PHARMACOGNOSTIC, PHYTOCHEMICAL AND INVITRO PLATELET AGGREGATION INHIBITORY ACTIVITY OF *PSIDIUM GUAJAVA LINN.*

Dissertation submitted to
The Tamilnadu Dr. M.G.R. Medical University, Chennai
In partial fulfillment of the requirement for the award of Degree of

MASTER OF PHARMACY

MARCH – 2008

DEPARTMENT OF PHARMACOGNOSY
MADURAI MEDICAL COLLEGE
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CERTIFICATE

This is to certify that the dissertation entitled **"Pharmacognostic, Phytochemical and Invitro Platelet Aggregation Inhibitory Activity Of *Psidium Guajava Linn.*"** was done by **Mr. S. GOPALAKRISHNAN** in Department of Pharmacognosy, Madurai Medical College, Madurai – 20, in partial fulfillment of the requirement for the Degree of Master of Pharmacy in Pharmacognosy, under my guidance and supervision.

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ACKNOWLEDGEMENT

To my parents I should be grateful because they struggled their present today's for the wealth of my tomorrows. They encouraged and supported me throughout the period for the completion of work.

I sincerely thank ***Dr. M. Shanthi, MD***, Dean Incharge, Madurai Medical College, Madurai for providing the infrastructure to carry out the project work.

Any work of this nature needs the help of many heads and hands. The efficient, friendly, cheerful guidance of ***Mr. Periyanayagam K., M.Pharm., P.G. Diploma in Clinical Pharmacy (Australia),*** Professor Incharge, Department of Pharmacognosy, Madurai Medical College, Madurai have done much to improve and complete this work. I express immense gratitude to him for having enriched me in the field of research and his interest shown in bringing this book.

I wish to place on record here my indebtedness and heartfelt thanks to ***Mr. T. Venkatarathinakumar, M. Pharm.,*** Assistant Reader, Department of Pharmacognosy, Madurai Medical College, Madurai.

I extend my thanks to **Ms. R. Gowri, (M.Pharm.,) Assistant Reader in Pharmacognosy.**

I extend my sincere thanks to ***Mrs. Abarna, DMLT***, ***Mrs. Revathy, DMLT***, ***Mrs. Banumathi***, Lab Supervisor, Mrs. Ellayee, Basic Servant, Department of Pharmacognosy, Madurai Medical College, Madurai, for their help during this work.

I extend my sincere thanks to ***Mrs. A. Sethuramani, M.Pharm.,*** ***Mrs.***

A.Krishnaveni, M.Pharm., Tutor in Pharmacy, Department of Pharmacognosy, Madurai.

I thank Professor ***Mr. M. Chandran, M.Pharm.,*** Head of the Department, Department of Pharmaceutical Chemistry for their help during this work.

I thank Professor ***Mr. M. Sekar Babu, M.Pharm.,*** Head of the Department, Department of Pharmaceutics.

I am extremely thankful to my precious colleagues Mrs. ***M.Vijayalakshmi, (M.Pharm.), Miss. T. Sangeetha (M.Pharm.)*** and to my junior jewels ***Mr. N. Ramasamy(M.Pharm.), Mrs. Uma(M.Pharm.), Mrs. Chitra(M.Pharm.)*** and ***Ms. Sangami Bharathi(M.Pharm.).***

I will have long lasting gratitude and special word of thanks to ***Mr. M. Ismail, M.Sc.*** Senior Entomologist, Madurai Medical College, Madurai.

I extend my sincere thanks to ***Prof. Dr. Uma, M.D.,*** Institute of Microbiology, Madurai Medical College, Madurai.

I extend my special thanks to ***Mr. S. Pandi, B.Sc., M.B.A., M.C.S.,*** Statistician, Government Rajaji Hospital, Madurai for his help in arriving the statistical results.

My sincere thanks to ***Dr. S. Narasiman, Ph.D.,*** Director, Asthagiri herbal research foundation, Chennai for their help and support in carrying out HPLC analysis.

I am very much thankful to ***Mr. S. Kumaravel***, Scientist, PPRC, Tanjore for carrying out GC – MS of volatile oil.

I extend my thanks to ***Prof. P. Jeyaraman, Director***, PARC, Medicinal plant Research Unit, Chennai for the authentication of my plant.

I also extend my thanks to ***Prof. A. Sadasakthi***, Head, Department of Horticulture, Agricultural College, Madurai for providing me the plant specimens.

I am very much thankful *to* ***Mr. Mahalingam***, Venus Computers, ***Mr. Tamizh Selvan, Mrs.Anitha, Miss. M. Munieswari, Miss. J. Jasmine, Miss. N. Dhanalakshmi*** and all other staffs for their help in compilation of my project book.

Chapter – I

INTRODUCTION

Importance of Medicinal Plants

Herbal Medicine, also known under the names of phytotherapy and herbalism, is a well defined branch of the ancient alternative medicinal system that involves the use of plants and plant extracts to cure diseases and alleviate symptoms.

The great healing powers of many medicinal herbs and herbal supplements can never be contested – for thousands of years, plants have been successfully used to fight against a wide spectrum of diseases and disorders, and many of these ancient natural cures are still being used today, sometimes in combination with modern medical treatments.

According to recent archeological findings, herbal medicine dates back ever since man had first walked on this earth, more than 60,000 years ago. For ages, man has continued to perfect the ways of herbal medicine and has been permanently looking for new medicinal herbs with strong curative effects.

Anthropologists speculate that over the ages, early tribal communities had acquired a good level of knowledge on herbal supplements and medicinal herbs through simple processes of trial and error.

This accumulated knowledge was passed from one generation to the other and has been gradually expanded and polished, carrying the print of many distinctive cultural influences. Western, based on Greek and Roman Medicinal wisdom, the

Ayurvedic from India and oriental, particularly Chinese influences.

Unfortunately, many secrets of ancient herbal medicine had been lost, and nowadays herbalists are constantly aiming to recover reminiscences of our ancestors medicinal wisdom.

At present herbalists, botanists, pharmacologists and microbiologists from many corners of the world have joined forces in an attempt to discover lost medicinal herbs and herbal supplements, as well as new specimens of plants which could be developed for treatment of various diseases in the near future.

In recent years, herbal supplements and medicinal herbs have been extensively used for treating diseases in non – industrialized societies of the world, but also in well developed urban societies. Many people have realized the great efficiency of herbal supplements and medicinal herbs and have decided to use them as substitutes for synthetic drugs (or) as enhancements for modern medication treatments. Nature has supplied us with so many natural ingredients with great curative effects[1].

Medicinal plants have always been the principle source of medicine in India since ancient past and presently they are becoming popular throughout the developed countries. Besides, they also play an important role in the lives of tribal and rural people, particularly in remote part of developing countries. Obviously, these plants help in alleviating human suffering.

These plants are being integrated to the field of foods as additives, beverage and cosmetics. There has been a rapid extension of allopathic system of medical

treatment in our country during the past century. However, these drugs have adverse effect on human health and people are going back to nature with hope of safety and security.

On the other hand, the drug obtained from the medicinal plants are safe, cheaper, easily available and with no fear of any side effects. Moreover, these are more compatible to the human body constitution and suits to the local and cultural need of the people. The indigenous method of preparation maintains the purity of the drug.

Medicinal plants generated commercial demand for pharmacopoeial drugs and their products in India. It is evident that many valuable herbal drugs have been discovered by knowing that a particular plant was used by the ancient folk healers for the treatment of some kind of ailments.

Moreover, the medicinal plant wealth are our national heritage and it seems to be the first and foremost line of defence for the treatment of various diseases mostly tribal and rural communities[2].

WHO views on Medicinal Plants

The world health organization has estimated that nearly 80% of the population of the developing countries meet their primary health care needs through plant based traditional medicine. Towards the end of the 20th century there began a revival of interest in traditional medicine not only in developing countries, but also in the developed countries. The revival of interest in herbal drugs started mainly because of the widespread belief that ‘green medicine’ is healthier than synthetic products. Now

the increasing popularity of plant - based drugs are felt all over the world leading to fast growing market for herbal drugs, pharmaceuticals, nutraceuticals, functional foods and even cosmetics[3].

Phytonutrients – The Natural Drugs of the Future

For over 80 years now the allopathic industry, combined with the might and power of the pharmaceutical giants, has been trying to combat degenerative diseases. Though many advances in the diagnosing of diseases and the discovery of a myriad of new names to diseases has occurred, the battle to increase quality of life and the overcoming, curing and proper treatment of degenerative diseases has been all but lost. Allopathic medicine has not changed its treatment of cancer in over 40 years. Though new drugs keep popping up, the results are still the same and the most effective treatment for cancer is still surgery. Heart disease, diabetes, arthritis, leukemia, Alzheimer's, Parkinson's, Hodgkin's and dozens of other disease names strike fear into our minds and as we age, many of us contemplate... ". Which one will take me from this life and how will I fight it?" Today with the current standard of allopathic medicine it is only a matter of time before you are beaten by one of the many known or even unknown degenerative diseases. There are no known cures to any of these degenerative diseases.

Currently your only option is a long list of prescription drugs that may alleviate symptoms but slowly eat away your body's immunity and quality of life.

There is hope however. For more than 20 years now scientists have been researching plants and discoveries made within the last 15 years may hold some of

the answers to combating many of the degenerative diseases[4,5].

Facts about Cardiovascular Diseases and Global Burden of CVD

- Cardiovascular diseases are the number one cause of death globally : more people die annually from cardio vascular diseases than from any other cause.
- An estimated 17.5 million people died from Cardiovascular diseases in 2005, representing 30% of all global deaths. Of these deaths, an estimated 7.6 million were due to coronary heart disease and 5.7 million were due to stroke.
- Over 80% of cardiovascular disease deaths take place in low and middle income countries and occur almost equally in men and women.
- By 2015, almost 20 million people will die from cardiovascular diseases, mainly from heart disease and stroke. These are projected to remain the single leading causes of death[6,7].

India's Killer Diseases

The 2002 data below from the world Health organization, based on figures from the Government of India, would seem to indicate that heart disease is the most common killer of Indians, just as it was for Americans in the 1950s and 1960s.

Most Common Causes of Death in India 2002(in%)[8]

Cardiovascular diseases	-	27
Infectious and parasitic diseases	-	20
Including		
Tuberculosis	-	4

HIV / AIDS	-	3
Diarrheal	-	4
Meningitis	-	1
Childhood cluster diseases	-	5
Respiratory infections	-	11
Unintentional injuries	-	8
Prenatal Conditions	-	7
Cancer	-	7
Respiratory diseases	-	6
Digestive diseases	-	3
Intentional injuries	-	3

Flavonoids and Cardiovascular diseases

Flavonoids occur naturally in fruit, vegetables, and beverages such as tea and wine. Research in the field of Flavonoids has increased since the discovery of the French Paradox, ie, the low cardiovascular mortality rate observed in mediterranean populations in association with red wine consumption and a high saturated fat intake. several other potential beneficial properties of flavonoids have since been ascertained.

Quercetin

One of the best described flavonoids, quercetin, is a member of this group. quercetin is found in abundance in onions, apples, broccoli, and berries[9].

A high dietary intake of this compound and others of the same family has been correlated with a decreased risk of coronary heart disease. This is believed to be

mediated through a wide spectrum of pharmacological effects.

Quercetin has been shown to be able to inhibit low-density lipo protein oxidation in vitro and cause vasodilation in rat aortic strips. Further more, quercetin has been shown invitro to inhibit platelet aggregation. However, a mechanism for this action has not been elucidated[10].

It is obvious that plants containing quercetin may inhibit the platelet aggregation. Reviewing of many plants possessing quercetin indicates that the leaves of ***Psidium guajava*** family : Myrtaceae is one of the plants which is widely cultivated for its fruits. So it is decided to study ***Psidium guajava*** in various aspects to exploit it for medicinal purposes.

Chapter – II

REVIEW OF LITERATURE

LEAVES

Ethnomedical Information

The leaves of Psidium guajava are used as astringent, anodyne, febrifuge, antispasmodic and tonic[11].

The leaves of guava are used for wounds, ulcers and as an astringent for bowels. The young leaves are used as a tonic in the diseases of the digestive functions.

A decoction of the young leaves is prescribed in febrifuge and Antispasmodic baths. The decoction of leaves has been used in cholera with some success in arresting vomiting and diarrhoea, leaf decoction when gargled relieves tooth ache and gum boils.

An Infusion of the leaves is a popular astringent drink in Ghana. Infusion of leaves are used in cerebral affections, nephritis and cochexia.

The pounded leaves are locally applied in rheumatism, and an extract is used in epilepsy and chorea[12].

Locally decoction of the leaves is applied with much benefit to the prolapsus ani of children ; is employed in scurvy. Leaves when ground make excellent poultice[13].

Tender leaves are chewed for bleeding gums and bad breath, and it is said to prevent hang overs (if chewed before drinking). Leaf decoction used as a gargle for

mouth sores (or) as a douche for vaginal discharge and to tighten and tone vaginal walls after child birth.

Guava leaves are in the Dutch pharmacopoeia for the treatment of diarrhoea and still used in Latin America, central and West Africa, and South East Asia for diarrhoea.

Brazilian researchers reported that guava leaf extracts have numerous effects on the cardiovascular system which might be beneficial in treating irregular heart beat (arrhythmia). Newer in the market are guava leaf extracts that are used in various herbal formulas for a myriad of purposes; from herbal antibiotic and diarrhoea formulas to bowel health and weight loss formulas.

Worldwide Ethno medical Uses

Amazonia	-	For diarrhoea, dystentery, menstrual disorders, stomach ache, vertigo.
Brazil	-	For anorexia, cholera, diarrhoea, digestive problems, dysentery, gastric insufficiency, inflammed mucous membranes, laryngitis, mouth (swelling), skin problems, sore throat, ulcers, vaginal discharge.
Cuba	-	For Colds, dysentery, dyspepsia
Ghana	-	Coughs, diarrhoea, dysentery, tooth ache
Haiti	-	For dysentery, diarrhoea, epilepsy, itch, piles, scabies, skin sores, sore throat, stomach ache, wounds, as antiseptic and astringent.
India	-	For anorexia, cerebral ailments, child birth, chorea, convulsions, epilepsy, nephritis.
Malaya	-	For dermatosis, diarrhoea, epilepsy, hysteria, menstrual disorders.

Mexico	-	For deafness, diarrhoea, itch, scabies, stomach ache, swelling, ulcer, worms, wounds.
Peru	-	For conjunctivity, cough, diarrhoea, digestive problems, dysentery, edema, gout, hemorrhages, gastroenteritis, gastritis, lung problems, vaginal discharge, vertigo, vomitting, worms.
Phillippines	-	For sores, wounds and as astringent
Trinidad	-	Bacterial infections, blood cleansing, diarrhoea, dysentery[14]

A leaf infusion is taken in Ghana and Nigeria for stomach complaints e.g. constipation and in Adamawa, leaf infusion is taken with ”red potash” for dysentery. The leaf infusions are used in the cape for diabetes. The young leaves are used for inflammation of the kidney and kidney problems.

The leaves are used as part of the pot herb in steam treatment for Malaria. The main ethno therapeutic use in Africa is said to be for Malaria.

Pulped leaves are made up into a suppository in Congo for treating piles. The pounded leaves in India are used for rheumatism. Crushed leaves are boiled in water and the infusion is either taken orally as a tea (or) as an enema[15].

Decoction of the leaves are taken externally as a lotion for skin complaints, ringworm, wounds and ulcers.

Leaf decoction with salt (or) sugar taken orally for digestive tract ailments, cold and high blood pressure. Leaf decoction used for hoarse throat, varix[16].

People in China use guava leaf as anti-inflammatory and haemostatic agent[17].

Leaves are antiemetic, blister in mouth, cold, cough, diarrhoea, dysentery, fever, head ache, gonorrhea, menstrual disorders, stomach ache[18].

Pharmacognostical Studies

Microscopic study, phytochemical preliminary test, qualitative evaluation of ***P. guajava*** Linn leaves were studied.

Characteristic tissue of Guava leaves were unicellular trichomes, paracytic and Anomocytic stomata, Rosette aggregate, Prism shaped calcium oxalate crystals and epidermis cells with thicken walls.

Stomatal index **–** 19.14 – 22.02 (Average 20.58) phytochemical preliminary test gave positive result with Shinoda's test, Ferric chloride, Liebermann - Burchard test, Froth Test.

Loss on drying (%) – Not more than 10.4%. Ethanol soluble extractive(%) – Not less than 16.4%. Water soluble extractive (%) – Not less than 19.6%. The characters and respective values were used for identification. **(Phisutthanan. S et al. 2000)**[19].

Pharmacognostic studies of leaf comprising Macro and microscopic characters, organoleptic and powder microscopic features, besides physico-chemical constants viz. ash and extractive values and chromatographic profiles were evaluated.

Physico – Chemical Studies :

TLC study on chloroform extract of mother tincture reveals 3 prominent spots on spraying with 10% $Fecl_3$ solution and heating at 110^0C, solvent system – Chloroform and benzene with few drops of acetic acid (1:1V/V), Adsorbent : Silicagel–G

Moisture content (L.O.D at 105^0C)	-	12.7 % w/w
Ash value (Total)	-	11.827 % w/w
Acid insoluble ash	-	1.59 % w/w

Water soluble ash	-	3.135 % w/w
Water soluble extractive	-	15 % w/w
Alcohol soluble extractive	-	23.5 % w/w

(Subramanian .P et al)[20]

Phytochemical Studies

Methanolic leaf extract of ***P. guajava*** subjected to bio-assay-guided isolation of spasmolytic constituents. Six fractions were separated on PVP column using a water methanol – gradient. Trace of Quercetin aglycone, guajavarin, isoquercetin, hyperin, quercetrin, quercetin 3-0-gentobioside were identified **(Lozoya .X et al 1994)**[21]**.**

From the methanol extract of guava leaves (+) – gallo catechin was isolated **(Matsuo .T et al 1994)**[22]**.**

Two new triterpenoids, guajavolide and guavenoic acid along with one known triterpene oleanolic acid were isolated from ***Psidium guajava*** fresh leaves. Their structure elucidation and stereochemistry were determined by spectroscopic experiments including 2D – NMR techniques **(Begum S. et. al 2002)**[23]**.**

Two triterpenoids, guavanoic acid and guava coumaric acid along with six known compounds 2 alpha – hydroxy ursolic acid, Jacoumaric acid, isoneriucoumaric acid, asiatic acid, ilelatifol D and Beta – sitosterol – 3 – 0 – beta – D – glucopyranoside have been isolated from ***Psidium guajava*** leaves.

Their structures were determined through spectroscopic methods **(Begum S. et al 2002)**[24]

Four antibacterial compounds were isolated from leaves of psidium guajava L.

and the structures of these compounds were established on the basis of chemical and spectroscopic evidence.

Two new flavonoid glycosides, morin – 3 – 0 – alpha – L – lyxopyranoside and morin – 3- 0 – alpha – L – arabo pyranoside and two known flavonoids, guijavarin and quercetin were identified **(Asima H et. al 2002)**[25]

Two methods developed based on microwave assisted extraction (MAE) and microwave assisted acidic hydrolysis (MAAH) for sample preparation of Guava leaves prior to GC determinations of aglycone quercetin and total aglycone quercetin, respectively. Ethanol was selected as solvent.

Microwave assisted techniques compared with reflux heating.

Average aglycone Quercetin yield

(MAE) (5 min) - 1.84 mg g^{-1}

Average aglycone Quercetin yield

(MAAH) (5 min) - 4.21mg g^{-1}

Quercetin yield by Reflux heating - 1.17 mg g^{-1}

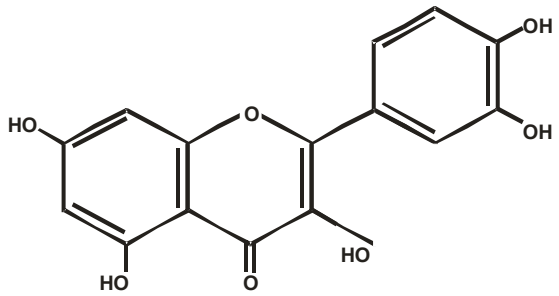
(5 min)

Microwave assisted techniques are better than reflux heating, because the yield of aglycone quercetin and total aglycone quercetin are higher in microwave assisted techniques. **(Huang J. et al 2004)**[26]

Five constituents including one new pentacyclic triterpenoid guajanoic acid and four known compounds beta - sitosterol, uvaol, oleanolic acid and ursolic acid have

been isolated from psidium guajava leaves. The new constituent guajanoic acid has been characterized as 3 beta – P – E – Coumaroyloxy – 2 alpha – methoxyurs – 12 – en – 28 – oicacid through 2D NMR techniques and chemical transformations. This is the first report of isolation of compound uvaol from the genus ***Psidium*** **(Begum S. et. al 2004)**[27].

Phytochemical studies of ***P. guajava*** plant were reviewed. Three flavonoids quercetin, avicularin and guaijaverin have been isolated from the leaves **(He. Q. et. al 2004)** [17].



Quercetin

Identification of flavonoids and flavonoid glycosides were carried out on ***P. guajava*** Linn leaves by means of HPLC – UV analysis and HPLC mass spectrometry.

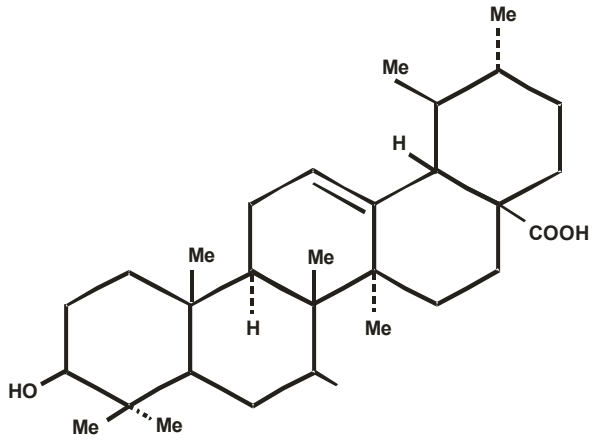
By using HPLC – UV, two known phenolics (gallic acid and quercetin) and five newly reported ones (procatechuic acid, chlorogenic acid, caffeic acid, kaempferol, ferulic acid) were identified in alcohol guava leaf extract. Structural information about the compounds obtained from retention times, UV Spectra and mass spectra.

Two flavonoids (Quercetin and Kaempferol) and four flavonoid glycosides (Quercetin 3 – 0 – alpha – L – arabinoside, Quercetin 3 – 0 – beta – D – glucoside, Quercetin 3 – 0 – beta – D – galactoside, along with one novel compound, kaempferol

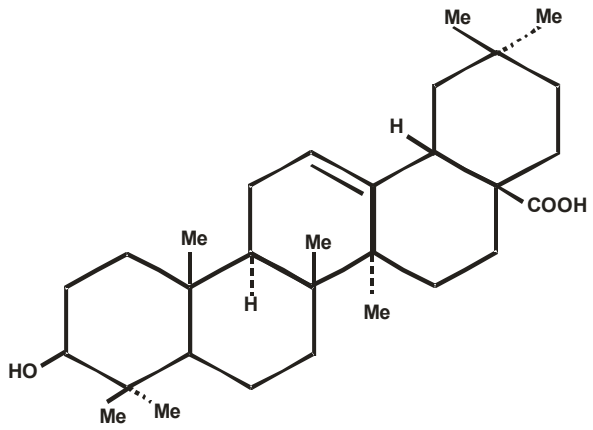
– glycoside) have been identified in the fractions **(Liang Q. et. al 2005)**[28].

One new pentacyclic triterpenoid, psidiumoic acid along with 4 known compounds beta – sitosterol, obtusol, oleanolic acid and ursolic acid have been isolated from ***P. guajava*** leaves.

The new constituent psidiumoic acid has been characterized as 2 alpha – glycolyl – 3 beta – hydroxy olean – 12 – en – 28 – oic acid through 2D NMR techniques. This is the first report of isolation of compound obtusol from the genus ***Psidium*** **(Begum S. et. al 2007)**[29].



Ursolic acid

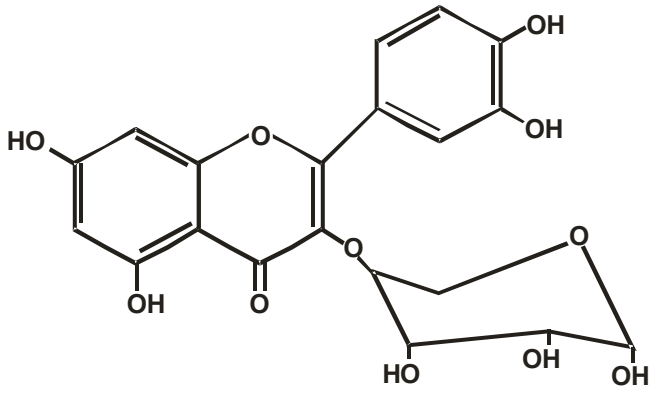


Oleanolic acid

Leaves of ***P. guajava*** contains the following chemicals. (+) – Gallo catechin,

amritoside, aromadendrene, avicularin, betasitosterol, caryophyllene oxide, crataegolic acid, ellagic acid, foeniculin, guaijavarin, hyperoside, guajivolic acid, guavin – A, B, C, D, Iso-quercetin, leucocyanidin, Maslinic acid, Nerolidiol, pyrogallol – Tannins, Quercetin, Quercitrin, Tellimagrandin–I,Ursolic acid **(Dr. Dukes phytochemical and Ethnobotanical data base)**[30].

Leaves contain resin, fat, cellulose, tannin, volatile oil, chlorophyll and mineral salts. In addition, the leaves contain an essential oil rich in cineol and four triterpenic acids as well as three flavonoids; quercetin, its 3–L– 4–4– arabino furanoside (avicularin) and its 3 – L – 4 – pyranoside with strong antibacterial action[15].



Avicularin

Studies on Volatile oil

Volatile constituents of guava leaves were obtained by super critical fluid extraction and analysed by Gas chromatography – mass spectrometry (GC – MS).

A total of 17 compounds accounting for 86% of the aroma were identified. α - selinene (23.7%), α - caryophyllene (18.8%) and δ selinene (18.3%) were the major compounds identified. The volatile fraction was rich in sesquiterpene compounds. **(Nieves L.S et. al 1993)**[31]**.**

The constituents of essential oils from the leaves of ***P. guajava*** were analyzed by GC – MS qualitatively and quantitatively.

Sixty compounds of the essential oils were identified at rate 90.56%. The major components were carophyllene (18.81%), Copaene (11.80%), 1aR – (1a alpha, 4a alpha, 7 alpha, 7a beta, 7b alpha)] – decahydro – 1,1,7 – trimethyl – 4- methylene – 1H – cyclo prop[e] azulene (10.27%), eucalyptol (7.36%) **(Li J. et al 1999)**[32]**.**

Hydro – distilled volatile oil from the leaves of ***P. guajava*** grown in India analyzed by GC and GC/MS.

Leaf volatile oil found to contain 18 constituents (91.1%). Quantitative characterisation of volatile oil shows 13 sesquiterpenes (85%) which consist of 8 sesquiterpene hydrocarbons (52%), 4 alcohols (32.8%) and an oxide (0.3%).

Oil contained only 3 monoterpenic constituents (2.3%). The important component was β – caryophyllene (35.7%), trans-nerolidol (19.2%), caryophyllenol (7.8%), α - longipinene (5.2%) and aromadendrene (4.1%) **(Mukhtar H.M et al 2005)**[33].

The composition of the essential oil obtained from the hydrodistillation of the leaves of ***P. guajava*** L was analyzed by capillary GC and GC/MS.

A total of 50 compounds were identified. The major constituents identified in the oil were β - Caryophyllene (27.7%), α - pinene (14.7%), 1, 8 – cineole (12.4%) **(Chun H. et. al 2007)**[34].

The leaves contain essential oil with the main components being α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene,

caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene. The essential from the leaves has been shown to contain nerolidiol, β-sitosterol, ursolic, crategolic and guayavolic acids have also been identified[15].

Pharmacological Screening

Leaves

The electrically stimulated guinea – pig ileum and spontaneously contracting guinea pig ileum preparations were employed in studies of alcoholic extract, quercetin and quercetin – 3- arabinoside, extracted from leaves of ***P. guajava.***

The extract showed a morphine like inhibition of acetyl – choline release in coaxially stimulated ileum, found to be due to quercetin at concentrations of 1-6 micrograms/ml. The glycoside did not show any such action at concentration of upto 1-28mg/ml. The extract inhibited spontaneous contractions in unstimulated ileum **(Lutterodt GD 1989)[35].**

The anti-inflammatory, analgesic, antipyretic activity, gastro intestinal and CNS effects of methanolic extract of ***P guajava*** leaves was evaluated to provide a basis for the folkloric use of the plant.

The extract shows anti inflammatory activity by inhibiting oedema induced by carrageenan in a dose dependent manner.

At the dose of 200 mg / kg, the extract exhibited analgesic effect to same degree as 150 mg / kg acetyl salicylic acid.

Extract showed a marked antipyretic activity by reducing yeast induced fever to

same degree as Indomethacin.

In Intestinal motility test, extract significantly reduced charcoal meal transit in a dose related manner, comparable to 1 mg / kg atropine.

The extract shows CNS depressant activity as seen in potentiation of phenobarbitone sleeping time in mice **(Olajide OA et al 1999)**[36].

The anticough activity of ***P. guajava*** Linn (guava) leaf extract was evaluated in rats and guinea pigs. The results showed that water extract at doses 2 and 5g/kg, p.o decreased the frequency of cough induced by capsaicin aerosol by 35 & 54% respectively as compared to control. So, guava leaf extract is recommended as a cough remedy **(Jaiaraj P. et al 1999)**[37].

The guava leaf extract effects on bleeding time and 3 main mechanisms of haemostasis: vasoconstriction, platelet aggregation and blood coagulation were investigated.

The water extract did not shorten bleeding times in rats, potentiated vascular muscle contraction induced in rabbits and stimulates human platelet aggregation, in vitro in a dose dependent manner, prolonged blood coagulation **(Jaiaraj P. et al 2000)**[38]**.**

Inotropic effects of extracts of ***P. guajava*** L. (guava) leaves on guinea pig atrium were studied by comparing crude extract (water – alcohol) with acetic acid extract.

Crude extract depresses guinea pig atrial contractility in a concentration dependent fashion. The acetic acid fraction of ***P. guajava*** reversibly decreased myocardial force in a concentration dependent fashion, increased the atrial relaxation time, abolished the positive staircase effect.

The acetic acid extract was 20 times more potent than crude extract **(Conde G.E.A et al 2003)**[39]**.**

Aqueous extracts of ***P. guajava*** was tested for their cardioprotective effects against ischemia – reperfusion injury using isolated perfused rat hearts

The extracts significantly attenuated ischemic contracture, and increases malondialdehyde in reperfused hearts.

The results indicate that ***P. guajava*** have cardio protective effects primarily through radical scavenging actions **(Yamashiro S et. al 2003)**[40]**.**

Water extract of ***P. guajava*** leaves was screened for hypoglycemic activity on alloxan induced diabetic rats. In both acute and sub – acute tests, the water extract, at an oral dose of 250 mg/kg, showed statistically significant hypoglycemic activity **(Mukhtar HM et. al 2004)**[41].

Antioxidant activity of lyophilized extracts was determined at ambient temperature by means of a 2,2 diphenyl – 1 – picrylhydryzyl (DPPH) colorimetry with detection scheme at 515nm, activity was evaluated by decrease in absorbance as a result of DPPH colour change from purple to yellow.

Higher the sample concentration used, the stronger was free radical scavenging effect. Commercial guava leaf extracts, ethanol guava leaf extracts showed almost same antioxidant power **(He. Q et. al 2004)** [17]**.**

Hypotensive and hypoglycemic effects of ***P. guajava*** leaf aqueous extract (50 – 800 mg/kg) in rat experimental paradigms were investigated. Hypoglycemic effect of plants extract was examined in normal and diabetic rats, using streptozotocin(STZ) induced diabetes mellitus model. Hypertensive Dahl salt sensitive rats used to

investigate hypotensive effect of plants extract. Chlopropamide (250 mg/kg P.O) used as reference hypoglycemic agent for comparison.

Acute oral administrations of plant's extract(50-800 mg/kg p.o) cause dose related, significant hypoglycemia in normal and STZ treated, diabetic rats.

Acute I.V administration of plant extract (50 – 800 mg/kg i.v) shows dose dependent reduction in systemic arterial and heart rates of hypertensive, Dahl salt sensitive rats **(Ojewole J.A 2005)**[42].

Anti diabetic effect of ***P. guajava*** leaf extract on mice was evaluated.

Significant blood glucose lowering effects of the extract were observed after intra peritoneal injection of extract at a dose of 10 mg/kg in both 1 and 3 month old mice.

The extract from ***P. guajava*** leaves possesses antidiabetic effect in Type 2 diabetic mice model and these effect atleast in part, mediated via the inhibition of protein tyrosine phosphatase 1B (PTP 1B) **(Oh W.K et. al 2005)**[43].

The anti inflammatory and analgesic properties of ***P. guajava*** leaf aqueous extract in some experimental animal paradigms were studied.

Anti inflammatory property investigated in rats, using fresh egg albumin induced paw edema and Diclofenac (100 mg / kg, i.p) used as standard. Analgesic effect evaluated by "hot plate" and acetic acid test models of pain in mice. Morphine (10 mg / kg, i.p) used as standard.

The aqueous extract produced dose dependent and significant inhibition of fresh egg albumin induced acute inflammation and produced analgesic effects against thermally and chemically induced nociceptive pain in mice **(OJewole JA 2006)**[44]**.**

Evaluation of hepatoprotective activity of ***P. guajava*** (aqueous leaf extract) in acute experimental liver injury induced by CCl_4, paracetamol (or) thio acetamide and chronic liver damage induced by CCl_4. The effects observed were compared with a known hepatoprotective agent, silymarin.

In acute liver damage, leaf extract (250, 500mg/kg, p.o) significantly reduced elevated serum levels of aspartate amino transferase, bilirubin. Higher dose prevented increase in liver weight compared to lower dose.

In chronic liver injury caused by CCl_4, higher dose (500mg/kg, p.o) more effective than lower dose.Aqueous extract of guava leaves possesses good hepato protective activity **(Roy CK et al 2006)**[45]**.**

Effects of aqueous extract of guava leaves on labelling of blood constituents (BC) with Technetium – 99m (^{99m} TC) was evaluated.

Blood samples of wistar rats were incubated with different concentrations of guava extract and labelled with ^{99M} TC after percentage of incorporated radio activity (% ATI) in BC was determined.

The results suggest that aqueous guava extract could present antioxidant action and alters the membrane structures, thus decreasing radio labelling of BC with ^{99m} TC. **(Abreu PRC et. al 2006)**[46]**.**

Antioxidant activity and free radical scavenging effects of extracts from guava leaves were studied. The results demonstrated that scavenging effects of guava leaf extracts on $ABTS^{\cdot+}$ radicals and super oxide anion increased with increasing concentrations and inhibits 94.4 – 96.2% of Linoleic acid oxidation at a concentration of 100 μg/ml **(Chen H.Y. et al 2007)**[47].

The effects of α dicarbonyl compounds on coagulation parameters in vitro and anticoagulant activities of aqueous extracts from guava leaves were examined. Incubation of plasma with glyoxal (or) methyl glyoxal at 0.1mM showed significant decrease in thrombin clotting (TT) ($P<0.05$), slight prolongation of prothrombin Time (PT) at 0.5mM and no effect on activated partial thrombosplastin time (APTT). Methyl glyoxal inhibited Antithrombin III activity.

Guava leaf extracts and its active phenolic compounds including ferulic acid, gallic acid and quercetin displayed a protective effect against Methyl glyoxal induced loss of activity of Antithrombin III.

Guava leaf extracts are a potent Anti glycative and anticoagulant, which is of great value in preventive glycation associated cardiovascular diseases in diabetes **(Hsieh CL et al 2007)**[48].

contractile effect of the aqueous extract of ***P. guajava*** leaves on aortic rings in rat was evaluated.

The effect was evaluated also in presence of Nifedipine (L-type calcium channel blocker) and phentolamine (α - adreno receptor blocker). The sensitivity of the aortic rings to cumulative doses of P. guajava was significantly enhanced in the presence of phentolamine suggesting that the effect of P. guajava was to a large extent mediated by activation of α - adrenoreceptor and to a lesser extent by acting via calcium ion channel.

Aqueous leaves extract of ***Psidium guajava*** significantly and dose dependently (0.25 – 2 mg/ml) contracted aorta rings. These findings may contribute to knowledge

on vasoconstrictor effect of plant P. guajava **(Bello Ilo et al 2007)**[49]**.**

Antiulcer potential and antioxidant activity of hydroalcoholic extract of leaves of ***P. guajava*** were studied to validate ethnobotanical claims regarding the plant use in stomach diseases.

Gastro protective potential of the leaves extract (200 and 400 mg / kg) was studied on aspirin – induced ulcer in rats. The free radical scavenging activity of hydroalcoholic extract was assessed using two methods, DPPH radical scavenging activity and super oxide scavenging activity.

Results of present study showed that the extract possessed gastro protective activity by inhibiting aspirin induced ulcers with a maximum of 70% curation (400 mg/kg). The plant exhibited very good antioxidant effect by prevention of free radical formation.

These findings justify inclusion of this plant in the management of gastric disorders in traditional medicine **(Edwin S et al 2007)**[50].

Volatile Oil

Anti inflammatory activity

P. guajava leaf oil obtained by steam distillation given orally to study its effects on exudative and proliferative phases of inflammatory reaction using carrageenan induced paw edema and cotton pellet granuloma technique in male albino rats and anti inflammatory activity compared with ketorolac tromethamine.

In carrageenan induced paw edema, 0.8ml/kg of volatile oil showed anti inflammatory activity. The oil found to be effective in cotton pellet granuloma studies

(Kavimani S et al 1997)[51].

Microbiological Studies

Anti streptococcus mutans activity and in vitro effects of subminimal inhibitory concentrations of guaijaverin isolated from ***P. guajava*** linn on cariogenic properties of streptococcus mutans was investigated.

Bio – autography directed chromatographic fractionation, yield biologically active compound, guaijaverin from Crude methanol extract of P. guajava.

The antistreptococcus mutans activity of guaijaverin was found to be bacteriostatic, both heat and acid stable and alkali labile with the minimum inhibitory concentration (MIC) of 4 mg ml (-1) for MTCC 1943 and 2mg ml(-1) for CLSM 001.

The active flavonoid compound, guaijaverin demonstrated high potential antiplaque agent by inhibiting growth of streptococcus mutans which led to acceptance of Natural products as alternative form of health care **(Prabu GR et. al 2006)[52].**

Broad spectrum antibacterial activity against 1) E. Coli 2) S. aureus 3) S. typhi of Ethyl acetate soluble fraction of aqueous extract of dried leaf powder than $CHCl_3$, n – butanol and residual aqueous fraction was proved. Lower concentration is more effective than crude extract by Disc diffusion method using the concentration of 100, 200, 400 mg/ml. E.Coli is more susceptible. It was also suggested that the activity may be due to the presence of flavonoids, Tannins and the narrow activity of crude extract may be due to the presence of carbohydrate which facilitates the growth **(Geidam Y.A et. al 2007)[53].**

Flowers

Ethnomedical information

The flowers are cooling, laxative and tonic, and are useful in bronchitis, ophthalmodynia, colic and ulemorrhagia[11].

Flowers are said to cool the body and are used in bronchitis. They are also applied to eye sores[12].

Flowers are mashed and applied to painful eye conditions such as sun strain, conjunctivitis or eye injuries[14].

Flowers are applicable in eye products for their soothing effect[15].

Fruits

Ethnomedical information

The fruits are sweet, astringent, sour, cooling, aphrodisiac, laxative and tonic. They are useful in vitiated conditions of pitta, dipsia, burning sensation, colic, ulemorrhagia, diarrhoea, dysentery and general debility[11].

The fruit is tonic, cooling and laxative. It is good in colic and for bleeding gums. The fruit and its conserve are astringent and used in diarrhoea and dysentery[12].

Fruit forms, when stewed, the well – known guava jelly (or) preserve. Jelly is tonic to the heart and good for constipation. Ripe fruit is a good aperient. Unripe fruit is employed in diarrhoea. Fruits are recommended for gout. Water in which the fruit is soaked is good for thirst in diabetes[13].

The fruit and juice is freely consumed for its great taste, nutritional benefit and nutrient content, as well as an effective children's diarrhoea remedy[14].

Fruit is used as laxative[15].

All parts of the young fruit are astringent. The dried ripe fruits are recommended

as a remedy for dysentery. Fruits used as a cure for diarrhoea[16].

Fruit is used in jaundice, as a tonic [18].

Phytochemical Studies

Fruits are the richest natural sources of vitamin – C and contains 4 to 10 times more of this vitamin than the citrus fruits. It also contains considerable amount of pectin. Leucocyanidin and ellagic acid are the polyphenolic compounds identified in the ripe fruit.

The red skin of apple guava type is found to contain a cyanidin diglucoside, probably mecocyanin.

Quercetin, its 3 – arabopyranoside guaijaverin, gallic acid, and arabinose ester of ellagic acid, besides leucocyanidin, have been isolated from the unripe fruit.

Guava is reported to contain a bound form of the vitamin ascorbigen, amounting to about 15% of the total Vitamin – C content. citric acid is the major acid in guava. Tartaric and l-malic acids being present in smaller amounts. Carbohydrates occur chiefly in the form of sugars of which reducing sugars form the major part[12].

This fruit contains vitamin C, Vitamin A, iron, calcium and phosphorus. Guavas are upto 5 times richer in vitamin C than oranges. The fruit contains saponin combined with oleanolic acid, morin – 3- 0 - α - L –lyxopyranoside and morin – 3- 0 - α - L –arabopyranoside and flavonoids, guaijavarin and quercetin.

The occurrence of pentane – 2 – thiol was found in the fruits. New components were described for the first time as active aromatic constituents in pink guava fruit (3 – penten – 2-0l and 2 – butenyl acetate)[15].

Pharmacological Screening

The methanolic fraction of psidium guajava fruit extract was found to possess

significant inhibitory activity against carrageenan, kaolin and Turpentine – induced oedema formation and protein exudation.

The proliferative form of inflammation was counteracted following cotton pellet – induced granuloma formation in rats.

The fraction significantly inhibits acetic acid – induced analgesia (writhing) and shows antipyretic activity following yeast – induced pyrexia in rats, and antiarthritic activity was observed against formaldehyde – induced chronic arthritis in rats**. (Sen T et al 2006)**[54]**.**

Seeds

Phytochemical Studies

The seeds which are very small but abundant in the fruit and have been reported to contain 14% oil on dry weight, with 15% proteins and 13% starch.

Ten phenolic and flavonoid compounds including one new acylated flavonol glycoside were isolated. The structures of the new compound Quercetin – 3 – O - β – D (2 " – O – galloyl glucoside) – 4' – 0 – Vinyl propionate and of the known compounds were elucidated[15].

Bark

Ethnomedical information

The bark is valued for its astringent properties and has been employed in diarrhoea in children. It is generally administered in the form of a decoction. The bark is tonic[12].

A decoction of the bark is used topically for wounds, ulcers and skin sores[14].

Bark is astringent ,febrifuge. In Amazon, decoction of bark used topically for wounds, ulcers and skin sores[15].

Bark is used as antiemetic, in dysentery, stomach ache[18].

Phytochemical studies

The bark contains considerable amount of Tannins (11-27%)[12].

Bark contains tannin 27.4 p.c., resin and crystals of calcium oxalate. There is a high percentage of carbohydrates and salts[13].

The bark contains 12 – 30% of tannins [15].

Root bark

Ethnomedical information

Root bark is successfully employed in chronic infantile diarrhoea in the form of concentrated decoction (1 in 12) or “2 ounces of the bark in a pint of water boiled down to ½ pint”. Dose is 1 drachm or 1 to 2 teaspoonfuls two or three times daily”. It is administered in cholera for arresting vomiting and diarrhoeic symptoms (especially those of the red variety)[13].

A decoction of the root bark is recommended as a mouth wash for swollen gums[15].

Roots

Ethnomedical information

The roots are astringent, haemostatic, constipating and antiemetic, and are useful in haemorrhages, diarrhoea and dysentery especially in children, ulcers,

gingivitis, proctoptosis and vomiting[12].

Phytochemical Studies

The roots are rich in Tannin. The plant also contains Leucocyanidins, sterols, and gallic acid in the roots. There is a high percentage of carbohydrates and salts. Roots contain a large percentage of tannic acid[15].

Microbiological Studies

Antibacterial activities of aqueous and Ethanol : Water extracts from leaves, roots and stem bark of ***Psidium guajava*** L. was evaluated by Microdilution assay.

Aqueous extracts active against Gram positive bacteria S. aureus (MICS = 500, 125, 250 µg respectively) and B. Subtilis (MICS = 500µg/ml), inactive against gram (-)ve bacteria E-Coli and pseudomonas aeruginosa (MICS>1000µg/ml).

The ethanol : Water extracts showed higher antimicrobial activity compared to aqueous extracts, and fractionated on silica gel column chromatography in a bio-assay guided fractionation affording Flavonoid mixture, triterpenes (α and β – amyrin) and sterol (β - sitosterol). Flavonoid mixture showed good activity on S. aureus with MIC of 25 µg/ml.

In conclusion, the results of antibacterial property of P. guajava extracts showed a good correlation between reported uses of this plant in Brazilian Folk medicine against infectious diseases **(Sanches NR et. al 2005)**[55]**.**

Twigs

Phytochemical Studies

Twigs contain calcium (0.30-1.00%), Magnesium (0.06-0.30%), phosphorus (0.10 – 0.38%), potassium (0.21 – 0.39%) and sodium (0.03 – 0.20%). The concentration of fluoride ranged from 0.02 to 0.11 ppm, copper (0.02 – 0.14 ppm), iron (2.86 – 5.14 ppm), Zinc (0.31 – 0.57 ppm), Manganese (0.00 – 0.26 ppm) and lead (0.00 – 0.11 ppm)[15].

Methods to inhibit Platelet Aggregation

Invitro

Platelet Aggregation in Platelet Rich Plasma (Born Method)

Platelet aggregation is induced in platelet rich plasma (PRP) by the addition of aggregating agents such as ADP. Arachidonic acid, Collagen, Thrombin (or) PAF with stirring. The formation of platelet aggregates leads to changes in optical density which are monitored photometrically. The test is used to evaluate quantitatively the effect of test compounds on induced platelet aggregation in vitro.

The transmission maximum serves as a scale for platelet aggregation

(0% = Transmission of platelet Rich plasma

100% = Transmission of Platelet poor plasma)

percent inhibition of platelet aggregation is determined in concentration groups relative to vehicle controls.

Statistical significance is evaluated by means of the unpaired student t-test. IC_{50} values are determined from the concentration effect curves. **(Vogel HG et. al. 1997)**[56].

Washed platelets Method

Washed platelets are prepared by the method of Rho et al and platelet aggregation is determined by a standard turbidometric method using an aggregometer.

The effective concentration (EC 100, g/ml) which produces a 100% inhibition of platelet aggregation induced by arachidonic acid is considered as pharmacological response by the test drug.

This relatively simple method for determining the platelet aggregation inhibitory activity.

Whole Blood Aggregometry

In Vitro platelet aggregation is measured with a whole blood electrical impedance aggrego meter (chrono-log corp). There is a change in the impedance of whole blood, which is proportional to the amount of platelet aggregation occurring in the cuvette.

Vascular injury necessary for platelet aggregation is achieved by exposing the blood to high shear flow and subendothelial components. (**Gupta SK 2004**)[57].

Anti – Platelet Activity of the Plant Products[3]

The inhibitory effects of five flavonoids on the aggregation and secretion of platelets were studied. These flavonoids inhibited markedly platelet aggregation and ATP release of Rabbit platelets induced by arachidonic acid as collagen, and slightly those by platelet – activating factor.

The IC_{50} on arachidonic acid – induced platelet aggregation wass fisetin, 22 μm; Kaemp ferol, 20 μm; quercetin 13μm; Morin, 150 μm less than IC_{50} less than 300 μm.

(Tzeng S.H et. al 1991)[58]**.**

A fraction isolated from a crude aqueous extract of Galega officinalis L and purified by column chromatography inhibit platelet aggregation in platelet rich plasma.

Aggregation of platelets initiated by 25μm ADP was inhibited 50 per cent by 11.2μg/ml of the Fraction. Aggregation of platelets initiated by 100μg/ml collagen and 0.8U/ml thrombin was completely inhibited by 16μg/ml and 18.3μg/ml, respectively. **(Atanasov AT et al 2002)**[59].

In vivo effects of Glycyrrhizin upon two experimental models of induced thrombosis in rats were studied

Intravenous administration of Glycyrrhizin caused a dose – dependent reduction in thrombus size on a venous thrombosis model that combines stasis and hypercoagulability. It was observed that Glycyrrhizin doses of 180mg/kg body weight produced 93% decrease on thrombus weight. Glycyrrhizin was also able to prevent thrombosis using an arterio venous shut model. Glycyrrhizin doses of 180 and 360mg/kg decreased the thrombus weight by 35 and 90% respectively **(Silva WM et. al 2003)**[60]**.**

Antiplatelet properties of lavender oil towards platelet aggregation induced by arachidonic acid, U 46619, collagen and ADP (IC_{50} = 51,84,191 and 640 μg/ml, respectively) on guinea – pig platelet rich plasma (PRP) and its ability to destabilize clot retraction (IC_{50} = 149 μg/ml) induced by thrombin on rat PRP was demonstrated. **(Ballabeni. V et al 2004)**[61]**.**

Effects of aqueous extract of ocimum basilicum (OBL) on platelet aggregation and experimental thrombus were studied.

Platelet aggregation induced by ADP (5μm) and thrombin (4UI), and thrombus weight in an arterio venous thrombosis (AVT) model were tested after 2 weeks treatment with 15, 75 and 375 mg/kg OBL orally in rats, compared to 8.8 mg/kg/day aspirin.

OBL (15, 75, 375 mg/kg) dose – dependently inhibits platelet Aggregation by ADP and thrombin, with 75 mg/kg/ day having approximately the same effect as 8.8mg/kg/day aspirin. **(Tohti I et al 2006)**[62]**.**

The antiplatelet activity of different fractions of melothria maderaspatana was studied using platelet rich plasma in presence of ADP using increasing polarity of solvents (Hexane, $CHCl_3$, Ethyl acetate and methanol). Each extract was tested in concentrations of 100 μg/ml, 200μg/ml, 400μg/ml and 500μg/ml for the study.

The ethyl acetate extract showed a dose – dependent antiplatelet activity. Hexane and methanol extracts showed antiplatelet activity only at 200μg/ml and 400 μg/ml concentrations. Chloroform extract showed negligible antiplatelet activity.

However, the inhibition of platelet aggregation was only 50% when compared to the standard aspirin. **(Iman R.A et al 2006)**[63].

Chapter – III

AIM AND OBJECTIVE

Cardiovascular and cerebro vascular disorders continue to be the leading cause of death globally. Arterial and venous thrombosis might lead to acute coronary syndrome (ACS), myocardial infarction (MI), stroke, Venous thromboembolism (VTE), peripheral artery occlusion (PAO), deep vein thrombosis (DVT) or disseminated intravascular thrombosis (DIT)[3].

Platelets play a major role in thrombosis, and platelet aggregation is promoted at sites of atheroma formation. The modulation of platelet activity using specific pharmacological agents has proven to be a successful strategy for the prevention of thrombosis. In recent years a number of dietary sources of inhibitors of platelet function have been reported[64].

Flavonoids belong to a group of natural substances with variable phenolic structures and are found in fruit, vegetables, grains, bark, roots, stems, flowers, tea, and wine[65].

Research on flavonoids received an added impulse with the discovery of the French paradox, i.e., the low cardiovascular mortality rate observed in Mediterranean populations in association with red wine consumption and a high saturated fat intake. The flavonoids in red wine are responsible, atleast in part, for this effect[66].

Furthermore, epidemiologic studies suggest a protective role of dietary flavonoids against coronary heart disease[67].

The association between flavonoid intake and the long term effects on mortality

was studied subsequently[68] and it was suggested that flavonoid intake is inversely correlated with mortality due to coronary heart disease[69].

One of the best described flavonoids, quercetin, is a member of this group. Quercetin is found in abundance in onions, apples, broccoli, and berries.

Quercetin appears to have many beneficial effects on human health, including cardiovascular protection, anti-cancer activity, anti-ulcer effects, anti-allergy activity, cataract prevention, antiviral activity, and anti-inflammatory effects[70].

Since potential beneficial properties of Quercetin have been ascertained against coronary heart diseases, we prompted as to study the effect of a medicinal plant having quercetin as important phytoconstituent. Review of literature on ***Psidium guajava*** revealed the presence of quercetin in the leaf part.

The available literature reveals that no study was performed on its effect on platelet aggregation, the literature survey of screening procedures for the platelet aggregation inhibitory activity. The methods using aggregometer are expensive and time consuming. So there is a need to develop a simple, cost effective in vitro procedure for preliminary screening in laboratory to facilitate the preliminary screening using plant extracts.

Review of literature also reveals that there are various varieties of ***P.guajava*** are available.

Aim

To study pharmacognostical, phytochemical and invitro effect on human platelet aggregation of leaf extract of ***P. guajava***.

Objective

The objective of the study was divided into three parts.

Part – I

Pharmacognostic Studies

- Collection and Authentification of the various varieties of the plant ***P. guajava*** in Madurai.
- Detailed pharmacognostical study on the leaves of all the above varieties of ***P. guajava*** including quantitative microscopy and other parameters to obtain the best possible structural details to assist in the solution of taxonomic problem to avoid misleading of diagnostic features, to check the presence of any specific cell characters pertaining to any variety which will support the powder analysis of pure mature leaves.

Part – II

Preliminary Phytochemical studies

- Preliminary phytochemical analysis on the crude powder and on the different extracts of leaf of high yielding and widely growing variety of ***P. guajava*** (Lucknow 49).
- Isolation of volatile oil and to study the composition and proportion by GC-MS analysis to subject the ethanolic extract to HPLC analysis to find out the

presence and percentage of Quercetin in the high yielding variety.

Part – III

Pharmacological Screening

- To design a simple laboratory scale preliminary procedure to identify the presence of platelet aggregation inhibitory activity without any specialized expensive instruments like aggregometer.
- To evaluate the effect of alcoholic extract of high yielding variety ***P.guajava*** effects on platelet aggregation in vitro.

Chapter – IV

PHARMACOGNOSTIC STUDIES

SECTION - A

MACROSCOPICAL STUDIES OF THE LEAVES OF *Psidium guajava Linn.*

Psidium guajava Linn is a large dicotyledonous shrub belonging to the family **Myrtaceae[16].**

Taxonomy[71]

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magno liophyta
Class	:	Magno liopsida
Sub Class	:	Rosidae
Order	:	Myrtales
Family	:	Myrtaceae
Genus	:	***Psidium***
Species	:	***Psidium guajava***

Synonym[16,72]

Psidium Cujavillus Burm. f

Guajava Pyrifera (L.) Kuntze

Guajava Pyriformis Gaertn

Psidium guayava Raddi

Psidium Pomiferum L.,

Psidium Pyriferum L.

Psidium Sapidissimum Jacq.

Psidium aromaticum L.

Psidium fragrans Mac fadyen

Vernacular Names[11,12,72,73]

English	:	Common guava, Guava tree
Hindi	:	Amrud, Saphed Saphari
Kannada	:	Keli
Malayalam	:	Pera, Atakkappalam
Sanskrit	:	Perukah
Tamil	:	Koyya
Telugu	:	Jampandu, Goyyapandu
Bengali	:	Goaachhi, Peyara
Marathi	:	Jamba, tupkel
Gujarathi	:	Jamrud, Jamrukh
Arabic	:	Guwafah Safra
Burmese	:	Melaka pen
Chinese	:	Fan shi liu, Fan tao
Dutch	:	Goeajaaba, Guyaba
French	:	Gouyave, Goyavier
Italian	:	Guiava, Psidio
Japanese	:	Banjirou
Nepalese	:	Ambaa, Ambaka
Portuguese	:	Goiaba, goiabeiro
German	:	Guavenbaum, guayave, Guave

Geographical Distribution[16,12,73]

Native

Colombia, Mexico, Peru, United States of America.

Exotic

Australia, Bangladesh, Brunei, Cambodia, Cameroon, China, Costa Rica, Cote d'Ivoire, Cuba, Dominican Republic, Ecuador, Eritrea, Ethiopia, Fiji, Gabon, Gambia, Greece, Guyana, Haiti, India, Indonesia, Israel, Kenya, Laos, Malawi, Malaysia, Myanmar, Nigeria, Pakistan, Panama, Philippines, Puerto Rico, Samoa, Senegal, South Africa, Srilanka, Sudan, Tanzania, Thailand, Togo, Uganda, Venezuela, Vietnam.

India

Uttar Pradesh, Bihar , Maharashtra, Assam, West Bengal, Andhra Pradesh, Madras.

Mesoamerica

Bellize, Costarica, Elsalvador, Guatemala, Honduras, Nicaragua; Panama.

Northern South America

French Guiana; Guyana ; Suriname; Venezuela

Brazil

Brazil (S.e.)

Western South America

Bolivia, Colombia, Ecuador

Southern South America

Argentina (n.), Paraguay

***Habit and Habitat*[16]**

Psidium guajava is a large dicoty ledonous – shrub, or small ever green tree, generally 3-10m high, many branches, crooked stems, root system generally superficial and very extensive, frequently extending well beyond the canopy, there are some deep roots but no distinct tap root (Plate - 1, Fig - 1).

P. guajava appears to have evolved in relatively open areas, such as savannah / shrub transitional zones, or in frequently distributed areas where it is a strong competitor in early secondary growth. In some areas it is found in large thickets. The guava is a hardy tree that adapts to a wide range of growing conditions.

The highest yields are recorded at mean temperatures of 23 – 28^{0}C. In the subtropics quiescent trees withstand light frost and 3.5 – 6 months (depending on the cultivar) of mean temperatures above 16^{0}C suffice for flowering and fruiting. It fruits at altitudes up to 1500m and survives upto 2000m. Guava is more drought resistant than most tropical fruit crops. For maximum production in the tropics, however it requires rainfall distributed over the year. If fruit ripens during a very wet period it loses flavour and may split.

Description of the Plant

Bark

Light to Reddish brown, thin, smooth, continuously flaking.

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, some what thick and leathery, dull grey to yellow-green above, slightly downy below, veins prominent, gland dotted (Plate - 2).

Morphology of the leaves showed no difference in all the varieties.

Flowers

1 to 3 flowered, Infilorescence, axillary, pedicles about 2 cm long, bracts two, linear (Plate - 3)

Calyx : Splitting irregularly into 2-4 lobes, whitish and sparsely hairy within.

Petals : 4-5, white, Linear - Ovate 2 cm long, delicate.

Stamens : numerous

Filaments : Pale white, about 12mm long, erect (or) spreading

Anther: Straw coloured

Ovary : inferior

Ovules : numerous

Style : 10 cm long

Stigma : Green - Capitate

Fruits

Ovoid or pear – shaped berry, 4 – 12 cm long, weighing upto 500g; skin, yellow when ripe, sometimes flushed with red; pulp juicy, creamy – white (or) creamy – yellow to pink or red: Mesocarp thick, edible, the soft pulp enveloping numerous, cream to brown, kidney – shaped or flattened seeds. The exterior of the fruit is fleshy, and the centre consists of a seedy pulp. (Plate - 4).

Seeds[74]

Yellowish, Reniform

Section – B

MICROSCOPICAL STUDIES OF THE LEAVES OF 9 VARIETIES OF *P.guajava Linn.*

Materials and Methods[75,76,77,78,79]

Collection of Specimens

The plant specimens were collected from Agricultural College, Department of Horticulture, Madurai during August 2007. Care was taken to select healthy plants and for normal organs. The leaves were cut and removed from the plant and fixed in FAA (Formalin – 5ml + Acetic acid – 5 ml + 70% ethyl alcohol – 90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary – butyl alcohol. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60^{0}c), until TBA solution attained super saturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. After dewaxing the section were stained with toluidine blue. Since toluidine blue is a polychromatic stain, the staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered pink color to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc., Where ever necessary, sections were also stained with safranin and fast-green and potassium iodide (for starch).

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs where ever

necessary. Photographs of different magnifications were taken with Nikon labphot 2 microscope unit. For normal observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background.

MICROSCOPY OF LEAF

The leaf is dorsiventral with distinction of adaxial and abaxial faces. The leaf surface is smooth. (Fig. 2)

Midrib

1. Anakapalli (AP)

Thickness in vertical plane- 1.25 mm

Horizontal Plane - 2.2 mm

Adaxial side is broadly concave. Abaxial part is semicircular, even, smooth surface. Laterally spreading lamina. (Plate – 5).

2. Bangalore (BAN)

Thickness in vertical plane- 1mm

Horizontal plane - 1.75 mm

Adaxial side is broadly concave. Abaxial part is slightly undulate in outline. Slightly vertical lamina. (Plate - 8).

3. Chittidhar (CD)

Thickness in Vertical plane - 1.25 mm

Horizontal plane - 2.45 mm

Adaxial side is concave and abaxial side is semicircular and slightly undulate outline.

slightly vertical lamina. (Plate - 11).

4. Hafsi (HF)

Thickness in Vertical Plane - 1.4 mm

Horizontal Plane - 2.7 mm

Adaxial concavity is wide and rectangular. Abaxial side is wavy with shallow ridges and furrows and the lamina being vertical in orientation. (Plate - 14).

5. Lucknow – 46

Thickness in Vertical Plane - 1.3 mm

Horizontal Plane - 2.3 mm

Adaxial side is widely concave. The abaxial part is tangentially oblong and semi circular Lamina is laterally spreading. (Plate - 17).

6. Lucknow – 49

Thickness in Vertical Plane - 1.5 mm

Horizontal Plane - 2.4 mm

Adaxial concavity is wide, U shaped. Abaxial part slightly wavy, semi circular Lamina is vertically oriented. (Plate - 20).

7. Nagpur Seedless

Thickness in Vertical Plane - 1.1 mm

Horizontal Plane - 2.45 mm

With wide adaxial concavity with the lamina extending outward.Abaxial part is horizontally widened with uneven circumference. (Plate - 23).

8. Red fleshed

Thickness Vertical Plane - 1.1 mm

Horizontal Plane - 2.3 mm

Adaxial part has conical concavity with the laterally spreading lamina. The abaxial part is horizontally extended and semi circular. (Plate - 26).

9. Smooth green

Thickness in Vertical Plane - 1.1 mm

Horizontal Plane - 1.7 mm

The adaxial part has shallow wide concavity with spreading lamina. The lower midrib is semi circular with more or less even outline. (Plate - 29).

VASCULAR BUNDLE

1. **Anakapalli**

Broadly bowl shaped, Vertical - 250μm and 1.6 mm horizontal. Xylem – compact, parallel radial rows thin walled, **less lignified** with narrow, **Uniseriate**, intervening layers of **thick walled fibres.** Metaxylem – 30 μ wide. Phloem - 50μm wide, continuous all along the lower part of xylem arc. phloem zone ensheathed wide band of dark tannin filled small compact parenchyma cells. (Plate - 6)

2. **Bangalore**

Wide, shallow arc shaped xylem, phloem strand – 1mm wide, 150 μm thick.

Xylem – dense parallel lines, thin walled containing dark elliptical sclerenchyma cells in between the lines.

Phloem is seen in small cluster at the end of each xylem row (30μm wide) (Plate - 9).

3. **Chittidhar**

Vascular strand is broad and bowl shaped, 1.5mm horizontal and 250μm in thickness. Xylem elements are narrow, thick walled angular, parallel with narrow intervening dark sclerenchyma cells. Metaxylem elements are 20μm in diameter. Phloem occurs in small strips opposite to each xylem row. (Plate - 12).

4. **Hafsi**

Vascular strand is wide, thin deeply bowl shaped. Xylem thin walled, angular in outline occur in short parallel lines (1.9mm in horizontal plane and 150 μm thick) **Phloem elements occur at the ends of xylem lines as small nests.** (Plate - 15).

5. **Lucknow – 46**

Vascular strand 1.6mm in horizontal plane and 700μm in thickness. **Xylem fairly wide thin walled angular in outline occur in compact radial lines**. Metaxylem 30μm wide. Phloem encircled around the xylem row. (Plate - 18).

6. **Lucknow – 49**

The vascular strand is broad and bowl shaped, 1.3mm wide, 60μm thick. Xylem elements are narrow thin walled. Metaxylem 30μm wide. (Plate - 21).

7. **Nagpur seedless**

The vascular strand is 1.8mm wide and 170 μm thick. Xylem elements are thick walled

and in long parallel rows. Metaxylem is 25 μm wide. Phloem is seen in thin dark layer beneath the xylem. (Plate - 24).

8. **Red fleshed**

The vascular strand is deep, wide and urn shaped 1.6mm wide, 250μm thick. Xylem thin walled, arranged in **dense parallel rows**. Metaxylem 2μm wide. Phloem parenchyma cells have dense content of tannin (Plate - 27).

9. **Smooth Green**

Vascular strand is broad and arc shaped measuring 1mm across and 200μm thick. Xylem thin walled and 20μm wide. Phloem zone is narrow and occurs opposite to the metaxylem element. (Plate - 30).

GROUND TISSUE OF THE MIDRIB

1. **Anakapalli**

Outer narrow zone of tannin filled parenchyma cells and inner wider circular turgid cells with minute intercellular spaces. Secretory cavities are sporadically seen in the outer part.

2. **Bangalore**

Homogenous parenchymatous compact tanniniferous. Secretory cavities are circular, sporadically seen outer zone (80μm in diameter).

3. **Chittidhar**

Homogenous parenchymatous less tanniniferous thin walled circular and compact cells. Secretory cavities are fairly common and occur along the periphery of the abaxial part

(50μm wide).

4. Hafsi

Parenchymatous, thin walled less compact, less tanniniferous. Secretory cavities are more frequent in the abaxial part.

5. Lucknow – 46

Parenchymatous, thin walled compact and **tannin content not evident**. Secretory cavities occur in the outer zone of abaxial midrib. **Calcium oxalate druses are common within dilated ground cells.**

6. Lucknow – 49

Parenchymatous, thin walled, circular, less compact with intercellular spaces with, most of them with small masses of tannin body. **Narrow Secretory** cavities are seen in the periphery.

7. Nagpur seedless

Circular, thick walled and compact and is **lacking of tannin**.

8. **Red fleshed**

Wide and Numerous secretory cavities along the periphery of the parenchymatous ground tissue are seen (100μm) with reduced content of tannin in the ground parenchyma.

9. Smooth Green

Parenchymatous circular thin walled compact, wide circular secretory cavities are frequently seen in the periphery (100μm wide).

LAMINA

1. Anakapalli

230μm thick, smooth uniformly even. Adaxial epidermis multiple (80 100μm), consist of oblong, spindle shaped outer layer and 2 or 3 layers of wider rectangular cells. The abaxial epidermis is thin, elliptical narrow cells (15μm thick). (Plate - 7).

2. Bangalore

200 μm thick, 4 layered adaxial epidermis (80μm) which consist of elliptical darkly staining cells. (Plate - 10).

3. **Chittidhar**

190 μm thick, surfaces even and smooth. Adaxial epidermis is four layered. Upper most layer of cells spindle shaped. Rest of the cells are rectangular and wide. Wide crystal bearing cells are frequently seen in this zone. Abaxial epidermis is thin, Unistratose with spindle shaped cells. (Plate - 13).

4. Hafsi

240μm thick, smooth and even. The adaxial epidermis is thin with narrow tabular cells. The subepidermal layers of cells are dilated, squarish or rectangular without tannins, four layered (70μm). Calcium oxalate druses in the dilated cells are frequently seen in the adaxial epidermis. (Plate - 16).

5. Lucknow – 46

200μm thick, smooth and even. Adaxial epidermis three layered, thin walled tabular in

shape. Subepidermal layers are wider and spindleshaped (70μm). Calcium oxalate druses are frequent. (Plate - 19).

6. Lucknow – 49

250μm thick. The adaxial epidermal zone consist of an outermost thin layer of small rectangular thin walled cells followed by 4 layers of dilated polyhedral compact cells with prominent wall. The abaxial epidermis is thin with small semi circular cells.(Plate - 22).

7. Nagpur seedless

210μm thick, even except in the region of the lateral vein. The adaxial epidermis is thin, oblong. The subepidermal layers have wide 3 rows of polyhedral cells. (Plate - 25).

8. **Red fleshed**

200μm thick, even, smooth. The adaxial epidermis is thin, narrowly cylindrical. 2 rows of wide and rectangular sub epidermal layers of cells are present with frequent druses. (Plate - 28).

9. Smooth Green

Smooth and even except in the raised lower side of the lateral vein. The adaxial epidermis is narrow with thin walled elongated cells. 3 layers of sub epidermal cell which are slightly dilated and square shaped. (Plate - 31).

MESOPHYLL

1. Anakapalli

Mesophyll differentiated into adaxial zone of 2 layers of compact cylindrical palisade cells (70-100μm in height). Lower part consist of 4 layers of compact spongy parenchyma.

2. Bangalore

Two horizontal rows of narrow compact palisade cells (70μm in height) 3 or 4 layers of small less compact darkly staining spongy parenchyma.

3. **Chittidhar**

Two layers of palisade cells (50μm in height) 4 horizontal layers of rectangular compact spongy cells.

4. Hafsi

Palisade zone is 1 or 2 layered, short, cylindrical, compact cells (30-60μm in height) 5 layers of short vertically oblong compact cells showing stratified arrangement of spongy mesophyll. **Dilated cells contain calcium oxalate druses both in palisade and spongy pareachyma cells.**

5. Lucknow – 46

3 layers of narrow compact cylindrical parenchyma cells. Spongy parenchyma is not distinct, two layered vertically elongated compact cells. Tannin content is abundant. **Calcium oxalate druses are frequent in the dilated cells.**

6. Lucknow – 49

2 layers of narrow cylindrical compact palisade cells(70μm in height) and 2 to 3 layers of vertically oblong palisade like spongy parechyma cells. **Tannin content is less.**

7. Nagpur seedless

The palisade cells are 2 layered with narrow cylindrical compact cells (80μm in height) and short vertically oblong cylindrical spongy mesophyll cells similar to palisade cells.

8. **Red fleshed**

Narrow cylindrical and compact **single row** palisade cells (60μm). 4 or 5 layered small lobed and loosely arranged spongy parenchyma cells. Mesophyll tissues are having **dark tannin accumulation.**

9. Smooth Green

Short compact 2 layers of palisade cells (50μm in height). Short 3 layers of palisade like spongy parenchyma.

LATERAL VEIN

1. Anakapalli

Less prominent

2. Bangalore

Vascular strands of lateral vein are prominent collateral with thick adaxial and abaxial sclerenchyma sheath.

3. **Chittidhar**

Less prominent

4. Hafsi

Lateral veins have **elliptical** collateral vascular bundle with **parenchymatous bundle sheath.**

5. Lucknow – 46

Vascular bundle of the lateral veins vertically stretched, collateral with sclerenchyma bundle caps.

6. Lucknow – 49

Vascular bundle of the lateral veins are small, collateral having a thin layer of parenchymatous bundle sheath.

7. Nagpur seedless

The lateral vein bundles project prominently below the surface, having thick mass of xylem, small groups of phloem and thick pillar of bundle sheath extensions.

8. **Red fleshed**

Lateral veins have vertically oblong collateral vascular strand with sclerenchymatous caps.

9. Smooth Green

The vascular strand of the lateral vein is vertically elongated and collateral with parenchymatous small bundle caps.

Secretory Cavity

1. **Anakapalli**
4. **Hafsi (50μm)**
5. **Lucknow – 46(100μm)**
7. **Nagpur seedless**
8. **Red fleshed(70μm)**

are having secretory cavities in the lower side. Smooth green varieties having 80μm wide in the upper portion. Lucknow - 49 varieties have secretory cavities in the periphery of the midrib. In Bangalore, Chittidhar secretory cavities are absent.

Stomata

There were no differences in stomatal type in all of the varieties.

General Powder Microscopy for the leaves of *P. guajava* Linn. (Fig – 3)

Colour : Light dull green

Nature : Smooth or finely coarse

Odour : No characteristic

Taste : No characteristic

The powder Microscopy of leaf powder reveals the following characters.

1. Fragments of Epidermis
2. Secretory cavity (absent in Bangalore, Chittidhar varieties)
3. Calcium oxalate crystals (present in Hafsi and Lucknow 46 varieties)
4. Fragments of palisade mesophyll
5. Conical and Flagellate Trichomes
6. Dark and greenish pieces of leaves
7. Parenchyma cells
8. Paracytic stoma

SECTION – C

QUANTITATIVE MICROSCOPY FOR LEAVES OF 9 VARIETIES OF *P.guajava Linn.*

Microscopic Schedules

The vein islet number, vein terminal number, stomatal number and stomatal index were determined on fresh leaves for 9 different varieties of P.guajava using standard procedures[80,81,82,83,84,85]

A. *Vein islet number and Vein terminal number*

The term vein islet in used to denote the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands.

The number of vein - islet mm^{-2} calculated from four contiguous square millimeters in the central part of the lamina, midway between the midrib and the margin, is termed the vein – islet number.

Veinlet termination number defined of veinlet as the number terminations per mm^2 of leaf surface.

Determination of Vein Islet Number and Vein Termination Number

Pieces of leaves were cut from the various regions of the leaves between midrib and margin, cleared in chloral hydrate, stained and mounted on a slide.

A camera lucida is set up and by means of a stage micrometer the paper in divided into squares of $1mm^2$ using a 16mm objective. The stage micrometer is then replaced by the cleared preparation and the veins are traced in four contiguous squares, either in a square

2mm x 2mm (or) rectangle 1mm x 4 mm.

When counting, it is convenient to number each vein-islet on the tracing. Each numbered area must be completely enclosed by veins, and those which are incomplete are excluded from the count if cut by the top and left-hand sides of the square (or) rectangle but included if cut by the other two sides.

Ten readings for vein islet and vein termination number were recorded.

TABLE – 1

VEIN ISLET NUMBER AND VEIN TERMINATION NUMBER FOR

9 DIFFERENT VARIETIES OF *P. guajava LEAVES*

No.	Anakapalli		Bangalore		Chittidhar		Hafsi		Lucknow - 46		Lucknow - 49		Nagpur Seedless		Red fleshed		Smooth Green	
	VI	VT	VI	VT	VI	VT	VI	VT	VI	VT	VI	VT	VI	VT	VI	VT	VI	VT
1	3	5	3	5	4	4	4	5	3	4	3	4	3	3	2	3	3	4
2	2	4	3	4	4	5	3	4	4	4	2	5	4	4	2	4	3	4
3	3	5	3	5	3	5	3	5	4	5	2	4	3	3	3	5	4	5
4	3	4	4	4	4	5	3	4	3	4	2	4	2	4	2	3	3	5
5	3	4	3	5	3	5	4	5	4	5	2	5	4	4	3	4	4	4
6	4	5	3	4	3	4	3	5	3	4	3	5	2	3	2	3	3	5
7	3	4	2	5	3	5	3	4	4	5	3	4	2	3	3	5	3	4
8	3	4	3	4	3	5	4	4	3	4	3	4	3	4	2	4	4	4
9	4	4	3	5	4	5	3	5	3	4	2	4	3	3	3	4	3	4
10	2	4	3	4	4	5	3	4	4	4	3	4	2	4	3	5	3	4
Range																		
Min.	2	4	2	4	3	4	3	4	3	4	2	4	2	3	2	3	3	4
Ave.	3	4.3	3	4.5	3.5	4.8	3.3	4.5	3.5	4.3	2.5	4.3	2.3	3.5	2.5	4	3.3	4.3
Max	4	5	4	5	4	5	4	5	4	5	3	5	4	4	3	5	4	5

VI – Vein Islet Number

VT – Vein Termination Number

Stomatal number

Stomatal number is defined as the number of stoma present in one square mm area of the photosynthetic tissues.

Using fresh leaves, replicas of leaf surface may be made which are satisfactory for the determination of stomatal number and stomatal index.

An approximate 50% gelatin and water gel is liquefied on a water bath and smeared on a hot slide. The fresh leaf is added, the slide is inverted and cooled under a tap and after about 15 – 30 min the specimen is stripped off. The imprint on the gelatin givens a clear outline of epidermal cells, stomata.

With the help of camera lucida and stage micrometer, 1mm square was drawn on a paper. The stage micrometer was replaced by the preparation. Then the preparation was observed and the stomata were marked in that unit area. Number of stomata present in that unit area was calculated. Ten readings were taken and the range of stomatal number was calculated separately for both upper and lower epidermis.

TABLE – 2

STOMATAL NUMBER FOR 9 DIFFERENT VARIETIES OF *P.guajava* LEAVES

No.	Anakapalli		Bangalore		Chittidhar		Hafsi		Lucknow - 46		Lucknow - 49		Nagpur Seedless		Red fleshed		Smooth Green	
	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE
1	29	41	32	40	36	42	38	42	34	40	28	46	31	38	30	40	32	48
2	32	44	33	41	37	43	39	43	35	41	31	47	33	40	31	42	34	49
3	31	43	35	42	36	45	40	42	36	42	29	46	35	39	32	46	36	50
4	30	42	32	44	38	42	38	44	35	44	30	48	32	41	30	45	32	48
5	33	45	34	43	37	45	39	46	36	43	32	46	34	42	32	47	36	49
6	31	42	36	44	36	42	41	45	37	44	30	47	32	40	33	48	34	51
7	30	41	35	41	39	46	40	47	38	41	31	48	31	38	30	41	36	52
8	32	43	36	42	40	44	42	46	37	42	29	49	34	39	31	43	37	48
9	29	44	35	43	39	45	41	43	36	43	28	48	35	41	32	48	38	50
10	30	45	35	44	40	46	38	48	38	44	29	46	32	42	33	47	36	52
Range																		
Min.	29	41	32	40	36	42	38	42	35	40	28	46	31	38	30	40	32	48
Ave.	30.7	43	34.3	42.4	37.8	44	39.6	44.6	36.2	42.4	29.7	47.1	32.9	40	31.4	44.7	35.1	49.7
Max	43	45	36	44	40	46	42	48	38	44	32	49	35	42	33	48	38	52

UE - Upper Epidermis LE - Lower Epidermis

B. Stomatal Index

It is the percentage, which the numbers of stomata from the total number of epidermal cells, each stoma being counted as one cell.

I, Stomatal index = S/S+E x 100

Where S = Number of Stomata per unit area

E = Number of epidermal cells in the same unit area

Determination of Stomatal Index

The procedure adopted in the determinations of stomatal number was observed under high power (45 x). The epidermal cells and the stomata were counted. From these values the stomatal index was calculated using the above formula.

TABLE – 3

STOMATAL INDEX FOR 9 DIFFERENT VARIETIES OF *P. guajava LEAVES*

No.	Anakapalli		Bangalore		Chittidhar		Hafsi		Lucknow - 46		Lucknow - 49		Nagpur Seedless		Red fleshed		Smooth Green	
	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE	UE	LE
1	20.0	19.2	21.0	19.4	21.2	19.6	21.0	19.2	20.1	19.2	19.8	19.4	20.8	18.8	20.1	19.2	20.1	19.0
2	20.1	19.5	21.1	19.2	21.4	19.4	21.2	19.0	20.0	19.3	19.9	19.5	20.9	19.0	20.5	19.3	20.2	19.1
3	20.3	19.6	21.1	19.2	21.6	19.7	21.1	19.1	20.2	19.1	20.1	19.6	20.8	19.1	20.3	19.5	20.4	19.1
4	20.5	19.4	20.9	19.4	21.7	19.8	21.3	19.0	20.3	19.4	20.2	19.2	21.0	19.2	20.2	19.2	20.5	19.0
5	20.4	19.5	21.0	19.5	21.8	19.9	21.6	19.1	19.9	19.5	20.0	19.3	21.2	18.7	20.4	19.4	20.6	19.2
6	20.2	19.3	21.2	19.6	21.5	20.0	21.7	19.1	20.0	19.2	19.8	19.5	21.1	18.9	20.5	19.4	20.2	19.0
7	20.1	19.1	21.0	19.3	21.6	20.0	21.5	19.2	20.1	19.1	20.2	19.6	21.0	19.1	20.6	19.5	20.1	19.1
8	20.3	19.6	20.9	19.5	21.8	19.8	21.8	19.0	20.2	19.3	20.1	19.4	20.8	19.0	20.5	19.4	20.4	19.2
9	20.0	19.2	21.1	19.5	21.8	19.6	21.6	19.1	20.3	19.4	19.9	19.6	20.9	19.1	20.3	19.6	20.1	19.0
10	20.5	19.1	21.2	19.6	21.6	19.9	21.8	19.2	20.2	19.5	20.2	19.5	21.2	19.2	20.6	19.5	20.6	19.2
Range																		
Min.	20	19.1	20.9	19.2	21.2	19.4	21.0	19.0	19.9	19.1	19.8	19.2	20.8	18.7	20.1	19.2	20.1	19.0
Ave.	20.2	19.3	21.0	19.4	21.6	19.8	21.4	19.1	20.1	19.3	20.0	19.5	21.0	19.0	20.4	19.4	20.3	19.1
Max	20.5	19.6	21.2	19.6	21.8	20.0	21.8	19.2	20.3	19.5	20.2	19.6	21.2	19.2	20.6	19.6	20.6	19.2

UE - Upper Epidermis LE - Lower Epidermis

QUANTITATIVE SCHEDULES FOR LEAVES OF 9 VARIETIES OF *P. guajava Linn.*

Determination of Ash Values

Ash Value

The ash values were determined by using air dried powder of the leaf as per the official method.

Total ash

Two grams of the air dried leaf powder was accurately weighed in a platinum crucible separately. The powder was scattered into a fine even layer on the bottom of the crucible and incinerated by gradually increasing the temperature not exceeding 450°c, until free from carbon. Then it was cooled and weighed for constant weight. The percentage of ash with reference to the air dried powder was calculated.

Water soluble Ash

The ash obtained from the total ash procedure was boiled with 25 ml of water for 5 minutes and the insoluble matter was collected on an ash less filter paper and washed with hot water. Then it was ignited for 15 minute at a temperature not exceeding 450°c. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight represents the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried powder.

Acid insoluble ash

The ash obtained from the total ash was boiled for five minutes with 25 ml

of dilute hydrochloric acid. The insoluble matter was collected in a tarred sintered glass crucible. The residue was washed with hot water, dried and weighed. The percentage of acid insoluble ash with reference to the air dried drug was calculated.

Table - 4

ASH VALUES FOR THE LEAVES OF *P. guajava (9 different varieties)*

ANAKAPALLI

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.45	1.46	-
2	11.26	1.52	-
3	10.90	1.50	-
4	11.12	1.53	-
5	11.49	1.56	-
6	10.80	-	2.96
7	11.20	-	2.60
8	10.91	-	2.52
9	11.12	-	3.02
10	10.78	-	2.90
Range			
Minimum	10.78	1.46	2.52
Average	11.10	1.51	2.8
Maximum	11.49	1.56	3.02

TABLE – 5

BANGALORE

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)

1	11.46	1.47	-
2	11.20	1.50	-
3	10.92	1.48	-
4	11.16	1.49	-
5	11.50	1.52	-
6	10.82	-	2.97
7	11.18	-	2.97
8	10.88	-	2.53
9	11.08	-	3.04
10	10.76	-	2.88
Range			
Minimum	10.76	1.47	2.53
Average	11.09	1.49	2.80
Maximum	11.50	1.52	3.04

TABLE – 6

CHITTIDHAR

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.40	1.47	-
2	11.19	1.52	-
3	10.84	1.46	-
4	11.08	1.48	-
5	11.52	1.52	-
6	10.78	-	2.98
7	11.18	-	2.62
8	10.92	-	2.48
9	11.12	-	3.01
10	10.77	-	2.88
Range			
Minimum	10.77	1.46	2.48
Average	11.08	1.49	2.79
Maximum	11.52	1.52	3.01

TABLE – 7

HAFSI

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.42	1.50	-

2	11.20	1.48	-
3	10.85	1.54	-
4	11.10	1.52	-
5	11.50	1.46	-
6	10.76	-	2.94
7	71.18	-	2.68
8	10.86	-	2.52
9	11.06	-	3.08
10	10.74	-	2.94
Range			
Minimum	10.74	1.46	2.52
Average	11.06	1.50	2.83
Maximum	11.50	1.54	3.08

TABLE – 8

LUCKNOW - 46

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.46	1.48	-
2	11.20	1.50	-
3	10.86	1.53	-
4	11.10	1.55	-
5	11.42	1.50	-
6	10.76	-	2.94
7	11.10	-	2.62
8	10.80	-	2.50
9	11.10	-	3.01
10	10.70	-	2.88
Range			
Minimum	10.70	1.48	2.50
Average	11.05	1.51	2.79
Maximum	11.46	1.55	3.01

TABLE – 9

LUCKNOW – 49

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.48	1.49	-
2	11.24	1.51	-

3	10.96	1.52	-
4	11.14	1.50	-
5	11.58	1.54	-
6	10.84	-	2.99
7	11.22	-	2.66
8	10.90	-	2.54
9	11.10	-	3.06
10	10.80	-	2.92
Range			
Minimum	10.80	1.49	2.54
Average	11.12	1.51	2.83
Maximum	11.58	1.54	3.06

TABLE – 10

NAGPUR SEEDLESS

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.44	1.45	-
2	11.23	1.49	-
3	10.81	1.52	-
4	11.14	1.56	-
5	11.44	1.51	-
6	10.70	-	2.95
7	11.08	-	2.60
8	10.82	-	2.48
9	11.12	-	3.02
10	10.74	-	2.84
Range			
Minimum	10.70	1.45	2.48
Average	11.05	1.50	2.77
Maximum	11.44	1.56	3.02

TABLE – 11

RED FLESHED

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.49	1.46	-
2	11.28	1.53	-
3	10.94	1.54	-

4	11.10	1.48	-
5	11.48	1.50	-
6	10.48	-	2.90
7	11.16	-	2.68
8	10.82	-	2.50
9	11.12	-	3.08
10	10.72	-	2.88
Range			
Minimum	10.72	1.46	2.50
Average	11.10	1.50	2.81
Maximum	11.49	1.54	3.08

TABLE - 12

SMOOTH GREEN

Observation Number	Total Ash (%)	Acid Insoluble ash (%)	Water soluble Ash (%)
1	11.50	1.52	-
2	11.20	1.49	-
3	10.98	1.54	-
4	11.10	1.47	-
5	11.60	1.52	-
6	10.79	-	2.95
7	11.19	-	2.67
8	10.85	-	2.51
9	11.06	-	3.02
10	10.82	-	2.90
Range			
Minimum	10.79	1.47	2.51
Average	11.11	1.51	2.81
Maximum	11.60	1.54	3.02

Determination of Loss on Drying

For the determination of loss on drying, the method described by wallis was followed.

One gram of the powdered leaf was accurately weighed in a tarred Petri dish, previously dried under the conditions specified in IP ’96. The powder was

distributed as evenly as practicable, by gentle sidewise shaking. The dish was dried in an oven at 100 – 105^0 c for 1 hour. It was cooled in a desiccator and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

PERCENTAGE LOSS ON DRYING FOR THE LEAVES OF *P. guajava (9 DIFFERENT VARIETIES)*

TABLE - 13

ANAKAPALLI

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		9.9	
2		10.5	
3		9.4	
4		9.3	
5		10.0	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.3	9.8	10.5

TABLE - 14

BANGALORE

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		10.0	
2		10.1	
3		9.6	
4		9.8	
5		10.2	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.6	9.9	10.2

TABLE - 15

CHITTIDHAR

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		10.1	
2		9.8	
3		9.7	
4		9.2	
5		10.1	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.2	9.8	10.1

TABLE – 16
HAFSI

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		9.6	
2		10.0	
3		9.4	
4		9.2	
5		10.3	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.2	9.7	10.3

TABLE – 17

LUCKNOW – 46

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		9.9	
2		10.0	
3		9.1	
4		9.8	
5		9.9	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.1	9.7	10.0

TABLE - 18

(LUCKNOW – 49)

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		9.7	
2		10.3	
3		9.5	
4		9.6	
5		10.1	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.5	9.8	10.3

TABLE – 19

NAGPUR SEEDLESS

Observation Number	Loss on Drying (%) W/W
	Leaf
1	10.1
2	10.4
3	9.6
4	9.3
5	9.8

Material	Minimum	Average	Maximum
Leaf	9.3	9.8	10.4

TABLE – 20

RED FLESHED

Observation Number	Loss on Drying (%) W/W
	Leaf
1	10.2
2	10.0
3	9.7
4	9.2
5	10.4

Material	Minimum	Average	Maximum
Leaf	9.2	9.9	10.4

TABLE – 21

SMOOTH GREEN

Observation Number		Loss on Drying (%) W/W	
		Leaf	
1		10.1	
2		10.4	
3		9.3	
4		9.8	
5		10.3	
Material	**Minimum**	**Average**	**Maximum**
Leaf	9.3	10.0	10.4

EXTRACTIVE VALUES

Petroleum Ether Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of petroleum ether in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of petroleum ether. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105^{o}C and weighed. The percentage of the petroleum ether soluble extractive value was calculated with reference to the air dried powder.

Benzene Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of benzene in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of benzene. 25 ml, of the filtrate was evaporated to

dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the benzene soluble extractive value was calculated with reference to the air dried powder.

Ethyl Acetate Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of ethyl acetate in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethyl acetate. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the ethyl acetate soluble extractive value was calculated with reference to the air dried powder.

Chloroform Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of chloroform in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the chloroform soluble extractive value was calculated with reference to the air dried powder.

Ethanol Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of ethanol in a closed flask for 24 hrs. It was frequently shaken for the first

6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of ethanol. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the ethanol soluble extractive value was calculated with reference to the air dried powder.

Water Soluble Extractive Value

Five gram of the coarsely powdered leaf was macerated separately with 100 ml of chloroform water in a closed flask for 24 hrs. It was frequently shaken for the first 6 hours and allowed to stand for 18 hours. Thereafter it was filtered rapidly taking precaution against loss of chloroform water. 25 ml, of the filtrate was evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°C and weighed. The percentage of the chloroform water soluble extractive value was calculated with reference to the air dried powder.

TABLE - 22

EXTRACTIVE VALUES (INDIVIDUAL SOLVENTS)

(9 DIFFERENT VARIETIES OF *P. guajava* LEAVES)

ANAKAPALLI

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.63
Benzene	4.26
Ethyl acetate	5.55
Chloroform	5.24
Ethanol	18.66
Water	20.10

TABLE – 23

BANGALORE

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.64
Benzene	4.20
Ethyl acetate	5.68
Chloroform	5.30
Ethanol	18.90
Water	21.20

TABLE – 24

CHITTIDHAR

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.62
Benzene	4.18
Ethyl acetate	5.66
Chloroform	5.28
Ethanol	19.12
Water	20.62

TABLE – 25

HAFSI

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.69
Benzene	4.40
Ethyl acetate	5.66
Chloroform	5.18
Ethanol	18.18

Water	23.24

TBALE – 26

LUCKNOW - 46

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.61
Benzene	4.42
Ethyl acetate	5.61
Chloroform	5.20
Ethanol	17.98
Water	20.46

TABLE – 27

LUCKNOW-49

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.66
Benzene	4.31
Ethyl acetate	5.74
Chloroform	5.36
Ethanol	19.99
Water	25.15

TABLE – 28

NAGPUR SEEDLES

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.70
Benzene	4.40
Ethyl acetate	5.66
Chloroform	5.24
Ethanol	18.76
Water	22.26

TABLE – 29

RED FLESHED

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.68
Benzene	4.32
Ethyl acetate	5.70
Chloroform	5.28
Ethanol	19.44
Water	25.06

TABLE – 30

SMOOTH GREEN

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	2.65
Benzene	4.40
Ethyl acetate	5.66
Chloroform	5.30
Ethanol	18.42
Water	20.16

Extractive Values

By using solvents successively with increasing order of polarity

Five grams of the coarsely powdered leaf was extracted continuously in soxhlet apparatus for six hours individually, separately with solvents of increasing order of polarity. After six hours the solvents was removed and evaporated to dryness in a tarred flat bottomed shallow dish, dried at 105°c and weighed. The percentage of the individual solvent soluble extractive value was calculated with

reference to the air dried powder.

TABLE - 31

EXTRACTIVE VALUES (SUCCESSIVE SOLVENTS)
(9 DIFFERENT VARIETIES OF *P. guajava* LEAVES)

ANAKAPALLI

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.24
Benzene	2.02
Ethyl acetate	1.90
Chloroform	0.90
Ethanol	12.40
Water (reflux)	37.44

TABLE – 32
BANGALORE

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.20
Benzene	2.01
Ethyl acetate	1.90
Chloroform	0.88
Ethanol	11.80
Water (reflux)	37.22

TABLE – 33

CHITTIDHAR

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.21
Benzene	2.00

Ethyl acetate	1.86
Chloroform	0.86
Ethanol	12.26
Water (reflux)	38.14

TABLE – 34

HAFSI

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.20
Benzene	2.06
Ethyl acetate	1.86
Chloroform	0.87
Ethanol	12.16
Water (reflux)	36.88

TABLE – 35
LUCKNOW - 46

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.18
Benzene	2.03
Ethyl acetate	1.81
Chloroform	0.91
Ethanol	11.96
Water (reflux)	37.62

TABLE – 36
LUCKNOW – 49

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.23
Benzene	2.04
Ethyl acetate	1.92
Chloroform	0.94
Ethanol	12.58

Water (reflux)	38.66

TABLE – 37
NAGPUR SEEDLESS

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.22
Benzene	2.01
Ethyl acetate	1.82
Chloroform	0.91
Ethanol	11.99
Water (reflux)	38.60

TABLE – 38
RED FLESHED

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.21
Benzene	2.02
Ethyl acetate	1.87
Chloroform	0.90
Ethanol	12.50
Water (reflux)	37.74

TABLE – 39

SMOOTH GREEN

Solvents	Extractive Value (%)
	Leaf
Petroleum ether	4.24
Benzene	2.03
Ethyl acetate	1.94
Chloroform	0.96

Ethanol	12.11
Water (reflux)	36.62

Chapter – V

PHYTOCHEMICAL STUDIES

SECTION – A

PHYTOCHEMICAL STUDIES FOR THE LEAF POWDER OF *P. guajava*[86,87] *(Lucknow – 49)*

Test for Alkaloids

Various procedures to liberate alkaloids

- Powdered drug was mixed thoroughly with 1 ml of 10% ammonia solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was mixed thoroughly with 1 ml of 10% sodium carbonate solution and then extracted for 10 minutes with 5 ml methanol, under reflux. The filtrate was then concentrated.
- Powdered drug was ground in a mortar for about 1 minute with 2 ml of 10% ammonia solution and then thoroughly mixed with 7 gram basic aluminum oxide. The mixture was then loosely packed in to a glass column and 10 ml chloroform was added, eluted, dried and methanol was added.
- Powdered drug was shaken for 15 minute with 15 ml of 0.1 N sulphuric acid and then filtered. The filter was washed with 0.1 N sulphuric acid to a volume of 20 ml filtrate; 1 ml concentrated ammonia was then added. The mixture was then shaken with two portions of 10 ml diethyl ether. The ether was dried over anhydrous sodium sulphate, filtered and evaporated

to dryness and the resulting residue was dissolved in methanol.

- Powdered drug was mixed with one gram of calcium hydroxide and 5 ml of water, made into a smooth paste and set aside for 5 minutes. It was then evaporated to dryness in a porcelain dish on a water bath. 20 ml of 90% alcohol was added, mixed well and then refluxed for half an hour on a water bath. It was then filtered and the alcohol was evaporated. To that dilute sulphuric acid was added.

The above made extracts were tested with various alkaloid reagents and the results were as follows.

1. Mayer's reagent	-	No cream color precipitate
2. Dragendorff's reagent	-	No reddish brown precipitate
3. Hager's reagent	-	No yellow precipitate
4. Wagner's reagent	-	No reddish brown precipitate

Test for purine group (Murexide test)

The residue obtained after the evaporation of chloroform was treated with 1 ml of hydrochloric acid in a porcelain dish and 0.1g of potassium chlorate was added and evaporated to dryness on water bath. Then the residue was exposed to the vapours of dilute ammonia solution.

No purple colour was obtained indicating the **absence** of purine group of alkaloids.

Test for Carbohydrates

- ***Molisch's test***

The aqueous extract of the powdered material was treated with alcoholic

solution of α - naphthol in the presence of sulphuric acid**.**

Purple colour was obtained indicating the **presence** of carbohydrates.

❖ ***Fehling's test***

The aqueous extract of the powdered material was treated with Fehlings I and II solution and heated on boiling water bath.

Reddish brown precipitate was obtained indicating the **presence** of free reducing sugars.

❖ ***Benedict's test***

The aqueous extract of the powdered drug was treated with Benedict's reagent and heated over a water bath.

Reddish brown precipitate was obtained indicating the **presence** of reducing sugars.

Test for Glycosides

General test

❖ ***Test A***

200 mg of the powdered drug was extracted with 5 ml of dilute sulphuric acid by warming on a water bath, filtered and neutralised with 5% sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it becomes alkaline and heated on a water bath for 2 mts.

❖ ***Test B***

200 mg of the powdered drug was extracted with 5 ml of water instead of sulphuric acid. Boiled and equal amount of water was added instead of sodium hydroxide solution. Then 0.1 ml of Fehlings solution A and B were added, until it

becomes alkaline and heated on a water bath for 2 mts.

The quantity of red precipitate formed in test A is greater than in test B indicating the **presence** of glycosides.

❊ ***Anthraquinones***

❖ ***Borntrager's test***

The powdered leaf was boiled with dilute sulphuric acid, filtered and to the filtrate benzene was added and shaken well. The inorganic layer was separated and ammonia solution was added slowly.

No color reaction was observed in ammoniacal layer indicating the **absence** of anthracene derived glycosides.

❖ ***Modified Borntrager's test***

About 0.1 gram of the powdered leaf was boiled for two minutes with dilute hydrochloric acid and few drops of ferric chloride solution was added, filtered while hot and cooled. The filtrate was then extracted with benzene and the benzene layer was separated. Equal volume of dilute ammonia solution was added to the benzene extract and shaken well.

No color was observed in ammoniacal layer indicating the **absence** of anthracene derived glycosides.

❊ ***Test for cyanogenetic glylosides***

Small quantity of the powdered leaf was placed in a stoppered conical flask with just sufficient water to cover it. A sodium picrate paper strip was inserted through the stopper so that it was suspended in the flask and it was set aside for 2

hours in a warm place.

No change in the colour of the sodium picrate paper was observed indicating the **absence** of cyanogenetic glycosides

❊ ***Test for cardiac glycosides***

❖ ***Keller Killiani test***

About 1 gram of the powdered leaf was boiled with 10 ml of 70% alcohol for two minutes, cooled and filtered. To the filtrate 10 ml of water and 5 drops of solution of lead sub acetate were added and filtered. The filtrate was then extracted with chloroform and the chloroform layer was separated and evaporated to dryness. The residue was dissolved in 3 ml of glacial acetic acid containing a trace of ferric chloride. To this 3 ml of concentrated sulphuric acid was added to the sides of the test tube carefully.

No reddish brown layer acquiring bluish green color after standing was observed indicating the **absence** of deoxy sugars of cardiac glycosides.

❖ ***Raymond Test***

To the alcoholic extract of the leaf, hot methanolic alkali was added.

No Violet color was produced indicating the **absence** of cardiac glycosides.

❖ ***Legal's Test***

To the alcoholic extract of the powdered drug, pyridine and alkaline sodium nitro pruside solution were added.

No blood red color was formed indicating the **absence** of cardiac glycosides.

❊ ***Coumarin glycosides***

A small amount of powdered leaf was placed in test tube and covered with a filter paper moistened with dilute sodium hydroxide solution. The covered test tube was placed on water bath for several minutes. Then the paper was removed and exposed to UV light.

No green fluorescence was observed indicating the **absence** of coumarin glycosides.

Test for Phytosterols

The powdered leaf was first extracted with petroleum ether and evaporated. The residue obtained was dissolved in chloroform and tested for sterols.

❖ ***Salkowski Test***

Few drops of concentrated sulphuric acid were added to the above solution, shaken well and set aside.

The chloroform layer of the solution turned red in color indicating the **presence** of sterols.

❖ ***Libermann – Burchard's Test***

To the chloroform solution few drops of acetic anhydride was added and mixed well 1 ml of concentrated sulphuric acid was added through the sides of the test tube and set aside for a while.

A brown ring was formed at the junction of the two layers and the upper layer turned green indicating the **presence** of sterols.

Test for Saponins

About 0.5 gram of the powdered leaf was boiled gently for 2 minute with 20 ml of water and filtered while hot and allowed to cool. 5 ml of the filtrate was then diluted with water and shaken vigorously**.**

Frothing occurred indicating the **presence** of saponins.

Test for Tannins

To the aqueous extract of the powdered leaf, few drops of ferric chloride solution were added.

Bluish black color was produced, indicating the **presence** of tannins.

❖ ***Gold beater's skin test***

2% hydrochloric acid was added to a small piece of gold beater skin and rinsed with distilled water and placed in the solution to be tested for five minutes. Then washed with distilled water and transferred to a 1% ferrous sulphate solution.

Formation of brown color indicates the **presence** of tannins.

Test for Proteins and Free Aminoacids

❖ ***Millon's test***

The aciduous alcoholic extract of the powdered leaf was heated with Millon's reagent.

The Colour was changed to red on heating indicating the **presence** of

proteins.

❖ ***Biuret test***

To the alcoholic extract of the powdered leaf 1 ml of dilute sodium hydroxide was added. Followed by this one drop of very dilute copper sulphate solution was added.

Violet color was obtained indicating the **presence** of proteins.

❖ ***Ninhydrin Test***

To the extract of the powdered drug, ninhydrin solution was added, and boiled.

Formation of violet color indicating the **presence** of Aminoacids

Test for Mucilage

To the aqueous extract of the powdered leaf, ruthenium red solution was added.

No Reddish pink color was produced indicating the **absence** of Mucilages.

Test for Flavonoids

❖ ***Shinoda Test***

A little amount of the powdered leaf was heated with alcohol and filtered. To the alcoholic solution a few magnesium turnings and few drops of concentrated hydrochloric acid were added, and boiled for 5 minutes.

Purple color was obtained indicating the **presence** of flavonoids.

❖ ***Alkaline reagent test***

To the alcoholic extract of the powdered leaf, few drop of sodium hydroxide solution was added.

Yellow color formed, turning to colorless on addition of few drops of dilute acid indicating the **presence** of flavonoids

❖ ***Zinc Hydrochloride Test***

To the alcoholic extract, mixture of zinc dust and concentrated hydrochloric acid was added.

Formation of red color indicating the **presence** of flavonoid

Test for Terpenoids

The powdered leaf was shaken with petroleum ether and filtered. The filtrate was evaporated and the residue was dissolved in small amount of chloroform. To the chloroform solution tin and thionyl chloride were added.

Pink color was obtained indicating the **presence** of Terpenoids.

Test for Volatile Oil

About 100 gram of fresh leaves, were taken in a volatile oil estimation apparatus (Cocking Middletor apparatus) and subjected to hydro distillation for four hours.

Golden yellow color Volatile oil was obtained indicating the **presence** of volatile oil.

Test for Fixed Oil

A small amount of the powdered leaf was pressed in between in the filter paper and the paper was heated in an oven at 105^{o}C for 10 minutes.

No translucent greasy spot occurred indicating the **absence** of fixed oil.

TABLE - 40

RESPONSE TO THE PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE CRUDE LEAF POWDER OF *P. guajava (HIGH YIELDING VARIETY LUCKNOW-49)*

S.NO	TEST	OBSERVATION
I.	**ALKALOIDS**	
	Mayer's reagent	-
	Dragendorff's reagent	-
	Hager's reagent	-
	Wagner's reagent	-
II	**CARBOHYDRATES**	
	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
III	**GLYCOSIDES**	
	General Test	+
	Anthraquinone	-
	Cardiac	-
	Cyanogenetic	-
	Coumarin	-
IV	**PHYTOSTEROLS**	
	Salkowski test	+
	Liberman Burchard's test	+
V	**SAPONINS**	+
VI	**TANNINS**	+
VII	**PROTEINS AND FREE AMINO ACIDS**	
	Millon's test	+
	Biuret test	+
VIII	**GUMS AND MUCILAGE**	-
IX	**FLAVONOIDS**	
	Shinoda test	+
X	**TERPENOIDS**	+
XI	**VOLATILE OIL**	+
XII	**FIXED OIL**	-

The above described tests were also performed on the different extracts of leaf powder of ***P. guajava*** and the results were as follows,

TABLE - 41

RESPONSE TO THE PRELIMINARY PHYTOCHEMICAL SCREENING FOR THE DIFFERENT EXTRACTS OF LEAF OF *P. guajava (HIGH YIELDING VARIETY LUCKNOW-49)*

Tests	Petroleum ether extract	Benzene extract	Ethyl acetate extract	Chloroform extract	Ethanolic extract	Aqueous extract
ALKALOIDS						
Mayers Reagent	-	-	-	-	-	-
Dragendorffs reagent	-	-	-	-	-	-
Hagers reagent	-	-	-	-	-	-
Wagners reagent	-	-	-	-	-	-
CARBOHYDRATES						
Molishch's Test	-	-	-	-	+	+
Fehlings Test	-	-	-	-	+	+
Benedicts Test	-	-	-	-	+	+
GLYCOSIDES						
General Test	-	-	-	-	+	+
Anthraquinone	-	-	-	-	-	-
Cardiac	-	-	-	-	-	-
Cyanogenetic	-	-	-	-	-	-
Coumarin	-	-	-	-	-	-
PHYTOSTEROLS						
Salkowski Test	+	+	+	+	-	-
Libermann Burchard Test	+	+	+	+	-	-
SAPONINS	-	-	-	-	+	+
TANNINS	-	-	-	-	+	+
PROTEINS & FREE AMINO ACID						
Millons test	-	-	-	-	+	+
Biuret test	-	-	-	-	+	+
Ninhydrin test	-	-	-	-	+	+
GUMS & MUCILAGE	-	-	-	-	-	-
FLAVONOIDS						
Shinoda test	-	-	-	-	+	+
Alkaline Reagent test	-	-	-	-	+	+
Zinc hydrochloric acid test	-	-	-	-	+	+
TERPENOIDS	+	+	+	+	-	-
FIXED OIL	-	-	-	-	-	-

"+" Indicate Positive reaction "-" Indicate Negative reaction

SECTION – B

ISOLATION AND GC-MS ANALYSIS OF VOLATILE OIL FROM THE LEAVES OF P. GUAJAVA (LUCKNOW – 49)

Materials and Methods

About 300 gm of fresh leaves were weighed and subjected to hydro distillation using cocking – Middleton apparatus used for the determination of volatile oil, yields 0.2%. The oil samples were dried over anhydrous sodium sulphate and stored in sealed glass bottles.

Analysis of Volatile Oil

Physical Analysis

Color : Golden Yellow

Odour : Aromatic

Taste : Pungent

Solubility : Toluene, Methanol

Analysis of Volatile Oil by Gas Chromatographic Mass Spectrometry[88,89]

Gas chromatography is one of the most widely used techniques for qualitative and quantitative analysis. In gas chromatography, the component of a vaporized sample are separated as a consequence of being partitioned between a mobile gaseous phase and a liquid (or) a solid stationary phase in a column. In performing a gas chromatographic separation the sample is vaporized and injected on to the head of a chromatographic column. Elution is brought about by the flow of an inert gaseous mobile phase. In contrast to most other types of chromatography, the mobile phase does not interact with the molecules of the analyte. Its only function is to transport the analyte through the column.

Gas chromatography is very efficient method for separating a complex mixture into its components. The high sensitivity of mass spectrometry provides the necessary information for either identification of compounds by comparison with available spectra (or) structure elucidation of a small quantity of compound. A combination of these techniques with the introduction of GC effluents after removal of most of the carrier gas into a mass spectrometer is finding increasing use in analytical and structural organic chemistry and biochemistry.

Gas chromatographic mass spectrometry is the single most important tool for the identification and quantitation of volatile and semivolatile organic compounds in complex mixtures. As such, it is very useful for the determination of molecular weight and the elemental composition of unknown organic compounds in complex mixtures. GC-MS is the most obvious method of combination to condense the fraction emerging from the GC column into a capillary or on to a small metal surface. The fractions collected in this way are introduced in the normal manner into the mass spectrometer source.

Gas Chromatography – Mass Spectrometry (Fig.4)

GC – MS data were obtained on GC clarus 500 perkin Elmer equipment.

Column	:	Elite-1 (100% Dimethyl Poly Siloxane), 30m x 0.25mm ID x 1μm df
Carrier gas	:	Helium 1ml/min
Detector	:	Mass detector – Turbomass gold – perkin Elmer
Software	:	Turbo mass 5.1

Sample injected	:	1ul
Split	:	25:1
Oven Temperature	:	110 deg. 2 min hold upto 280 deg at the rate of 5 deg/min-9min hold
Injector temperature	:	250 deg c
Total GC Time	:	45 min

Mass Spectrometry

Inlet line temperature	:	200 deg c
Source Temperature	:	200 deg c
Electron energy	:	70 ev
Mass scan	:	(m/z) 45 – 450
MS Time	:	46 min

SECTION – C

"TLC & HPLC ANALYSIS OF ALCOHOLIC EXTRACT OF LEAVES OF *P.guajava*" (LUCKNOW – 49)

Preparation of Alcoholic extract

The dried leaf powder of ***Psidium guajava*** was extracted in a soxhlet extraction apparatus and the extracted solution was concentrated under reduced pressure. A brown colour residue was obtained.

The extract was dissolved in Methanol. Small quantity was taken in a capillary tube and used for chromatography procedure.

Thin layer chromatography of the extract[90] (Plate No. 32)

Stationary Phase	:	Precoated Aluminium plate (Silica gel 60 F_{254})
Mobile Phase	:	Methanol : Chloroform (1:9)
		After derivatisation with $AlCl_3$
Detecting Agent	:	UV light (366nm)
R_f Value	:	Yellowish brown spot R_f(0.2)

Rf(0.2) coincides with the standard quercetin. Hence the spot may be due to quercetin.

Further the amount of quercetin present in the ***P.guajava*** leaves was quantified using High performance liquid chromatography.

High performance liquid chromatography[91]

HPLC was referred to as high pressure liquid chromatography, but nowadays the term high performance liquid chromatography is performed. Since it better describes the characteristics of the chromatography and avoids creating the impression that high pressures are an inevitable pre-requisite for high performance.

A typical HPLC unit consist of the following

- A solvent reservoir and mixing system
- A high pressure pump
- A sample inlet pump
- A column (stainless steel)

- A detector and recording unit

Procedure

The appropriate solvents (mobile liquid phase) from the reservoirs are allowed to enter the mixing chamber where a homogenous mixture is obtained. A pump capable of maintaining high pressures draws the solvent from the mixing chambers and pushes it through the column.

The sample is injected through a port into the high pressure liquid carrier stream between the pump and the column. The separation takes place on the columns which vary from 50 – 100cm in length and 2.3 m.m in i.d. Typical flow rates are 1-2ml/min with pressure up to several thousand psi. The column effluent passes through a non-destructive detector where a property such as ultraviolet absorbance, refractive index (or) molecular fluorescence is monitored, amplified, and recorded as a typical detector response vs retention time chromatogram. The effluent may be either discarded, recycled, or saved for the further studies in a fraction collector which is synchronized with the detector.

Practical Procedure

There are two methods which are generally used.

First method makes use of micro syringe designed to with stand high pressure. The sample is injected through a septum in an injection port, either directly on to the column packing (or) on to a small plug of inert material immediately above the column packing. This can be done while the system is under pressure, or the pump may be turned off before injection and when the pressure has dropped to near atmospheric, the injection is made and the pump

switched on again. This is termed a stop flow injection.

In the second method, the sample is introduced by use of a loop injector. This consists of a metal loop of small volume which can be filled with the sample. By means of an appropriate valve, the eluant from the pump is channeled through the loop, the outlet of which leads directly on to the column. The sample is thus flushed on to the column by the eluant, without interruption of solvent flow to the column.

Repeated application of highly impure samples such as sera, urine which have preferably been deproteinated may eventually cause the column to lose its resolving power.

To prevent this occurrence a guard column is installed between the injection and the analytical column.

HPLC Analysis of Alcoholic extract of P.guajava leaves (Lucknow-49) (Fig. 5,6)

Instrument	:	Shimadzu BM – 101
Liquid Chromatogram	:	10 ATVP Shimadzu liquid chromatography
Column Used	:	C-18 ODS Column
Detector	:	SPD – 10 AVP Shimadzu UV – Vis detector
Wave length	:	254 nm
Mobile Phase	:	ACN : H_2O : Methanol : Acetic acid
Flow rate	:	0.5ml/1min
Injection volume	:	25 µl
Sample	:	10 mg/ml

Standard	:	0.4mg/0.5ml
Quercetin	:	1.21% w/w

Result

The percentage of quercetin present in the extract is 1.2% w/w.

Chapter – VI

PLATELET AGGREGATION INHIBITORY ACTIVITY OF THE ALCOHOLIC EXTRACT OF THE LEAVES OF *PSIDIUM GUAJAVA* (LUCKNOW – 49)

INTRODUCTION

Formation of thrombus or clot is an integral part of haemostasis to prevent blood loss, due to injury, by maintaining a balance between thrombotic and thrombolytic system. Arterial and venous thrombosis are the principal causes for the evolution of myocardial infarction, thromboembolic stroke and deep vein thrombosis. Initiation of thrombosis is a complex process, which is primarily due to platelet and coagulation activation[92].

Platelets play a central role in haemostasis and thrombosis, participating in many biologic and pathologic processes including inflammation, atherosclerosis and immune reactions. These activities require platelet activation, a complex process involving shape change, release of granular constituents, adhesion and aggregation[93] (Fig – 7).

Platelet aggregation may be stimulated by collagen, thrombin, thromboxane A_2, serotonin, adenosine diphosphate (ADP), platelet activating factor (PAF), epinephrine or most probably by a combination of these two factors [57].

A number of synthetic drugs are available for the treatment of

thrombotic disorders such as aspirin, ticlopidine, clopidogrel, warfarin, hirudin, low molecular weight heparin etc.

Most of the anti thrombotic drugs, though effective in preventing thrombus formation, but are associated with multiple side effects and limitations. Search for newer drugs is therefore still being pursued with lot of vigor.

An ideal antithrombotic compound should therefore have the ability to inhibit the propagation of thrombus after vascular injury, inhibit platelet aggregation, promote fibrinolysis with minimal side effects on coagulation parameters, and should have a predictable dose response. Since herbal formulations usually have multiple ingredients to act synergistically with various targets, have better bioavailability and efficacy, scientific community is therefore interested in developing herbal drugs for various pathologies including intravascular pathologies.

Formulation / Extracts from plant sources

Various herbal formulations, their aqueous extracts, alcoholic extracts (or) active components have been investigated in various invitro test systems. Some of these herbs or spices have even been studied on human subjects for their efficacy. E.g.: Terminalia arjuna, Andrographis paniculata, Ocimum, guggul and amla.

Anti platelet activity of the plant products

Flavonoids found in plant products inhibit cyclic nucleotide phospho diesterase and hence platelet aggregation[94].

Invitro Screening Methods

Evaluation of invitro platelet aggregation in Aggregometry using platelet rich plasma, whole blood, washed platelets method are available[56,57].

We planned to develop a method, which is a simple laboratory scale preliminary procedure to identify the presence of platelet aggregation inhibitory activity without any specialized instruments like aggregometer. So we adapted a smear method, modified to allow the massive screening of plant extracts and or fractions for platelet antiaggregating activity so that to pre screen potential sources form plants.

As plants are being considered especially valuable in the empirical search for new drugs in such situations where one can rationally design new classes of molecules which could be expected to be effective, the method for screening plant material for this activity should be adaptable to materials which are highly coloured, tarry and poorly soluble in water and chemically complex.

The smear method seemed more suitable for testing plant derived samples, since the blood is smeared on a glass slide, stained and degree of

aggregation of platelets directly examined under a microscope. This method relates to inhibiting blood platelet aggregation in human and more particularly to the use of an extract of plant for effectively controlling said aggregation.

Materials and Method

1. Platelet rich plasma (PRP)
2. Sterilised microslides
3. Micropipettes
4. Microscope with CCTV and camera (Laboscope)
5. ADP - 10μm/ml (Sigma)
6. Collagen - 3μg/ml (Sigma)
7. Ethanol (Sd fine chemical)
8. JSB stain I and II
9. Ethanolic extract of ***P. guajava*** leaf in the concentration of 5,10,15,20,25 μg/ml.
10. Sterile Normal saline

Experimentation

Preparation of PRP using human Blood [56]

Whole blood is collected from human volunteers who have not received medication for 14 days. All the procedures are performed in plastic tubes. Whole blood is mixed with acid citrate dextrose (ACD).

Preparation of Slides

The following slides were prepared

Side 1 : $PRP_{(50\mu l)}$ + Normal saline

Slide 2 : $PRP_{(50\mu l)}$ + Normal saline + Collagen

Slide 3 to 7 : $PRP_{(50\mu l)}$ + Test drug of various + Collagen concentration

Slide 8 : $PRP_{(50\mu l)}$ + Normal Saline + ADP

Slide 9to13 : $PRP_{(50\mu l)}$ + Test drug of various + ADP concentration

Then collagen 20µl was added after 10 minutes to the slide 2 to 7. ADP 20µl was added after 10 mts to the slides 8 to 13 and kept at 37^0C for 5 mts.

Smears were prepared after fixing with ethanol, then dried. Stained with JSB I and II, washed and dried.

The smears then were subjected to examination under microscope using on oil immersion objective lens (100x).

The observations were tabulated (Table No. – 42)

Table – 42

Platelet aggregation Inhibitory Activity of Ethanolic Extract of Leaves

of *P. guajava* (Lucknow – 49) by smear method

Slides	Degree of Platelet Aggregation
Slide 1 (Control) (PRP)	(-)
Slide 2 (Agonist – Collagen)	(++)
Slide 3 (Test drug 5μg/ml, Collagen)	(++)
Slide 4 (Test drug 10μg/ml, Collagen)	(++)
Slide 5 (Test drug 15μg/ml, Collagen)	(+)
Slide 6 (Test drug 20μg/ml, Collagen)	(±)
Slide 7 (Test drug 25μg/ml, Collagen)	(-)
Slide 8 (Agonist – ADP)	(++)
Slide 9 (Test drug 5μg/ml, ADP)	(++)
Slide 10 (Test drug 10μg/ml, ADP)	(++)
Slide 11 (Test drug 15μg/ml, ADP)	(+)
Slide 12 (Test drug 20μg/ml, ADP)	(±)
Slide 13 (Test drug 25μg/ml, ADP)	(-)

The degree of Platelet aggregation was graded as follows (Plate – 33 to 36)

(-) No aggregation (control)

(±) Slight aggregation

(+) Moderate aggregation

(++) Major aggregation (as observed with Collagen or ADP)

(+++) Stronger aggregation (than any of the above)

PLATELET AGGREGATION INHIBITORY ACTIVITY USING PLATELET AGGREGOMETER

A typical aggregometer is basically a simple photometer consisting of a light source, usually white light from a low voltage, DC tungsten filament and a photoelectric cell to receive the light beam from passage through the sample[57].

Materials and Method

1. ADP - 10μM/10μl – Agonist (Sigma)
2. Collagen - 10μM/10μl – Agonist (Sigma)
3. Platelet rich plasma 2.0-3.0x10^5/microlitre
4. Sterile normal saline solution for blank
5. Test drug – Alcoholic extract of ***P. guajava*** leaf (5,10,15,25 μg/ml)
6. Chrono log Aggregometer – 560ca

Procedure

Step

1. The Aggregometer may be calibrated so that PRP gave 10% of light transmission, while PPP (Platelet poor plasma) gave 90% of light transmission[95].
2. PRP + Normal Saline (Blank)
   (350μl) (100μl)
   Incubation time is 37^0C for 10 minutes

3. PRP + Normal Saline + ADP (agonist)
   (350μl) (10μl)

Incubation time is 37^0C for 10 minutes

4. PRP + Test drug of various concentration
   (350µl)
   Incubation time is 37^0C for 10 minutes.
   Then add ADP.

5. PRP + Normal Saline + Collagen (agonist)
   (350µl) (10µl)
   Incubation time is 37^0C for 10 minutes

6. PRP + Test drug of various concentration
   (350µl)
   Incubation time is 37^0C for 10 minutes. Then add collagen

7. From step (2) to step (6), reaction was initiated by a magnetic stirrer at 1200rpm, and the reaction time was 5 mins and change in light transmittance was recorded over time.

Percentage Inhibition of aggregation was tabulated (Table – 43, Fig-8).

Statistical analysis

All results were presented as mean ± S.E.M. Data were analyzed by student 't' test.

TABLE – 43

PERCENTAGE INHIBITORY EFFECT OF ALCOHOLIC EXTRACT OF LEAVES OF *P.guajava* (LUCKNOW – 49) ON PLATELET AGGREGATION INDUCED BY AGONISTS - ADP AND COLLAGEN

Test Drug	Percentage inhibition of platelet aggregation (ADP)	Test Drug	Percentage inhibition of platelet aggregation (Collagen)
5 μg	21.32±0.2685	5 μg	14.546±0.2961
10 μg	65.296±0.2085	10 μg	93.163±0.1763
15 μg	97.243±0.2010	15 μg	96.46±0.2483
25 μg	99.836±0.09838	25 μg	99.82±0.1419

Each experiment was done in triplicate. Results are expressed as means ± S.E.M. P value <0.05.

Chapter – VII

RESULTS AND DISCUSSION

This dissertation covers the work on Pharmacognostic, phytochemical studies and demonstrated preliminary invitro studies on platelet inhibitory activity of the leaves of ***Psidium guajava*** Family: **Myrtaceae** in an attempt to rationalise its uses, in the development as new antithrombotic drug.

In **Chapter – 1 Introduction** : We discussed the importance of medicinal plants. → WHO views on medicinal plants → phytonutrients – the natural drugs of the future – Facts about cardio vascular disease and global burden of CVD → India's killer diseases most common causes of death in India 2002 → Flavonoids and cardio vascular diseases → quercetin reason for selection of the plant ***P.guajava*** Fam. Myrtaceae.

In **Chapter – 2 Review of Literature** : The various literatures available were categorized into ethnomedical, phytochemical and pharmacological screening of leaves, flowers, fruits, seeds, bark, root bark, roots, twigs including studies on volatile oil to review systematically.

Various methods to study the platelet aggregation inhibitory activity were also reviewed.

In **Chapter 3, Aim and Objective** was planned to study the pharmacognosy of various varieties, phytochemical and invitro effect on

human platelet aggregation of leaf extract of high yielding variety of ***P. guajava*** (Lucknow 49). Moreover to design a newer simple technique to identify the presence of platelet aggregation inhibitory activity without any specialized expensive instruments.

In **Chapter – 4, Section – A ; Pharmacognostic Study :** Macroscopical studies of the leaves includes taxonomical position, vernacular names, geographical distributions, habit and habitat to identify the plant with the support of photograph and line drawings as on establishment of authenticity (Plate 1 – 4) (Fig.1).

Leaves

Shape	–	Oblong to elliptic
Size	-	5 – 15 x 4 – 6 cm
Colour	-	Dull grey to yellow green
Margin	-	Entire
Apex	-	Obtuse to acuminate
Base	-	rounded
Odour	-	No characteristic
Taste	-	No characteristic

Morphology of the leaves showed no difference in all the varieties.

Flowers - 1 to 3 flowered, axillary

Fruits - Ovoid or pear shaped berry

Skin - Yellow when ripe

Mesocarp - Thick and edible

Section – B deals with the microscopical studies of leaves of 9 varieties namely Anakapalli, Bangalore, Chittidhar, Hafsi, Lucknow-46, Lucknow-49, Nagpur seedless, Red fleshed, Smooth green to ascertain the arrangement of tissues (Plate No. 5-31, Fig – 2).

Leaf : The T.S. of leaf through midrib and the powder analysis showed the following features (Table 44).

TABLE NO. – 44

MICROSCOPY OF 9 DIFFERENT VARIETIES OF *P. guajava* LEAVES SHOWING SPECIAL FEATURES

Anakapalli	Bangalore	Chittidhar	Hafsi	Lucknow - 46	Lucknow - 49	Nagpur seedless	Red fleshed	Smooth Green
Vascular bundle Xylem less lignified, uniseriate, thickwalled fibres	**Vascular bundle** xylem dense parallel lines, thinwalled containing dark elliptical sclerenchyma cells in between the lines Phloem – seen in small cluster at the end of each xylem row (30µm wide)		**Vascular bundle** phloem elements occur at the ends of xylem lines as small nests	**Vascular bundle** xylem fairly wide, thinwalled angular in outline occur in compact radial lines **Ground tissue of midrib** **Epidermis** Tannin content not evident calcium oxalate druses are common within dilated ground cells.	**Epidermis** Narrow Secretory cavities seen in periphery.	**Epidermis** Lacking of tannin	**Vascular strand** xylem – dense parallel rows	

Anakapalli	Bangalore	Chittidhar	Hafsi	Lucknow -46	Lucknow -49	Nagpur seedless	Red fleshed	Smooth Green
			Mesophyll Dilated cells contain calcium oxalate druses both in palisade and spongy parenchyma cells.	**Mesophyll** Calcium oxalate druses are fragment in the dilated cells.	**Mesophyll** Tannin content is less		**Mesophyll** Single row palisade cells. Dark tannin accumulation	
	Lateral vein vascular strands of lateral vain are prominent collateral with thick adaxial and abaxial sclerenchyma sheath		**Lateral veins** elliptical, parenchymatous bundle sheath	**Lateral vein** vascular bundle of the lateralveins vertically stretched collateral with sclerenchyma bundle caps.		**Lateral vein** bundles project prominently below the surface having thick mass of xylem, small groups of phloem, thick pillar of bundle sheath extensions.		
Secretory cavity present in lower side	Absent	Absent	Present in lower side	Present in lower side	Present in the periphery of the midrib	Present in Lower side	Present in Lower side	80µm wide in upper portion

A noteworthy feature of this family Myrtaceae is the presence of secretory cavities. **It was observed that secretory cavities were absent in Bangalore, Chittidhar** and it is present in the lower side in case of Anakapalli, Hafsi, Lucknow-46, Nagpur seedless and Red fleshed, **but it was 80 μm wide and present in upper portion in the case of smooth green**. Narrow secretory cavities seen in the periphery of the midrib in Lucknow – 49.

The plants belongs to the family **Lecythidaceae** (in which formerly the family **Myrtaceae** was included) differs from **Myrtaceae** in the absence of secretory cavities from the leaves and ground tissues.

Calcium oxalate druses in dilated cells are present in Hafsi and Lucknow-46 alone. Hypoderm stated to occur below the upper epidermis in several species of Psidium (and the genus Metrosideros). Here all the 9 varieties contains invariably multiple epidermis. According to Debore hypoderm is crystalliferous. But it was not noticed in all varieties.

Tannin is abundant in the tissues, but is very less in Lucknow-49 and dark tannin accumulation was in Red fleshed. Certain variation do occur in vascular bundle. Xylem less lignified, uniseriate thick walled in Anakapalli, thin walled containing dark elliptical sclerenchyma in Bangalore variety[96].

Section – C deals with the quantitative microscopy in forms of microscopic and physical parameters and the results were tabulated from table 1 to 39.

The combined results were tabulated as follows (Table 45-49).

TABLE – 45

COMPARATIVE REPORT OF VEIN ISLET AND VEIN TERMINATION NUMBER OF 9 VARIETIES OF *P. guajava* LEAVES

		Vein islet No			Vein Termination No		
S.No	**Varieties**	**Minimum**	**Average**	**Maximum**	**Minimum**	**Average**	**Maximum**
1.	Anakapalli	2	3	4	4	4.3	5
2.	Bangalore	2	3	4	4	4.5	5
3.	Chittidhar	3	3.5	4	4	4.8	5
4.	Hafsi	3	3.3	4	4	4.5	5
5.	Lucknow -46	3	3.5	4	4	4.3	5
6.	Lucknow – 49	2	2.5	3	4	4.3	4
7.	Nagpur Seedless	2	2.3	4	3	3.5	4
8.	Red fleshed	2	2.5	3	3	4	5
9.	Smooth Green	3	3.3	4	4	4.3	5

TABLE – 46

COMPARATIVE REPORT OF STOMATAL NUMBER AND STOMATAL INDEX OF 9 VARIETIES OF *P. guajava* LEAVES

S. No	Varieties	Stomatal Number						Stomatal Index					
		Upper Epidermis			Lower Epidermis			Upper Epidermis			Lower Epidermis		
		Min	Ave	Max	Min	Ave	Max	Min	Ave	Max	Min	Ave	Max
1.	Anakapalli	29	30.7	43	41	43	45	20	20.2	20.5	19.1	19.3	19.6
2.	Bangalore	32	34.3	36	40	42.4	44	20.9	21	21.2	19.2	19.4	19.6
3.	Chittidhar	36	37.8	40	42	44	46	21.2	21.6	21.8	19.4	19.8	20
4.	Hafsi	38	39.6	42	42	44.6	48	21	21.4	21.8	19	19.1	19.2
5.	Lucknow -46	35	36.2	38	40	42.4	44	19.9	20.1	20.3	19.1	19.3	19.5
6.	Lucknow – 49	28	29.7	32	46	47.1	49	19.8	20	20.2	19.2	19.5	19.6
7.	Nagpur Seedless	31	32.9	35	38	40	42	20.8	21	21.2	18.7	19	19.2
8.	Red fleshed	30	31.4	33	40	44.7	48	20.1	20.4	20.6	19.2	19.4	19.6
9.	Smooth Green	32	35.1	38	48	49.7	52	20.1	20.3	20.6	19	19.1	19.2

TABLE – 47

COMPARATIVE REPORT OF TOTAL ASH, ACID INSOLUBLE ASH AND WATER SOLUBLE ASH OF 9 VARIETIES OF *P. guajava* LEAVES

S. No	Varieties	Total Ash (%)			Acid insoluble ash(%)			Water soluable Ash (%)		
		Min	Ave	Max	Min	Ave	Max	Min	Ave	Max
1.	Anakapalli	10.78	11.10	11.49	1.46	1.51	1.56	2.52	2.8	3.02
2.	Bangalore	10.76	11.09	11.5	1.47	1.49	1.52	2.53	2.8	3.04
3.	Chittidhar	10.77	11.08	11.52	1.46	1.49	1.52	2.48	2.79	3.01
4.	Hafsi	10.74	11.06	11.5	1.46	1.5	1.54	2.52	2.83	3.08
5.	Lucknow -46	10.7	11.05	11.46	1.48	1.51	1.55	2.5	2.79	3.01
6.	Lucknow – 49	10.8	11.12	11.58	1.49	1.51	1.54	2.54	2.83	3.06
7.	Nagpur Seedless	10.7	11.05	11.44	1.45	1.5	1.56	2.48	2.77	3.02
8.	Red fleshed	10.72	11.1	11.49	1.46	1.5	1.54	2.5	2.81	3.08
9.	Smooth Green	10.79	11.11	11.6	1.47	1.51	1.54	2.51	2.81	3.02

TABLE – 48

COMPARATIVE REPORT OF LOSS ON DRYING (%) W/W OF 9 VARIETIES OF *P. guajava* LEAVES

S. No	Varieties	Loss on Drying (%) w/w		
		Min	Ave	Max
1.	Anakapalli	9.3	9.8	10.5
2.	Bangalore	9.6	9.9	10.2
3.	Chittidhar	9.2	9.8	10.1
4.	Hafsi	9.2	9.7	10.3
5.	Lucknow -46	9.1	9.7	10
6.	Lucknow – 49	9.5	9.8	10.3
7.	Nagpur Seedless	9.3	9.8	10.4
8.	Red fleshed	9.2	9.9	10.4
9.	Smooth Green	9.3	10	10.4

TABLE – 49

COMPARATIVE REPORT OF EXTRACTIVE VALUES IN INDIVIDUAL SOLVENTS AND SUCCESSIVE SOLVENTS OF 9 VARIETIES OF *P. guajava* LEAVES

S. No	Varieties	Extractive value (%) [individual solvents]						Extractive value (%) [Successive solvents]					
		P.E	B.Z	E.A.	C.H	E.T	W	P.E	B.Z	E.A.	C.H	E.T	W
1.	Anakapalli	2.63	4.26	5.55	5.24	18.66	20.10	4.24	2.02	1.9	0.9	12.40	37.44
2.	Bangalore	2.64	4.20	5.68	5.3	18.9	21.20	4.2	2.01	1.9	0.88	11.8	37.22
3.	Chittidhar	2.62	4.18	5.66	5.28	19.12	20.62	4.21	2.00	1.86	0.86	12.26	38.14
4.	Hafsi	2.69	4.4	5.66	5.18	18.18	23.24	4.2	2.06	1.86	0.87	12.16	36.83
5.	Lucknow -46	2.61	4.42	5.61	5.2	17.98	20.46	4.18	4.03	1.81	0.91	11.96	37.62
6.	Lucknow – 49	2.66	4.31	5.74	5.36	19.99	25.15	4.23	2.04	1.92	0.94	12.58	38.66
7.	Nagpur Seedless	2.70	4.40	5.66	5.24	18.76	22.26	4.22	2.01	1.82	0.91	11.99	38.6
8.	Red fleshed	2.68	4.32	5.7	5.28	19.44	25.06	4.21	2.02	1.87	0.9	12.5	37.74
9.	Smooth Green	2.65	4.4	5.66	5.30	18.42	20.16	4.24	2.03	1.94	0.96	12.11	36.62

P.E = Petroleum Ether
B.Z = Benzene
E.A = Ethyl Acetate
C.H = Chloroform
E.T = Ethanol
W = Water

From the above result, it was noticed that there was no distinct difference in leaf parameters, Ash values, Loss on drying, extractive values.

In **Chapter – 5, Section – A** deals with the preliminary phytochemical screening of the high yielding variety L-49. The results were tabulated (Table No. 40,41).

Constituents Present	Constituents Absent
Carbohydrates, glycosides, phytosterols, saponins, tannins, flavonoids, terpenoids, volatile Oil, Proteins and Free amino acids	Alkaloids, Anthraquinone, cardiac, cyanogenetic, coumarin glycosides, gums and mucilage, fixed oil.

Section – B deals with the Isolation and GC-MS analysis of volatile oil from the leaves of ***P. guajava*** Linn.

The percentage of oil obtained was 0.2%. Physical analysis of volatile oil was done.

Colour - Golden yellow

Odour - Aromatic

Taste - Pungent

Solubility - Toluene, Methanol

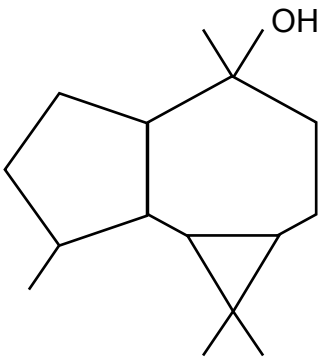
Refractive index - 1.49

GC-MS of the analysis of the oil shows presence of 37 compounds. List of compounds were as follows.

From the table it is obvious that caryophyllene (17.58%), 1-6,

10-Do-deca trien – 3- ol, 3,7,11 trimethyl, (E) (16.44%), (2-Methyl – cyclo hex – 2 enyl-idene) acetaldelyde (14.78%) were found to be major constituents of the high yield variety Lucknow-49. When compared to the already reported studies caryophyllene in various percentage was the major component. Aromadendrene (4.1%) was reported in only one study,[33] is present along with its oxide in this oil also. Cineole, Limonene etc. were found to be absent[34,15].

New compounds Ledol, 3 carene, muurolol are present. The review of literature of composition of volatile oil of ***P. guajava*** shows wide variation. 17 to 60 compounds have been reported. This difference may be due to climatic condition, soil, time of collection etc.

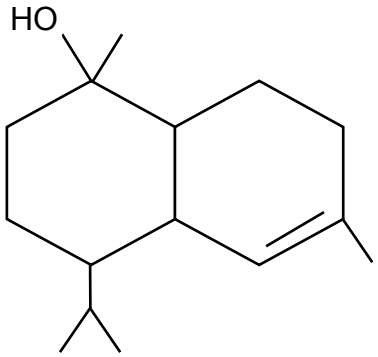


Name : Ledol

Formula : $C_{15}H_{26}O$

M.W. : 222

CAS #: 577-27-5

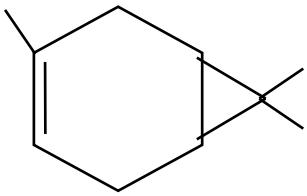


Name : Tau-Muurolol

Formula : $C_{15}H_{26}O$

M.W. : 222

CAS #: 19912-62-0



Name : 3-Carene

Formula : $C_{10}H_{16}$

M.W. : 136

CAS #: 13466-78-9

Section – C deals with the TLC and HPLC analysis of the alcoholic extract of leaf of ***P. guajava***. Co TLC of the extract using methanol : Chloroform (1:9) on silica Gel $60F_{254}$ shows the presence of quercetin. (R_f – 0.2).

The literature review also showed the presence of quercetin[12,17,26,28] Huang .J et.al reported high yield of quercetin by microwave assisted technique (1.85mg/gm) in 5 minutes in ethanol than compared to reflux heating for (1.57 mg/gm) for 20 minutes[26]. But in our extraction method we adapted reflux heating for 10 minutes. It was also reported that quercetin appears to have many beneficial effects on human health including cardio-vascular protection. Considering the beneficial properties of quercetin against heart diseases we selected this study and we planned to estimate the percentage of quercetin in the leaf part of high yielding variety of ***P.guajava***.

The report of HPLC analysis showed 1.2% w/v of quercetin content in the alcoholic extract of leaves of high yielding variety ***P.guajava*** (Lucknow – 49).

Chapter – 6 deals with the platelet aggregation inhibitory activity of alcoholic extract of the leaves of ***P.guajava*** variety (Lucknow-49). In this we discussed the central role of platelets in haemostasis and thrombosis. We reviewed the need for search for newer antithrombotic drugs, since the already existing drugs are associated with multiple side effects and limitations. Medicinal plants usually have multiple phyto-constituents to act synergistically with various targets, have better bio availability and efficacy. We are interested to study this extract for its platelet aggregation inhibitory activity.

Flavonoids found in plant products inhibit cyclic nucleotide phospho diesterase and hence platelet aggregation[94]. Since the extract showed the considerable quantity of quercetin it was presumed for its platelet aggregation inhibitory activity.

Methods for evaluation of invitro platelet aggregation using aggregometer were available. Since this instrument highly expensive and having limited use, we designed a method for screening plant material for this activity which is adaptable to natural and synthetic materials, simple, rapid, reliable microscopic technique. The smear method seemed more suitable for testing materials the degree of aggregation of platelets directly examined under a microscope and

relates to inhibiting blood platelet aggregation in human. More particularly to the use of an extract of plant for effectively controlling said aggregation.

Initially we standardised the micro slide method using few extracts. The observations made by using our ethanolic extract(25μg/ml) showed significant inhibition of platelet aggregation induced by both collagen and ADP (Table No. 42).

The volatile oil isolated from the leaves of ***P.guajava*** variety (Lucknow-49) showed no inhibition of aggregation. The inhibitory activity was further confirmed using platelet aggregometer. (Table No. 43).

It was observed that 99.8±0.098 and 99.82 ±0.1419 (Means±S.E.M $p<0.05$) were percentage inhibition of platelet aggregation against the aggregation induced by agonists ADP and collagen respectively. It was obvious that there was a sudden steep increase of activity between 5μg and 10μg/ml concentrations both in collagen and ADP (graph Fig. 8).

The results were statistically significant. The present results may be due to inhibition by quercetin of collagen stimulated platelet activation through inhibition of multiple components of the GPVI signaling pathway[10]. This preliminary search shows the possibility of rational design of new classes of molecules for anti-thrombotic drugs. Since platelet aggregation may be stimulated not only by collagen, ADP but also by Thromboxane A2, Serotonin, PAF, epinephrine or by combination of these factors[57], this evaluation should be tested with these agonists also to provide suitable information on mechanism of action. The chances for multifacet inhibition should be studied.

Chapter – VIII

CONCLUSION

This dissertation covers the pharmacognostic parameters of the leaves of ***Psidium guajava Linn***. Family : **Myrtaceae** such as macroscopical, microscopical, powder analysis, quantitative microscopy and physical standards like ash values, extractive values, which have been studied and presented.

- 9 varieties of ***P. guajava*** leaves namely **Anakapalli, Bangalore, Chittidhar, Hafsi, Lucknow – 46, Lucknow – 49, Nagpur Seedless, Red fleshed, Smooth green** were compared macroscopically and microscopically, to establish the botanical identity including anatomical variations.

- Specific anatomical characters pertaining to the leaf varieties were presented.

- No difference in quantitative microscopy and other physical parameters like ash values, extractive values were found.

- The preliminary phytochemical studies of high yielding variety (Lucknow-49) leaves reveals the presence of carbohydrate, glycosides, proteins, sterols, saponins, tannin, flavonoid, terpenoid, volatile oil both in powder as well as extracts of various solvents.

- The isolated volatile oil from leaves of ***P. guajava*** shows presence of 37 components. Caryophyllene, aromadendrene were major constituents.

- New components in V.O ledol, 3 carene, muurolol were reported and absence of cineole, nerolidol, limonene which were reported earlier were found.

- The quercetin content of ethanolic extract of high yielding variety ***P.guajava*** (Lucknow – 49) leaves was determined by HPLC (1.2%w/w).

- A simple rapid, economical, reliable, laboratory scale micro slide method for the determination of preliminary platelet aggregation inhibitory activity was standardised and presented.

- **Invitro Platelet aggregation inhibitory activity** : The ethanolic extract of leaves of high yielding variety of ***P.guajava*** (Lucknow – 49) showed significant concentration dependent platelet aggregation inhibitory activity induced by agonists collagen and ADP. It may be due to the presence of quercetin. But the volatile oil showed no inhibitory activity.

It can be optimistic that the present work represent that leaves of ***P.guajava*** likely to be a potential antithrombotic drug. The already reported anti-inflammatory, antidiabetic, hepatoprotective activity strengthens its beneficial therapeutic importance.

Its platelet aggregation inhibitory activity may be due to the presence of quercetin which found to inhibit collagen stimulated platelet activation through inhibition of multiple components of the GPVI signaling pathway.

The above study require further detailed investigation about its effect on thromboxane B_2 (TxB_2) and cGMP synthesis in order to elucidate its role in the risk of CVD.

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