

Development and Evaluation of Dosage Form Prepared from an Ethnomedicine-*Eriobotrya japonica*

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OF

MASTER OF PHARMACY (AYURVEDA)

(Rasashastra & Bhaishajya Kalpana)

By

Amrit Pal Singh

Reg. No.11606239

Under the guidance of

Dr. Manish Vyas

(Associate Professor)



Ayurvedic Pharmacy

Lovely School of Pharmaceutical Sciences

Lovely Faculty of Applied Medical Sciences

Lovely Professional University (Punjab)

Punjab 144411

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Statement by the Candidate

This is to submit that this written submission in my project report entitled “**Development and Evaluation of Dosage Form Prepared from an Ethnomedicine - *Eriobotrya japonica***” represents original ideas in my own words and where others 'ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have stuck to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be because of disciplinary action by the School and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when required. I assure and hold full responsibility for its genuineness.

Forwarded Through

Dr. Manish Vyas

Associate Professor

Amrit Pal Singh

Reg. No.11606239

Certificate by Supervisor

The work described in this project report entitled “**Development and Evaluation of Dosage Form Prepared from an Ethnomedicine - *Eriobotrya japonica***” has been carried out by **Amrit Pal Singh** (11606239) under my supervision. I certify that this is her bonafide work. The work described is original and has not been submitted for any degree to this or any other university.

Date:

Place:

Dr. Manish Vyas

Associate professor

Certificate by School

This is certified that the work described in this project report entitled “**Development and Evaluation of Dosage Form Prepared from an Ethnomedicine - *Eriobotrya japonica***” Has been carried out by **Amrit Pal Singh** at the School of Ayurvedic Pharmaceutical Sciences, Lovely Professional University, Punjab.

Mr. Saurabh Singh Baghel

(COD)

Ayurvedic Pharmacy

Dr. Monica Gulati

(Professor & Sr. Dean)

Sr. Dean and Head of School

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Chapter-I INTRODUCTION

Ethnomedicine is traditional medical practice of indigenous cultural that gave remedial and palliative effects of a complex multi-disciplinary system constituting the use of Herbs, spirituality and the natural environment and which having the ability to promote the health and healing potential for humanity. Ethnomedicinal treatment is not a part of any medical system but still it's having monumental impact and contribution during the evolution of various medicine systems i.e. Ayurveda, Siddha, Unani, Naturopathy As well as modern medicine on different time interval.

From last decade, scientific research tremendously enhance in field of ethnomedicine to know innumerable possibilities in field of health sciences. *Eriobotrya japonica* (E. japonica) is traditional medicinal plant and its different parts are used to treat diverse pathophysiology and as well as food products consumed in daily life. Mainly used in East Asian countries like Japan, China, Korea, India, Nepal, and Pakistan. In India, it is mainly used in Uttar-Pradesh, Punjab, and Himachal Pradesh. The traditional healers and Vaidyas of Punjab have been using Swarasa of leaves of *E. japonica* to treat the diabetes. In addition to this, the recent studies based upon Anti diabetes effect of *E. japonica* are suggesting its role to reduce blood glucose^{[1],[2],[3]}. Moreover, it also possesses therapeutic properties anti oxidant, anti viral^[2], neuroprotection^{[4],[5]}, cardiovascular health^[6], interactions with glucose metabolism^{[7],[8],[9],[10]}, obesity and fat mass^[11], bone and joint health^[12], inflammation and immunology^{[13],[14],[15],[16]}, interactions with hormones^{[17],[18]}, peripheral organ systems^{[19],[20],[21],[22],[23]} and interactions with cancer metabolism activities^{[24],[25],[26]} are shown.

Chapter-II

LITERATURE REVIEW

2.1 Review of ghana & ghanavati

2.1.1 Samhita Kala: Various references are available regarding raskriya in Charaka and Sushruta samhita which can be compared with ghana kalpana.

Classical text	Description	Reference
Charak Samhita ^[27]	Ghana is 1 st time mentioned in charak samhita. Charak has mentioned the ghana kalpana according to its method of preparation and consistency. (Dadhi)	Ch. Chi. 15/229
Sushruta Samhita ^[28]	Acharya Dalhana defined raskriya. In raskriya decoction is prepared from 8 or 16 times of water and evaporated till 1/8 or 1/16 parts of water remain. Then, subjected to further heating till it becomes thick.	Su. Su. 37/21 (Dalhana Tika)

Table 2.1: Description available in samhita kala

2.1.2 Formulations mentioned in Charak samhita:

S.No.	Formulations	References
1	Dravyadi raskriya ^[29]	Ch. Chi. 26/202
2	Pippalyaadi raskriya ^[30]	Ch. Chi. 26/258
3	Krishnasarparasadi raskriya ^[30]	Ch. Chi. 26/259
4	Dhatryadi raskriya ^[31]	Ch. Chi 26/260-261

Table 2.2: Rasakriya mentioned in Charaka samhita

2.1.3 Sangraha Kala

Various references are available in Astanga sangraha, Bhavaprakash, Ayurveda prakash and Sharangdhara samhita which can be correlated with Ghana kalpana.

Classical text	Description	References
Astanga Sangraha ^[32]	Rasakriya is one of the type of anjana kalpana.	Ref. 11
Bhavaprakash ^[33]	Description about rasanjana is available. Decoction of darvi added with an equal quantity of milk and heated till it becomes	6/203 Haritakyadi.

	thick termed as rasakriya.	
Ayurveda Prakash	The method of preparation of rasanjana with aja milk.	A.P. 2/234
Sharangadhara Samhita ^[34]	Examples of rasakriya under anjana kalpana is mentioned i.e. davryadi rasakriya, babul rasakariya.	Sha. Sam. Ut. Kha. 13/93 , 13/101

Table 2.3: Description available in Sangraha kala

2.1.4 Adhunik Kala

Sidha Yoga Samgraha:

Guduchi ghanavati is mentioned by Acharya Yadavaji Trikamaji as Samshani Vati in Jwaradhikar^[35].

2.1.5 Ghana

Ghana is a concentrated form of the liquid material of plant like swarasa and decoction etc. Ghana is actually a dried aqueous extract. The liquid content of 'kwatha' is evaporated with the help of heat. In ayurvedic literature, the Raskriya is considered as Ghana, Phanita, Avleha because the method of preparation of these dosage forms is similar except minor differences. Avleha is semi-solid which can be licked whereas 'Ghana' is a solid form.

2.1.5.1 Definition of Ghana

Swarasa and kwatha of plant parts when further heated to concentrate is known as rasakriya.^[36]

“Aqueous solutions of plants i.e. swarasa and kwatha when concentrated with the help of heat to get the semi-solid form is termed as Ghana.”

2.1.5.2 Synonyms of Ghana

There is no synonyms mentioned for Ghana but due to the common pharmaceutical procedure of Leha, alveha, and phanita,^[36] has mentioned as synonyms of raskriya by Acharya Sharangdhara. All these terms have their specific meaning and therapeutical value. Avleha and khanda are concentrated form liquids with sweet substance but Ghana and raskriya are concentrated forms of aqueous solution without sweet substance.

2.1.5.3 Advantages of Ghana:^[37]

- Highly concentrated.
- Reduced dose.
- More shelf life.

- More stable.
- Increased bioavailability.

2.1.6 Ghana Vati

Ghana vati is compressed pill form of ghana. Many formulations of ghana vati are available in classical texts like sarpagandha ghana vati and guduchi ghana vati. In charak samhita it is mentioned that vati is prepared from semisolid form.^[38]

2.1.6.1 Vati kalpana:

The pill form of the medicines is a convenient form for the patient as well as a physician in treatment ^[39]. Vati is made in the shape of flat circular mass hence it is similar to the tablet.

2.1.6.2 Definition: Medicines prepared in the form of tablet or pills are known as Vati or Gutika.^[40]

2.1.6.3 Synonyms of vati: Gutika, vati, vatika, modak, pindi, guda and varti.

2.1.6.4 Types of vati kalpana:

1. Agnisadhya Vati: Vati is prepared with the help of heat. The sugar or jiggery or guggulu is prepared like leha on mild heat and powder materials are added to leha and then vati is made by rolling, circular in shape
2. Anagnisadhya Vati: Vati is made without heat. Powder materials are triturated with guggulu, guda or suggested liquid or to make the vati.^[41]

2.1.6.5 Dose of ghanavati:

The dose of Ghana is not clearly mentioned in our classics but, there is reference regarding Samsamani Vati in Siddha Yoga Sangraha. The dose of Ghana is mentioned as 5 to 10 Vati of 2 Ratti (250 mg) four to five times a day. So, the dose of Ghana may be taken 5- 10 g per day.^[42]

2.1.6.6 Some formulations of ghanavati with references: ^[40]

S. No.	Formulation	Reference
1	Kutajaghana vati	Siddhayogasangraha, atisara-pravahika-grahanyadhikara
2	Sarpagandhaghana vati	Siddhayogasangraha, bhrama-anidra-unmadadhikara
6	Sarpagandhaghana Vati	Siddhayogasangraha, bhrama-Anidra-unmadadhikara
7	Samsamani Vati (Guduchi Ghana Vati)	Siddayogasangraha, jvaradhikara

Table 2.4: Examples of ghanavati with reference

2.2 LITERATURE REVIEW OF DRUG

2.2.1 Loquat (*Eriobotrya japonica*):

Loquat (*Eriobotrya japonica*) is indigenous plant of china. Loquat is a non classical plant in Ayurveda but in Unani known as lokaat and in siddha Ilakatta.

2.2.2 References found in different text about Loquat.

Sr no.	Name of text	References
1.	The Japanese Pharmacopoeia	The Japanese Pharmacopoeia seventeenth edition official from april 1,2016 English version by The Ministry of Health Labour and Welfare page no-1909
2.	Indian Medicinal Plants An Illustrated Dictionary	Khare C.P. Indian Medicinal Plants An Illustrated Dictionary published by Sringer Page no-242-243
3.	Indigenous Drugs of India Their Medical And Economic Aspects	Chopra R. N. Indigenous Drugs of India Their Medical And Economic Aspects published by The Art Press, Calcutta

Table 2.5: Showing the classification of *Loquat* according to different text

2.2.3 Taxonomic position of Loquat (*Eriobotrya japonica*)^[43]:

Kingdom – Plantae

Division – Magnoliophyta

Class- Magnoliopsida

Subclass - Rosidae

Order – Rosales

Family – Rosaceae

Genus – *Eriobotrya*

Species – *E. japonica*

Generic Group- Rose

2.2.4 Vernacular name ^[44]:

English : Loquat, Japanese Medlar, Japanese plum ^[45]

Tamil : Nokkotta

2.2.5 Description of plant ^[45]:

Eriobotrya japonica is a small, short-trunked, upward-branching, broadleaf evergreen tree that typically grows to 10-25' tall with a round form. It also often grows as a large spreading shrub. It is generally noted for its compact size, attractive foliage, fragrant flowers and edible fruit.



Figure: 2.1 Habitat of *Eriobotrya japonica* is a Evergreen plant.

a) Macroscopy:

- **Leaf** - Loquat Leaf is an oblong to wide lanceolate leaf, 12 – 30 cm in length, 4 – 9 cm in width; pointed at the apex and wedge-shaped at the base; roughly serrate leaf with short petiole; occasionally being cut into strips 5 – 10 mm in shorter diameter and several cm in longer diameter; upper surface green to green-brown in color, lower surface light green-brown with light brown woolly hairs; vein, light yellow-brown in color, raised out on the lower surface of the leaf. Odor, slight; practically tasteless. ^[46]
- **Flower** - Sweetly fragrant, five-petaled, white flowers in large panicles (to 6" long) bloom in late fall-early winter. ^[45]



Figure: 2.2 Leaves of Loquat

- **Fruits** – Small spherical to pear-shaped fruits (to 1-2" long), each with juicy flesh. Fruits typically ripen in spring. Fruits have smooth to downy, yellow to orange skin. [45]
- **Seed** - One to several large seeds. [45]

b) Microscopy: a transverse section of Loquat Leaf reveals thick cuticle on both surfaces; palisade tissue, mostly 4 to 5 layers with several large cells without chloroplast; at main vein, ring of collateral bundle partly cut by intruding fundamental tissue at xylem side, and group of fiber attaching to phloem; solitary and clustered crystals of calcium oxalate in mesophyll; woolly hair, unicellular and curved, about 25 μ m in thickness, and up to 1.5 mm in length [46].

2.2.6 Distribution: Cultivated mainly in Saharanpur, Dehradun, Muzaffarnagar, Meerut, Kanpur, Bareilly districts of Uttar Pradesh, Amritsar, Gurdaspur and Hoshiarpur districts of Punjab. [44]

2.2.7 Chemical constituents of Eriobotrya japonica:

Sr. no.	Active principle	Parts
1	Euscaphic acid ^[47]	Leaves
2	1 β -hydroxyeuscaphic acid ^[48]	
3	3-O-trans-feruloyl euscaphic acid ^{[49][50]}	
4	<u>Ursolic acid</u> ^[51]	
5	Corosolic acid ^[47]	
6	3-epicorosolic acid ^[48]	
7	Oleanolic acid ^[52]	
8	α -hydroxyoleanolic acid ^[53]	
9	δ -oleanolic acid ^[48]	

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10	Betulinic acid and methyl betulinat ^[48]		
11	Arjunic acid ^[55] and methyl arjunolat ^[48]		
12	Pomolic acid ^[52]		
13	Tormentic acid ^[51]		
14	Hyptadienic acid ^{[54] [51]}		
15	Nerolidiol glycosides ^{[56][57]}		
16	Cinchonain Id 7-O-glucoside ^{[58][59]}		
17	Cinchonain Id 7-O-glucoside ^{[58][59]}		
18	<u>Chlorogenic acid</u> and methyl chlorogenate, ^[60]		
19	Ferulic acid ^[58]		
20	β -sitosterol ^[61]		
21	Oleanolic acid ^[62]		Flower
22	Ursolic acid ^[62]		
23	Amygdalin ^[62]		
24	Proteins ^[63]		Seed
25	Lipids ^[63]		
26	Sugars (carbohydrate) ^[63]		
27	Amygdalin ^[63]		
28	Catechins ^[63]		
29	Chlorogenic acid ^[63]		
30	fructose , glucose, trace sorbitol and sucrose ^[64]	Fruit	
31	Vitamin A ^[65] and other carotenoids		
32	Epicatechin ^[66]		
33	Chlorogenic acid ^[66]		
34	Cyanidin glucosides ^[67]		
35	Caffeic acids ^[66]		
36	Hydroxybenzoic acid ^[66]		
37	Ferulic acid ^[66]		
38	Vitamin C ^[68]		

Table 2.6: Chemical constituents of Eriobotrya japonica in different parts

2.2.8 Part used : Leaf, Fruit, Flower and Seed

2.2.9 Reported pharmacological actions:

S. No.	Pharmacological activity	Part used	Extract	Model	Dose
1.	Anti-inflammatory activity ^[69]	Leaf	aqueous extract	The mouse paws edema model	-
			ethyl acetate-soluble	12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ear edema of mice	0.03–0.43 mg per ear
2.	Anti-Diabetic Activity ^[69]	Leaf	ethanol extract	Alloxan-diabetic mice	300 mg/kg
3.	Anti-Cancer Activity ^[69]	Leaf	aqueous extract	7,12-dimethylbenzoanthracene (DMBA)-induced breast cancer in rats	-
			ethanol extract		
4.	Antioxidant Activity ^[69]	Leaf	n-butanol, methanol and water extract	free radical generation using the dichlorofluorescein method	-
5.	Hepatoprotective Activity ^[69]	Seed	70% ethanol and methanol extracts	The dimethylnitrosamine-induced hepatopathic rats	-

Table 2.7: Pharmacological actions of Loquat

Chapter- III

SCOPE OF STUDY

E. Japonica is being in East Asian countries like Japan, China, Korea, India, Nepal, and Pakistan for the treatment of various disorders. Recent studies based on the *E. Japonica* showing its wide array of therapeutic activities like anti oxidant, anti viral neuroprotective activity, cardio protective, anti-obesity, and anti-diabetic. It has been observed that traditional healers and vaidyas of the Punjab are using *E. Japonica* in the form of swarasa for the treatment of diabetes. But, swarasa has some pharmaceutical limitations like stability, palatability, efficacy, inadequate dose and patient compliances. Therefore, in the present study an effort will be made to prepare the ghanvati of the *E. Japonica* to improve its drawbacks.

Chapter-IV

AIMS AND OBJECTIVES

4.1 AIMS

Pharmaceutical development and evaluation of Loquat ghanavati

4.2 OBJECTIVES:

- To authenticate the crude drug.
- To develop the method of preparation of Loquat ghanavati.
- To evaluate the prepared ghanavati by using analytical parameters.
- To evaluate anti-oxidant and antidiabetic activity of Ghanvati by *in-vitro* models.

Chapter -V
MATERIALS AND RESEARCH METHODOLOGY

5.1 List of Equipment used:

S.no	Material
1.	Beakers
2.	Spatula
3.	Crucible
4.	China dish
5.	Microscope
6.	Hot plate
7.	Water bath
8.	Hot air oven
9.	Magnetic stirrer
10.	Dessicator
11.	Funnel
12.	Iodine flask
13.	Muffle furnace
14.	Weighing balance

Table 5.1: List of Equipment.

5.2 Chemicals used:

S.no.	Chemicals used
1.	Fehling A,B
2.	Benedict's reagent

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3.	Iodine solution
4.	Sodium hydroxide
5.	Copper sulphate
6.	Million's reagent
7.	Sulphuric acid
8.	Ammonium hydroxide
9.	Lead acetate
10.	Ether
11.	Benzene
12.	Chloroform
13.	Ethanol
14.	Acetic anhydride
15.	Sodium nitroprusside
16.	Pyridine
17.	Glacial acetic acid
18.	Ferric chloride
19.	Ammonia
20.	Hydrochloric acid
21.	Magnesium turning
22.	Dragendorff's reagent
23.	Mayer's reagent
24.	Hager's reagent

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25.	Wager's reagent
26.	Nitric acid

Table 5.2: List of chemicals used.

5.3 RESEARCH METHODOLOGY:

- Identification and collection of raw drug.
- Authentication of raw drug.
- Physicochemical studies of raw drug.
- Preparation of Loquat ghanavati.
- Qualitative study of finished product.
- Quantitative study of finished product.
- Evaluation of Loquat ghanavati.
- In vitro antioxidant study of the Loquat ghanavati.

Chapter-VI

EXPERIMENTAL WORK

6.1 Collection of drug:

The leaves of loquat was collected from the from village kotla naudh singh Distt. Hoshiarpur (Punjab).

6.2 Authentication of drug:

Drug was selected on the basis of its macroscopic characters.

6.3 Organoleptic study:

The *Eriobotrya japonica* was observed for colour, odour, taste, texture etc.

6.4 Physicochemical Analysis of Loquat (*Eriobotrya japonica*):

6.4.1 Foreign matter: ^[70]

100 g of sample was taken and spreaded in a stainless-steel tray. The foreign matter was detected with the unaided eye. Remaining quantity of sample was weighed and percentage of foreign matter calculated.

$$\text{Foreign matter} = (\text{Weight of foreign matter} / \text{Weight of drug}) \times 100$$

6.4.2 Loss on drying: ^[70]

5-10 gm of sample was taken (without preliminary drying) in the accurately weighed dry petri dish and kept into the dry oven at 105°C for 5 hours. Then, petri dish was removed from the oven and placed into desiccator under vacuum till shelf cooling and weighed the reduced moisture content from the sample.

6.4.3 Total ash: ^[70]

Incinerated of the 2.5 gm of the sample into the crucible, at temperature of 450°C for 5 hours. After shelf cooling, kept in the desiccator under vacuum. The weight of obtained ash was measured and percentage of obtained ash was calculated.

$$\text{Total Ash} = (\text{Weight of ash} / \text{Weight of sample}) \times 100$$

6.4.4 Acid insoluble ash: ^[70]

Ash obtained from the above method was mixed with 25 ml dilute hydrochloric acid and boiled for 5 minutes. Then, mixture was filtered through ash less filter paper. The filtrate was subjected for the washing with hot water to make it chloride free and again ignited to constant weight. Percentage of acid insoluble ash was calculated after weighing obtained ash.

$$\text{Acid insoluble ash} = (\text{Weight of residue} \times \text{Volume made}) / (\text{Weight of sample} \times \text{Volume taken}) \times 100$$

6.4.5 Alcohol soluble extractive: ^[70]

5gm of the sample (coarse powder) was taken in a closed conical flask with 100ml of alcohol. Conical flask was shaken frequently for 6 hours and kept undisturbed for 18 hours. Then, it was filtered by using filter paper. 25 ml of filtrate was taken in the china dish and allowed the content to evaporate. Percentage was calculated after weighing the residue.⁶³

Alcohol soluble extractive value = (Weight of residue × Volume made) / (Weight of sample × Volume taken) × 100

6.4.6 Water soluble extractive: ^[70]

5gms of the sample (coarse powder) was taken in a closed conical flask with 100ml of water. Conical flask was shaken frequently for 6 hours and kept undisturbed 18 hours. Then, it was filtered by using filter paper. 25 ml of filtrate was taken in the china dish and allowed the contents to evaporate. Percentage was calculated after weighing the residue.

Water soluble extractive value = (Weight of residue × Volume made) / (Weight of sample × Volume taken) × 100

6.5 Qualitative analysis of *Eriobotrya japonica* :

6.5.1 Test for flavonoids

6.5.1.1 Lead acetate Test: Extracts were treated with few drops of lead acetate solution.

Formation of yellow colour precipitate indicates the presence of flavonoids.

6.5.1.2 Shinoda test:

The extract was dissolved in methanol (50%, 1-2 ml) by heating. To an alcoholic solution of each of the extract, three pieces of magnesium chips were added followed by a few drops of concentrated hydrochloric acid. Appearance of an orange, pink or red to purple colour indicates the presence of flavonoids.

6.5.2 Test for alkaloids

6.5.2.1 Mayer's test:

One ml of aqueous extract was acidified with 2-3 drops of 1M hydrochloric acid and treated with 4-5 drops of Mayer's reagent (Potassium Mercuric Iodide) Formation of a yellow or white coloured precipitates or turbidity indicate the presence of alkaloids.

6.5.2.2 Dragendroff's test:

Extract was dissolved individually in dilute Hydrochloric acid and filtered. Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

6.5.2.3 Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

6.5.3 Test for Tannin:

A small quantity of the extract was boiled with water and filtered. Two drops of ferric chloride were added to the filtrate. Formation of blackish green precipitates confirmed presence of tannins.

6.5.4 Test for Phenol (Ferric Chloride Test): Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

6.5.5 Test for Reducing Sugar (Fehling's test):

To a test tube 1 ml each a Fehling's A and B solutions were added and mixed. To this 2 ml of plant extract was added and heated on a boiling water bath for 10 minutes. Formation of brick red or orange precipitate indicates the presence of reducing sugar/ carbohydrates.

6.5.8 Test for Saponins (Foam Test):

0.5 g of extract was shaken with 2 ml of water. Foam produced persists for ten minutes it indicates the presence of saponins.

6.5.9 Test for Proteins (Xanthoproteic Test):

The extracts were treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins.

6.5.10 Test for Phytosterols

6.5.10.1 Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

6.5.10.2 Libermann Burchard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

6.5.11 Test for Glycosides (Keller-Killiani test)^[72]

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing trace amount of ferric chloride. Transfer to a small test tube, add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube. Acetic acid layer shows blue colour.

CHAPTER VII
RESULTS AND DISCUSSIONS

7.1 Analysis of raw drug:

7.1.1 Organoleptic study:

S. no.	Parameters	Observation
1	Colour	Dark Green (Dorsal surface) Light green (Ventral Surface)
2	Odour	Characteristic
3	Taste	Slight Bitter
4	Texture	Smooth

Table 7.1: Observation of organoleptic study of Loquat Leaves

7.1.2 Physicochemical study:

S. no.	Parameters (%)	Result percentage of different Batches						Average Mean %
		I	II	III	IV	V	VI	
1	Foreign matter	0	0	0	0	0	0	0
2	Loss on drying	7.5	7	7.5	7.5	6	7	7.08
3	Total ash	7.5	9	8	8	7.5	8	8
4	Acid insoluble ash	1	1.5	1	0.5	1	1	1
5	Water soluble extractive	22.4	18.4	20.8	21.4	24	22.4	21.6
6	Alcohol soluble extractive	12	9.6	9.6	11.2	11. 2	10.4	11.06

Table 7.2: Observation during pharmaceutical analysis of Loquat Leaves

Standards of the physicochemical analysis of Loquat were not found in Ayurvedic Pharmacopoeia of India. However, physicochemical parameters were performed during the study and results of the analysis are mentioned in **Table 7.2** Foreign Matter is zero because self collected sample is taken.

7.3 Phytochemical Screening for Loquat ghanavati:

S. No.	Components	Chemical tests	Oveservation	Results A,W	
				Alcohol Extract	Water Extract
1	Flavonoides	Lead acetate tests	Yellow colour	+ve	+ve
		Shinoda test	Orange colour	-ve	+ve
2	Alkaloids	Mayer's test	Red Precipitates	+ve	-ve
3		Dragendroff's test	Red precipitates	-ve	-ve
4		Wagner test	Brow Reddish precipitates	+ve	-ve
5	Tannins	Ferric chloride test	Greenish black colour	+ve	+ve
6	Phenol	Ferric chloride test	Greenish black colour	-ve	-ve
7	Reducing sugars	Fehling's test	Brick red colour	+ve	+ve
8	Saponins	Foam Test	Foam absent	-ve	-ve
9	Protein	Xanthoprotein test	Yellow colour	+ve	+ve
10	Phytosterols	Salkowski test	Golden Yellow color	-ve	-ve
11		Lieberman Burchardt test	Brown green red junction	+ve	+ve
12	Glycosides	Kellar Killani's test	Brown ring at the junction	+ve	+ve

Table 7.3: Observation of physicochemical study of Loquat Leaves

CHAPTER VIII

REFERENCES

1. Noreen W, Wadood A, Hidayat HK, Wahid S. Effect of *Eriobotrya japonica* on blood glucose levels of normal and alloxan–diabetic rabbit. *Planta Med* 1988; 54:196-199.
2. Bhogireddy N. et al Anti-inflammatory and anti-diabetic activities with their other ethnomedicinal properties of the plants, *Journal of Medicinal Plants Studies* 2013,vol. 1,87-96, ISSN:2320-3862
3. Shih CC, et al. Cell suspension culture of *Eriobotrya japonica* regulates the diabetic and hyperlipidemic signs of high-fat-fed mice. *Molecules*. (2013)
4. Kim MJ, et al. Neuroprotective effects of *Eriobotrya japonica* against β -amyloid-induced oxidative stress and memory impairment. *Food Chem Toxicol*. (2011)
5. Cha DS, Eun JS, Jeon H. Anti-inflammatory and antinociceptive properties of the leaves of *Eriobotrya japonica*. *J Ethnopharmacol*. (2011)
6. Tanaka K, et al. Hypotriacylglycerolemic and antiobesity properties of a new fermented tea product obtained by tea-rolling processing of third-crop green tea (*Camellia sinensis*) leaves and loquat (*Eriobotrya japonica*) leaves. *Biosci Biotechnol Biochem*. (2010)
7. Kotowaroo MI, et al. Screening of traditional antidiabetic medicinal plants of Mauritius for possible alpha-amylase inhibitory effects in vitro. *Phytother Res*. (2006)
8. Qa'dan F, et al. Cinchonain Ib isolated from *Eriobotrya japonica* induces insulin secretion in vitro and in vivo. *J Ethnopharmacol*. (2009)
9. Lü H, et al. Hypoglycemic effect of the total flavonoid fraction from folium *Eriobotryae*. *Phytomedicine*. (2009)
10. Shih CC, et al. Cell suspension culture of *Eriobotrya japonica* regulates the diabetic and hyperlipidemic signs of high-fat-fed mice. *Molecules*. (2013)
11. Zong W, Zhao G. Corosolic acid isolation from the leaves of *Eriobotrya japonica* showing the effects on carbohydrate metabolism and differentiation of 3T3-L1 adipocytes. *Asia Pac J Clin Nutr*. (2007)
12. Choi YG, et al. Protective changes of inflammation-related gene expression by the leaves of *Eriobotrya japonica* in the LPS-stimulated human gingival fibroblast: microarray analysis. *J Ethnopharmacol*. (2011)

13. Alshaker HA, et al. Eriobotrya japonica hydrophilic extract modulates cytokines in normal tissues, in the tumor of Meth-A-fibrosarcoma bearing mice, and enhances their survival time. BMC Complement Altern Med. (2011)
14. Uto T, et al. Eriobotryae folium extract suppresses LPS-induced iNOS and COX-2 expression by inhibition of NF-kappaB and MAPK activation in murine macrophages. Am J Chin Med. (2010)
15. Kim SH, et al. Effect of leaves of Eriobotrya japonica on anaphylactic allergic reaction and production of tumor necrosis factor-alpha. Immunopharmacol Immunotoxicol. (2009)
16. Yokota J, et al. Gastroprotective activity of Eriobotrya japonica seed extract on experimentally induced gastric lesions in rats. J Nat Med. (2008)
17. Kang SC, et al. Evaluation of oriental medicinal herbs for estrogenic and antiproliferative activities. Phytother Res. (2006)
18. Gumy C, et al. Inhibition of 11beta-hydroxysteroid dehydrogenase type 1 by plant extracts used as traditional antidiabetic medicines. Fitoterapia. (2009)
19. Yokota J, et al. Scavenging of reactive oxygen species by Eriobotrya japonica seed extract. Biol Pharm Bull. (2006)
20. Nishioka Y, et al. Effects of extract derived from Eriobotrya japonica on liver function improvement in rats. Biol Pharm Bull. (2002)
21. Huang Y, et al. Anti-oxidative effect of triterpene acids of Eriobotrya japonica (Thunb.) Lindl. leaf in chronic bronchitis rats. Life Sci. (2006)
22. Ge JF, et al. Anti-inflammatory effect of triterpenic Acids of Eriobotrya japonica (Thunb.) Lindl. Leaf on rat model of chronic bronchitis. Am J Chin Med. (2009)
23. Hamada A, et al. The effect of Eriobotrya japonica seed extract on oxidative stress in adriamycin-induced nephropathy in rats. Biol Pharm Bull. (2004)
24. Wu G, et al. Structural basis of IAP recognition by Smac/DIABLO. Nature. (2000)
25. The flavonoid kaempferol sensitizes human glioma cells to TRAIL-mediated apoptosis by proteasomal degradation of survivin.
26. Kim MS, et al. Loquat (Eriobotrya japonica) extracts suppress the adhesion, migration and invasion of human breast cancer cell line. Nutr Res Pract. (2009)
27. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 269.
28. Sushruta, Sushruta Samhita, Uttarantra, edited with Ayurveda TatvaSandipika Hindi

- commentary by Ambikadutta Shastri, 13th edition, Chaukhamba Sanskrit Sansthan, Varanasi, 2002, 24/20
29. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 445.
30. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 451.
31. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 452-453.
32. Vagbhata, Astanga Hridaya, Sutrasthana, Vidyotini Bhashatika by Kaviraj Atridev Gupta, 14th edition, Chaukhamba Sanskrit Sansthan, Varanasi, 2003, 27/41.
33. Bhavamishra, Bhavaprakasha, Part 2, Edited with Hindi commentary by Pandit Bhramashankar Misra, 11th edition, Chaukhamba Sanskrit Bhavan, Varanasi, 2007, 2/1
34. Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, utara khanda. Chaukhamba surbharti prakashan Varanasi; 437.
35. Vaidhya Yadavji Trikamaji, Siddha Yoga Sangraha, 11th Edition, Shri Baidhnath Ayurved Bhavan; 4.
36. Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, Mdhyam khanda. Chaukhamba surbharti prakashan Varanasi; 210.
37. Dhundi S N. Pharmaceutical standardization of Guduchi Ghana. International research journal of pharmacy 2011; 2(11):104.
38. Agniveshacharya, Charak Samhita, Elaborated by Charak and Drudhabala edited by Priyavrat Sharama Chikistha sthana, 26/206. Chaukhambha orientalia, Varanasi 2007; 446.
39. Tripathi Brahmanand. Sarangadhara Samhita of Pandit Sarngadhar Acarya, Mdhyam khanda. Chaukhamba surbharti prakashan Varanasi; 195.
40. The Ayurvedic Pharmacopoeia of India. Part II (Formulations) Volume-1. First edition; 97.
41. Panda P, Meher SK. Tablet & tableting in ayurveda (vati kalpana)- A review. IAMJ 2016; 07:1219.

42. The Ayurvedic Pharmacopoeia of India. Part II (Formulations) Volume-1. First edition; 146
43. <http://dhcrop.bsmrau.net/loquat/> (Accessed on 28 oct. 2017)
44. Khare C.P. Indian Medicinal Plants An Illustrated Dictionary published by Sringer Page no-242-243
45. <http://www.missouribotanicalgarden.org/PlantFinder/PlantFinderDetails.aspx?taxonid=286498>
46. The Japanese Pharmacopoeia seventeenth edition official from april 1,2016 English version by The Ministry of Health Labour and Welfare page no-1909
47. Li EN, Luo JG, Kong LY. Qualitative and quantitative determination of seven triterpene acids in *Eriobotrya japonica* Lindl. by high-performance liquid chromatography with photodiode array detection and mass spectrometry. *Phytochem Anal.* (2009)
48. Banno N, et al. Anti-inflammatory and antitumor-promoting effects of the triterpene acids from the leaves of *Eriobotrya japonica*. *Biol Pharm Bull.* (2005)
49. Ito H, et al. Megastigmane glycosides and an acylated triterpenoid from *Eriobotrya japonica*. *J Nat Prod.* (2001)
50. A new triterpene ester from *Eriobotrya japonica*.
51. Taniguchi S, et al. Production of bioactive triterpenes by *Eriobotrya japonica* calli. *Phytochemistry.* (2002)
52. Studies on constituents of triterpene acids from *Eriobotrya japonica* and their anti-inflammatory and antitussive effects.
53. Yang Y, et al. Antifibrosis effects of triterpene acids of *Eriobotrya japonica* (Thunb.) Lindl. leaf in a rat model of bleomycin-induced pulmonary fibrosis. *J Pharm Pharmacol.* (2012)
54. Wu L, et al. Processing technology investigation of loquat (*Eriobotrya japonica*) leaf by ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry combined with chemometrics. *PLoS One.* (2013)

55. Yang Y, et al. Antifibrosis effects of triterpene acids of *Eriobotrya japonica* (Thunb.) Lindl. leaf in a rat model of bleomycin-induced pulmonary fibrosis. *J Pharm Pharmacol.* (2012)
56. Lee MH, Son YK, Han YN. Tissue factor inhibitory sesquiterpene glycoside from *Eriobotrya japonica*. *Arch Pharm Res.* (2004)
57. Chen J, et al. Hypoglycemic effects of a sesquiterpene glycoside isolated from leaves of loquat (*Eriobotrya japonica* (Thunb.) Lindl.). *Phytomedicine.* (2008)
58. Ito H, et al. Antitumor activity of compounds isolated from leaves of *Eriobotrya japonica*. *J Agric Food Chem.* (2002)
59. Ito H, et al. Polyphenols from *Eriobotrya japonica* and their cytotoxicity against human oral tumor cell lines. *Chem Pharm Bull (Tokyo).* (2000)
60. Jung HA, et al. Antioxidant flavonoids and chlorogenic acid from the leaves of *Eriobotrya japonica*. *Arch Pharm Res.* (1999)
61. Ito H, et al. Megastigmane glycosides and an acylated triterpenoid from *Eriobotrya japonica*. *J Nat Prod.* (2001)
62. Zhou C, et al. Determination of oleanolic acid, ursolic acid and amygdalin in the flower of *Eriobotrya japonica* Lindl. by HPLC. *Biomed Chromatogr.* (2007)
63. Tanaka K, et al. Hypoglycemic activity of *Eriobotrya japonica* seeds in type 2 diabetic rats and mice. *Biosci Biotechnol Biochem.* (2008)
64. Xu HX, Chen JW. Commercial quality, major bioactive compound content and antioxidant capacity of 12 cultivars of loquat (*Eriobotrya japonica* Lindl.) fruits. *J Sci Food Agric.* (2011)
65. Tropical and subtropical fruits: composition, properties and uses.
66. Ding CK, et al. Metabolism of phenolic compounds during loquat fruit development. *J Agric Food Chem.* (2001)

67. Effect of loquat (*Eriobotrya japonica*) extracts on LDL oxidation.
68. Xu HX, Chen JW. Commercial quality, major bioactive compound content and antioxidant capacity of 12 cultivars of loquat (*Eriobotrya japonica* Lindl.) fruits. *J Sci Food Agric.* (2011)
69. Liu Y. et al Biological Activities of Extracts from Loquat (*Eriobotrya japonica* Lindl.): A Review *International Journal of Molecular Sciences* 2016, 17, 1983; doi:10.3390 /ijms 17121983
70. The Ayurvedic Pharmacopeia of India, Govt. of India, Controller of Publications, New Delhi, 1st ed.1999, part I, Vol.-II,Apx-2 Page no-140-141
71. Tiwari. P.Kumar B. et.al. Phytochemical screening and Extraction : A Review, *International Pharmaceutical Science* 2011;1: 98-106
72. Vyas B. (2012).Phytopharmacological action of *pergularia daemia* with special reference to its actions and mechanism of action as diuretic and anti-inflammatory agent.