract, propylene glycol, methyl paraben, propyl paraben and required amount of distilled water. The pH (6.8-7) was maintained by drop wise with addition of triethanolamine. The physicochemical Parameters of formulations were determined. Stability studies have carried out as per ICH guidelines. The present study was designated to evaluate the antifungal activities of ethanolic, methanolic, ethyl acetate and hot water extract from leaves of *Psidium guajava*. Compare to all parts, the leaves were showing the best activity and the zone of inhibition was obtained 25 mm in hydroalcoholic extract of leaves against *Aspergillus Niger*.

Key words: Antifungal property, *Psidium guajava* leaf, Hydroalcoholic Extract, Zone of inhibition.

2. INTRODUCTION:

Study of traditional medical practice, is an integral part of the culture and the interpretation of health by indigenous populations in many parts of the world. For example, Indian Ayurveda and traditional Chinese medicine are among the most enduring folk medicines still practiced. These systems try to promote health and improve the quality of life, with therapies based on the use of indigenous drugs of natural origin. Given that plants have been widely used as herbal medicines, several approaches are now being carried out to discover new bioactive compounds. *Psidium guajava* L., popularly known as guava, is a small tree belonging to the myrtle family (Myrtaceae). Native to tropical areas from southern Mexico to northern South America, guava trees have been grown by many other countries having tropical and subtropical climates, thus allowing production around the world. Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy. Moreover, other several uses have been described elsewhere on all continents, with the exception of Europe.^[1] Depending upon the illness, the application of the remedy is either oral or topical. *Psidium guajava* is evergreen shrub native to tropical. The *Psidium guajava* leaves and fruits were collected from America that has neutralized in South East Asia. The part of guava has been reported the wide range of activity against the human ailments 4,5, There are over 20 compounds have been reported present in leaves, stems, bark and roots of *P. guajava*. Guava leaves were used to treat diarrhoea and stomach. The leaves were used in TSA as an antibiotic in the form of poultice or decoction for wounds, ulcers and toothache. Guava fruits also contain vitamin C vitamin, iron calcium and phosphorus. Emulgel is a combination of emulsion and gel, which is a new approach for topical delivery of drugs. It has a double control release like emulsion and gel.^[2] Gel is new class of formulation, it releases the drug faster in comparison ointment, cream, and lotion. Incorporation of drug in emulgel formulation is suitable to treat skin disorders.^[3] Topical application of therapeutic agents provides various advantages over the other route of administration. The presence of a gelling agent in the aqueous phase converts a classical emulsion into an Emulgels. Within the major group of semisolid preparations, like use of transparent gels has expanded both in cosmetics and in pharmaceutical preparations.^[4] Emulgels have several complimentary properties for dermatological use such as being thixotropic, greaseless, easily spreadable, easily removable, emollient, non-staining, long shelf life, bio-friendly, transparent and pleasing appearance.^[5]

3.Plant Profile



fig.1 *Psidium guajava*

3.1Scientific Classification:

- Kingdom: Plantae
- Clade: Angiosperms
- Order: Myrtales
- Family: Myrtaceae
- Genus: *Psidium*
- Species: *P. guajava*

3.2 Botanical Name:

Psidium guajava

3.3 Synonyms:

Guava, *Guajava pyrifera*.

3.4 Description:

Leaves opposite, simple; stipules absent, petiole short, 3-10 mm long; blade oblong to elliptic, 5-15 x 4-6 cm, apex obtuse to bluntly acuminate, base rounded to subcuneate, margins entire, somewhat thick and leathery, dull grey to yellow-green above, slightly downy below, veins prominent, gland dotted. ^[6]

3.5 Habitat:

It is native to central America from Mexico to northern South America^[7]

3.6 Part used:

Leaves.

3.7 Traditional uses:

Powdered leaves are used for Anti-diarrhoeal, Antifungal. Other reported uses include gastroenteritis, dysentery, stomach, antibacterial colic pathogenic germs of the intestine. Its medicinal usage has been. An infusion of the leaves is used for bilious fever, eczema, and eruptions^[8]

4. Material and Methods

Plant material:

Collection and Drying

Fresh Leaves of *Psidium gujava* were collected from the fields of Kharadi goan Dist -Pune, Maharashtra India. cleaned and dried at a room temperature. The dried leaves were coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60 to 120 mesh to remove fines and larger particles and the powder was subjected for further study^[9]



fig.2 Collection of leaves

5. Pharmacognostic Study-

5.1 Macroscopy

Size: 5-15cm long leaves.

Shape: The leaf is elliptic to oblong in shape

Color: Dark-Green

Odor: Myrtates

Taste: Slightly bitter

5.2. Microscopy

Thin Transverse section of middle part of fresh leaf was taken, stained with Phloroglucinol- con. HCl (1:1), observed under 10X, 45X^[10]

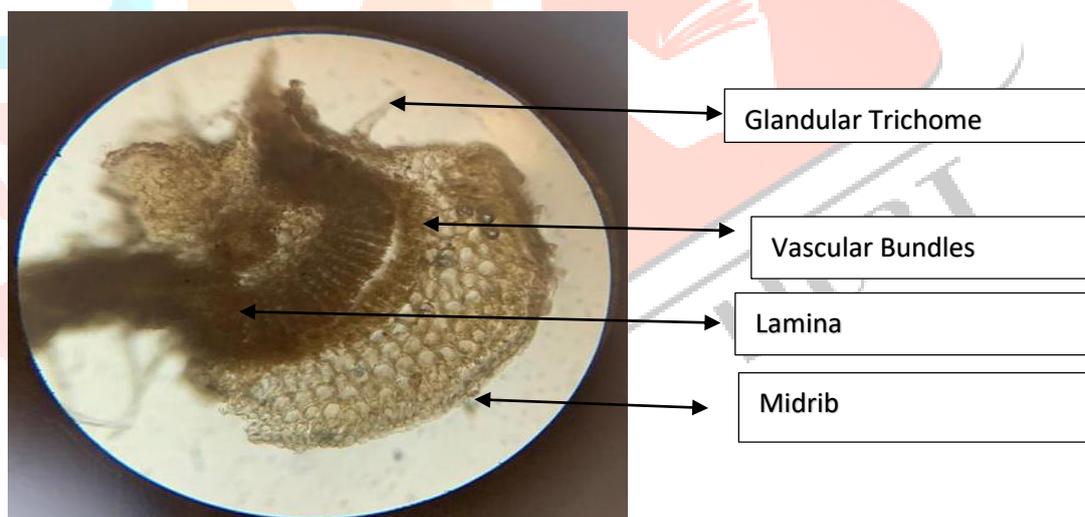


fig.4 TC of Leaves

5.3. Microscopic studies of Powder characteristics

The microscopic examination of powdered leaves material was performed to detect and to establish various peculiar microscopic characters in order to differentiate between the adulterated and the substituted powdered or intact leaves supply. Slides of powdered leaf material was prepared using formalin, glycerine and water (8:1:1 v/v/V) and were thus embedded and seen under microscope on different magnifications at 10x, 40x, and 100x after staining with phloroglucinol and conc HCL^[11]

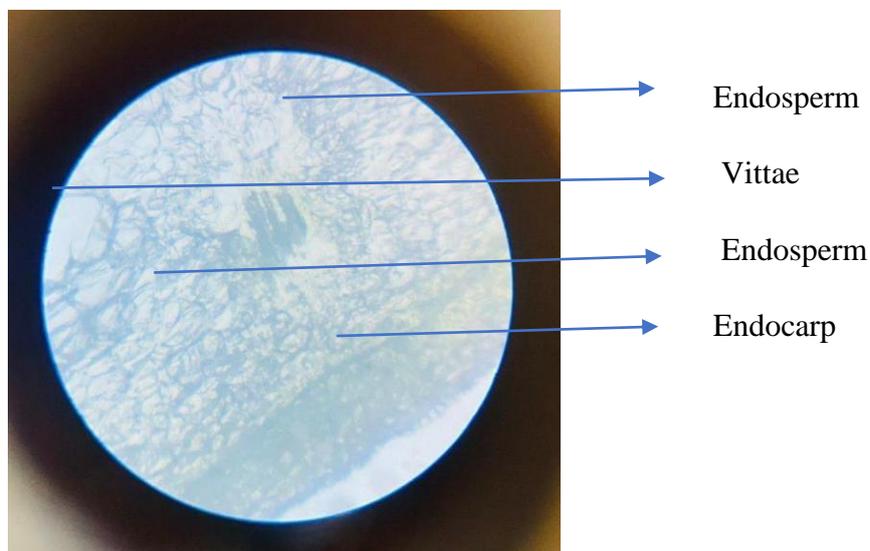


Fig.5 Powder Characteristics

5.4. Determination of Loss on Drying

Fresh leaves (3 gm) was taken without preliminary drying and was placed on a tarred evaporating dish and dried at 105°C for six hours and weighed. The drying was continued until two successive reading matches each other or the difference between two successive weighing after drying for 30 minutes in a desiccator, showed not more than 0.01 g difference.

Weighed and calculated the loss on drying in terms of percent w/ w.^[12]

Observation

Weights of powdered sample taken = 3 gm

Weights of powdered sample After drying = 2.18gm

Result:

The percentage of loss on drying = 72 % w/w

5.5. Determination of ash Value

Ash value is useful in determining authenticity and purity of drug and also these values are important quantitative standards. Ash value is used to determine quality and purity of crude drug. Ash value contains inorganic radicals like phosphates, carbonates and silicates of sodium, potassium, magnesium, calcium etc. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of the crude drug affects.^[26]

Procedure

2 gm of the air dried powdered drug was taken in a crucible and ignited in the furnace at 150°C for 45 minutes and weighed same procedure was followed for 4 times till constant weight of the ash, was formed weight of the ash was taken and calculated the percentage of total ash with reference to the air dried sample of the crude drug. ^[13]

Before ignition of powder:

- Weight of empty silica crucible = 15.78 gm
- Weight of powder = 3gm
- Weight of silica crucible + powder = 18.78gm

After ignition of powder:

- Weight of silica crucible + ash = 16.49 gm
- Total ash = 0.71 gm

- **Result**

The percentage of total ash obtained = 23 % w/w

5.5. Extraction Methodology:

Material and Methods:

Preparation of the plant extract:

Powder sample of leaves *Psidium gujava* measuring 100 gm was extracted using 500 ml of hydroalcoholic solvent by maceration method after filtration the extract was concentrated using a rotatory vacuum evaporated and the semidried extract were dried kept in air tight. ^[14]

5.6 Percentage yield of Extract:

It indicates the approximate measures of the chemical constituent plant. All the values were taken in triplicate and the mean average was taken. 50 gms of the powdered drug was taken in a weighing bottle and transfer it in 500 ml graduated flask filled with the solvent (90 % alcohol).

Flask was closed and set aside for 24 hours, shaking frequently. (Maceration) extract was filtered into a 50 ml cylinder. When sufficient filtrate has collected, 100 ml. of the filtrate was taken and evaporated to dryness on a water-bath and complete the drying in an oven at 100° C.it was cooled in a desiccator and weighed ^[15]

percentage w/w of extract was calculated with reference to the air-dried drug.

- Weight of powdered taken. = 50 gms
- Weight of Extract obtained = 7.45gms

Result:

Percentage yield of hydro-alcoholic extract was found to be 7.45% w/w.

5.7 Preliminary Phytochemical Screening**1. Test for Steroids^[16]****a) Salkowski test:**

One ml of concentrated sulphuric acid was added to 10 mg of extract dissolved in 1 ml of chloroform. A reddish brown colour exhibited by chloroform layer and green fluorescence by the acid layer suggests the presence of steroids.

b) Liebermann test:

2 mg of the residue few ml of acetic anhydride was added and gently heated. The contents of the test tube were cooled and 2 ml of concentrated sulphuric acid was added from the side of the test tube. Development of blue colour gave the evidence for presence of sterols.

c) Liebermann-Burchard test:

10 mg extract was dissolved in 1ml of chloroform and 1ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid from the sides of the test tube. Formation of reddish violet colour at the junction indicates the presence of steroids.

2. Test for Saponins^[17]**a) Foam formation test:**

1ml solution of the extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. The development of stable foam indicates the presence of saponins.

3. Test for Alkaloids^[18]**a) Dragendorff's test:**

0.1 ml dilute hydrochloric acid and 0.1 ml Dragendorff's reagent was added in 2 ml of extracts in test tube. Formation of orange brown precipitate indicates the presence of alkaloids.

b) Mayer's test:

0.2 ml of extract was taken in test tube. 0.2 ml of dilute hydrochloric acid and 0.1 ml of Mayer's reagent were added. Formation of yellowish buff precipitate indicates the presence of alkaloids.

c) Wagner's test:

2 ml of extract was treated with 0.2 ml dilute hydrochloric acid and 0.1 ml of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. Test for Glycoside^[19]

a) Modified Borntrager's test:

The extract was treated with ferric chloride solution and heated on Bolling water bath for 5 min. The mixture was cooled and shaken with equal volume of benzene. The benzene layer was separated and treated with half its volume of ammonia solution. The formation of rose pink or cherry red colour in the ammoniacal layer indicates the presence of anthraquinones.

b) Million's test:

The extract was treated with 2 ml of Million's reagent. The formation of white precipitate, which turns to red upon heating indicates the presence of proteins and amino acids,

5. Test for Tannins^[20]

a) Ferric Chloride test:

Five ml of extract solution was allowed to react with 1 ml of 5 per cent ferric chloride solution. Greenish black colouration indicates the presence of tannins.

b) Lead Acetate test:

5 ml of extract was treated with 1 ml of 10 per cent aqueous lead acetate solution. Development of yellow coloured precipitate indicates the presence of tannins.

c) Potassium Dichromate test:

Five ml of extract was treated with 1 ml of 10 per cent of aqueous potassium dichromate solution. Formation of yellowish-brown precipitate suggests the presence of tannins.

6. Test for Proteins^[21]

a) Biuret test:

The extract was treated with 1 ml of 10 per cent sodium hydroxide solution and heated. A drop of 0.7 per cent copper sulphate solution was added to the above mixture. The formation of purplish violet colour indicates the presence of proteins.

b) Xanthoproteic test:

A little test residue was taken in 2 ml of water and to it 5 ml of concentrated nitric acid was added. Formation of yellow colour indicates the presence of proteins.

c) Million's test:

The extract was treated with 2 ml of million's reagent. The formation of white precipitate, which turns to red upon heating, indicates the presence of proteins and amino acids.

7. Test for Amino Acids^[22]

a) Ninhydrin test:

The extract was treated with Ninhydrin reagent at pH range of 4-8 and boiled. Development of purple colour indicates the presence of amino acids.

8. Test for Carbohydrates^[23]

a) Molish test:

Two ml of extract solution was treated with few drops of 15 per cent ethanolic alpha-naphthol solution in a test tube and 2 ml of concentrated sulphuric acid was added carefully along the sides of the test tubes. The formation of a reddish violet ring at the junction of two layers indicates the presence of carbohydrates

b) Barfoeds test:

The test residue was dissolved in water and heated with a little quantity of Barfoed's reagent. Development of brick red precipitate within wt.0 minutes shows the presence of monosaccharide.

9. Test for Reducing sugars^[24]

a) Fehling's test:

Five ml of extract solution was mixed with 5 ml of Fehling's solution equal mixture of Fehling's solution A&B & boiled. Formation of brick red Precipitate indicates the presence of reducing sugars.

b) Benedict's test:

Equal volumes of Benedict's reagent and extract in test tube boiled for min solution appeared green, yellow or red depending upon the amount of reducing sugar present



Fig: preliminary test (1)



Fig: preliminary test (2)

Table:1 Preliminary Phytochemical Screening

Chemical tests	Hydroalcoholic Extract	Methanol
Test for Steroids	+	-
Test for Saponins	+	+
Test for Alkaloid	+	+
Test for Glycosides	+	+
Test for Reducing sugars	-	-
Test for Tannins	-	+
Test for Flavonoids	+	-
Test for Amino acids	+	-
Test for Carbohydrates	+	-

5.8 Formulation of emulgel:

Formulations with different quantity of ingredient were made as shown in Table 1. The gel portion of the emulgel was made by dissolving carbopol-934 in cold water with constant stirring at a moderate speed until uniform mixture was made. The pH was then adjusted to 6-6.5 using triethanolamine (TEA). Tween 80 was dissolved in distilled water to prepare the aqueous phase of the emulsion while for the preparation of the oil phase of the emulsion; span 80 was dissolved in liquid paraffin. To preserve the emulsion, methyl parabene was dissolved in propylene glycol and the extract was dissolved in ethanol then both solutions were mixed with the aqueous phase. Both the aqueous and the oil phase were heated in a water bath at 70 °C separately. Then the oil phase was added drop wise to the aqueous phase with continuous stirring using homogenizer at speed of 3000 rpm for 10 min then cold to room temperature. At the end the gel and emulsion portions were mixed in 1:1 ratio with moderately stirring to prepare emulgel [25]

Table: 3

Sr.No.	Ingredient	Quantity Taken
1	Hydroalcoholic extract	5gm
2	Carbopol 934	0.75 gm
3	Liquid paraffin	2.5ml
4	Span 60	0.45ml
5	Tween 80	0.50ml
6	Propylene glycol	3.5ml
7	Methyl paraben	0.01gm
8	Distilled water	Up to q.s.

5.9 Antifungal Assay:

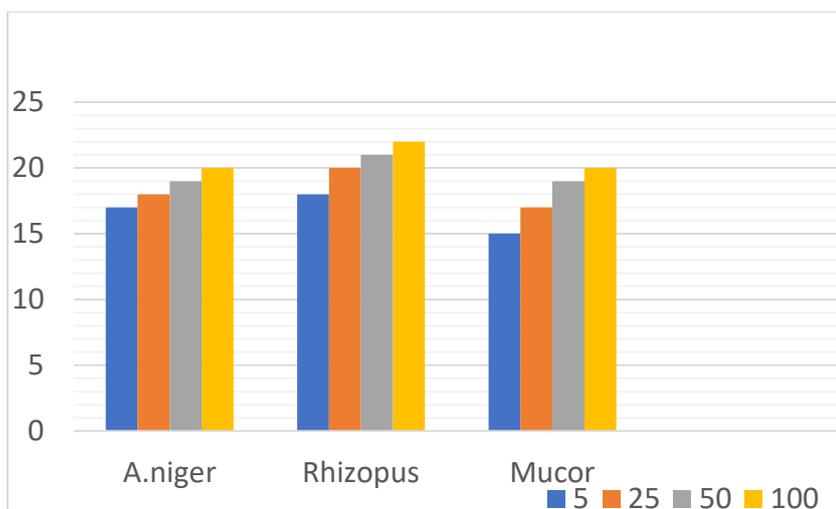
Three fungal strains (*Aspergillus niger*, *Rhizopus*, *mucor*) These strains have been selected for the basis of its application purpose of further formulation study. antifungal potential of emulgel was assessed in terms of zone of inhibition of bacterial growth. The results of the antifungal activities are presented in Table.2^[26]

Table:2

Antifungal activities of EMULGEL of leaves *Psidium gujava* against fungal organism

Antifungal Activity (zone of inhibition)			
Concn. In ($\mu\text{g/ml}$)	Zone of inhibition in mm <i>A . Niger.</i>	Zone of inhibition in mm <i>Rhizopus</i>	Zone of inhibition in mm <i>Mucor</i>
5	17	18	15
25	18	20	17
50	19	21	19
100	20	22	20

Antifungal activities of the extracts increased linearly with increase in concentration of extracting ($\mu\text{g/ml}$). As compared with standard drugs, the results revealed that in the extracts for fungal activity, *Rhizopus* shows good result as compare with *Aspergillus Niger* and *Mucor* The growth inhibition zone measured ranged from 11 to 20mm for all the sensitive bacteria, and ranged from 14 to 20mm for fungal strain

Table:3

5.10. Evaluation of Topical Gel Formulation

A. Physical Evaluation:

Physical parameters such as color and appearance were checked.

B. Measurement of pH:

pH of the gel was measured by using Ph meter.

C. Viscosity:

Viscosity of gel was measured by using Brookfield viscometer with spindle.

D. Spreadability:

Spreadability was determined by the apparatus which consists of a wooden block, which was provided by a pulley at an end. By this method spreadability was nearest on the basis of slip and drag characteristics of gel. An excess of gel (about 2g) under study was placed on the ground slide. The gel was then sandwiched between this slide and another glass side having the dimension of fixed ground slide and provided with the hook. A one kg weighted was placed on the top of the two sides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the edges. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5cm be noted. A shorter interval Indicate better spreadability. Spreadability was calculated using the following formula:

$$S=M/L/T$$

Where,

S=Spreadability,

M-Weight in the pan (tied in the upper slide)

L-Length moved by the glass side

T=Time (in sec.) takes to separate the sã de completely each other.

E. Stability Study:

The stability study was performed as per ICH guidelines & The Formulated gel were filled in the collapsible tubes and stored at different temperatures and humidity conditions, vix 250 C/20C/ 60% 5% RH 300 C 20C/65% /5% RH 400 C20C/ 75% 15% RH for a period of three months and studied for appearance, pH, and spread ability.

6.Result and Disussion:

Psidium guajava leaves under microscope T.S shows Midrib, lamina, vascular bundles. L.S. shows Xylem Phloem and Fibers. *Psidium guajava* leaves after drying calculation of loss of drying was to be obtained 72% w/w and powder under muffle furnace gives ash Value of 23% w/w. *Psidium guajava* leaves after maceration extraction and constant stirring in water bath gives the product, that product was calculated 14.90% w/w to be obtained. *Psidium guajava* showing Preliminary Phytochemical Screening Hydro-Alcoholic Extract shows presence of Steroids, Saponins, Flavonoids. Hexane Extract shows presence of flavonoids, glycosides are present.

7.Conclusion:

Emulgel of, *Psidium guajava* leaves showed prominent anti-fungal activity against human pathogenic, thus *Psidium guajava* leaves can be used as a potential antifungal drug against human pathogenic fungal infection. Lupeol and Taraxerone are measure constituents of Hydro alcoholic extract of *Psidium guajava* leaves. In-vivo studies performed for activities like anti-Bacterial, anti-microbial and anti-fungal showed the beneficial use of 20% w/w of hydroalcoholic extract of *Psidium guajava* leaves for the said activities. It was also supported by molecular docking study carried out for Lupeol and Taraxerone which gives better Dock score for mentioned activity.

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