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The Phytochemistry and Medicinal value of Psidium Guajava (Guava): Antiobesity Effect

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Abstract:

Obesity in society has reached epidemic proportions in the 21 st century. Being Overweight is not only appearance issue but also is actually a medical concern, because it is serious health condition. Diabetes and Heart diseases that can stem from obesity . The plant *Psidium guajava* Linn (Guava) is used not only as food but also as folk medicine. We subjected the leaves of these plant for Alcoholic extraction using solvent Ethanol and extract were prepared we infer that the oral administration of guava leaves extract shows Anti-Obesity effect (weight loss). So this study proves that the guava leaves extract helps to control obesity.

Key Points :

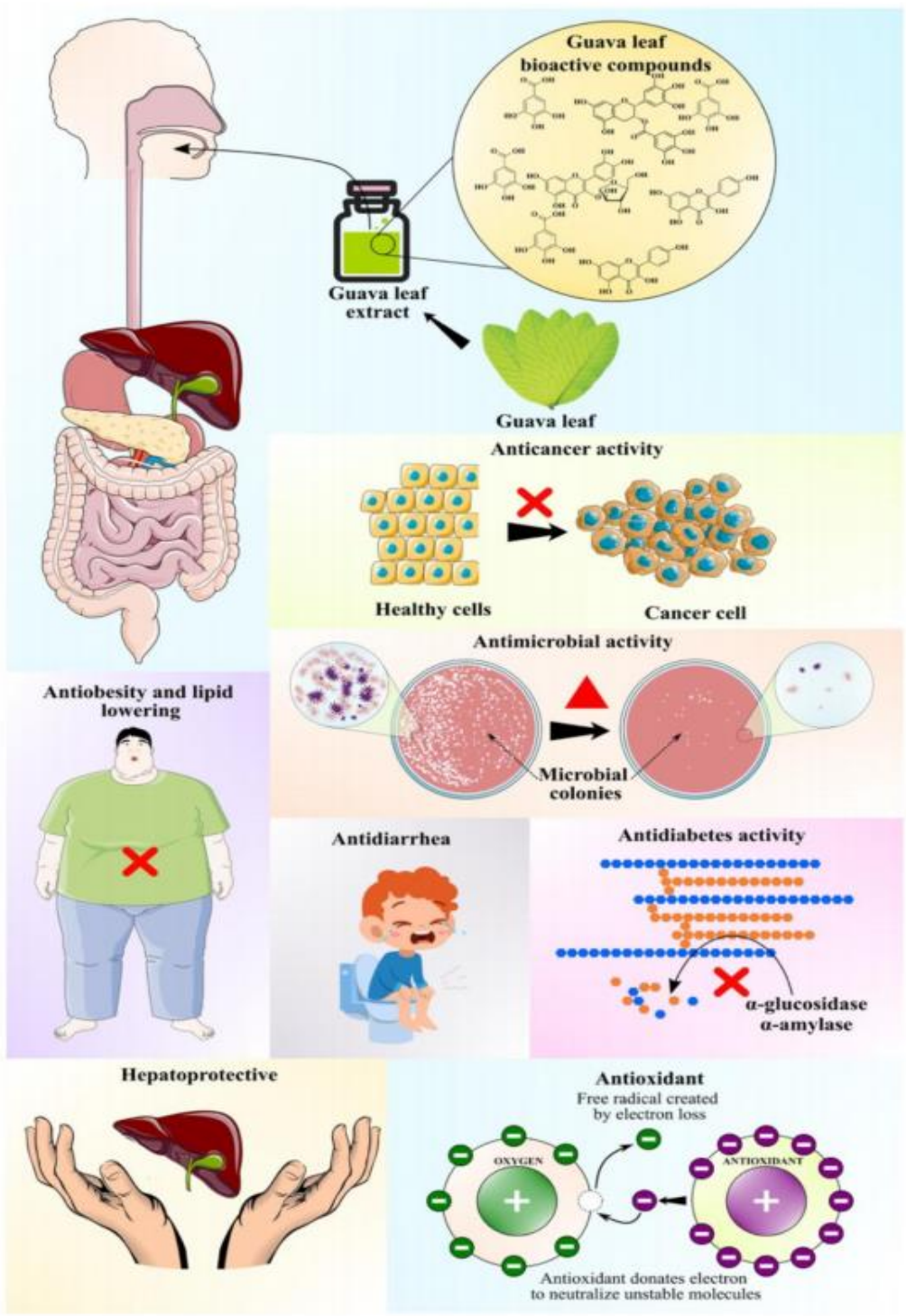
Antiobesity, overweight, Psidium guajava, Antimicrobial, Antinflammatary, Antihyperglycemic, Anticancer,Antitussive activity, Hepato protective activity, Cardiovascular effect,central nervous system related act

Introduction

Obesity in society has reached epidemic proportions in the 21 st century. Being Overweight is not only appearance issue but also is actually a medical concern, because it is serious health condition. Diabetes and Heart diseases that can stem from obesity . The plant *Psidium guajava* Linn (Guava) is used not only as food but also as folk medicine . we subjected the leaves of these plant for Alcoholic extraction using solvent Ethanol and extract were prepared . we infer that the oral administration of guava leaves extract shows Anti- Obesity effect (weight loss). So these study proves that the guava leaves extract helps to control obesity.

Guava leaf has pharmacological properties , that has been showed by many studies, i.e. exhibit antioxidant, hepatoprotection, anti-allergy, antigenotoxic, antiplasmodial, cytotoxic, cardioactive, anticough, antimutagenic, and as asthma cure, antidiabetic, antiinflamatory activities, supporting its traditional uses, and for the treatment of infantile rotaviral enteritis, diarrhoea and diabetes , antispasmodic and antimicrobial properties. The leaf as the raw material for simplicia is crucial for the good quality of the natural product. The leaves harvested should come from the guava tree with high leaf flavonoid content. Seedling will grow to its normal tree height, that made it difficult to harvest, and with smaller number of branches, so pruning is needed to control tree shape, maintain the tree shape , and balance between vegetative and reproductive growth , there is a need to understand and appreciate the processes involved in governing shoot and tree growth and development

Studies have demonstrated that the consumption of alcoholic extract of leaves of psidium guajava can be helpful to control obesity and prevent the risk factors of many diseases due to the bioactive compounds. Plant leaves have been used for the purpose of reducing risk factors associated with the occurrence of chronic disorders and for many other purposes .Psidium guajava) L. is a small medicinal tree that is native to South America. It is popularly known as guava (family Myrtaceae) and has been used traditionally as a medicinal plant throughout the world for a number of ailments. There are two most common varieties of guava: the red (P. guajava var. pomifera) and the white (P. guajava var. pyrifera).


Guava leaf
bioactive compounds
Guava leaf
extract
Guava leaf
Anticancer activity
Healthy cells
Cancer cell
Antimicrobial activity
Microbial
colonies
Antiobesity and lipid
lowering
Antidiarrhea
Antidiabetes activity
α-glucosidase
α-amylase
Hepatoprotective
Antioxidant
Free radical created
by electron loss
OXYGEN
ANTIOXIDANT
Antioxidant donates electron
to neutralize unstable molecules

Project Rationale

Obesity is a metabolic disorder characterized by an excess accumulation of fat in the body due to energy intake exceeding energy expenditure . Obesity is an increasingly common phenomenon all over the world. Body mass index (BMI) is the most commonly used measure to evaluate the degree of obesity. In 2016, the AACE (the American Association of Clinical Endocrinologists) released new diagnostic criteria of obesity based on BMI combined with obesity-related complications . The latest study, which analyzed data from 68.5 million persons between 1980 and 2015, found that a total of 107.7 million children and 603.7 million adults were obese in 2015 . Obesity has become a worldwide epidemic, and the trend is becoming increasingly serious. Obesity is an independent risk factor for metabolic syndrome; major medical problems associated with the development of hypertension, type 2 diabetes (T2DM), dyslipidemia, sleep apnea, and respiratory disorders; and ultimately life-threatening cardiovascular disease (CVD), stroke, and certain types of cancer. The number of obese patients is increasing globally . Reducing body weight by lifestyle alteration is advisable, but sometimes drug intervention is necessary Obesity drugs can be divided into five categories: central appetite suppressants, digestion and absorption blockers, metabolic promoters, obesity gene product inhibitors, and other drugs for the treatment of obesity . However, the weight loss drugs prescribed in conventional medicine induce many adverse reactions, primarily effecting monoamine neurotransmitters, and causing drug abuse or dependence . For example, sibutramine has been reported to commonly cause adverse events, including dry mouth, insomnia, anorexia, constipation, formation of thrombi, and neurological symptoms . Surgery is commonly used in morbidly obese patients (BMI $\geq$ 40 kg/m^2) or in patients with comorbidities, such as hypertension, diabetes, and obstructive sleep apnea . Common surgical complications include infection, postoperative anastomotic fistula, deep vein thrombosis, and long-term complications such as anemia and malnutrition . Given the dangers of obesity and the shortcomings of western medicine, alternative treatments should be further investigated. Objective of Research the potential role of herbal medicines in the treatment of obesity.

Plant profile : Psidium guajava Linn

Psidium guajava is a shrub or small tree usually growing 1- 6 m tall, but occasionally reaching 10 m in height. The older stems are covered in a light reddish-brown, smooth bark that peels off in flakes. This sometimes gives the trunks a mottled appearance, because the newly revealed bark is somewhat greenish-brown in colour. Younger stems are greenish in colour, hairy (pubescent), and somewhat fourangled (quadrangular). The flowers are usually borne singly in the upper leaf forks (axils). These flowers are about 25 mm across and are borne on a hairy stalk (pubescent peduncle) 1-2.5 cm long. The fruit is either rounded (globose), egg-shaped (ovoid) or pear-shaped (pyriform) and turns from green to yellowish in colour as it matures. The **leaves** are simply oppositely arranged along the stems and are borne on short stalks (petioles) 4-10 mm long. The leaf blades (7-15 cm long and 3-7 cm wide) are somewhat oval in shape (ovate-elliptic or oblong-elliptic) with rounded or pointed tips (obtuse or acute apices) and rounded (obtuse) bases. They have hairy (pubescent) undersides (especially when young are generally dull green in colour), and have entire margins. Each leaf has a prominent central vein (midrib) and 10-20 pairs of side veins (lateral veins).

- **Synonyms:** Psidium cujavillus burm.; Psidium pomiferum L.; Psidium pumilum Vahl; Psidium pyriferum Linn.
- **Common Names:** Guava, lemon guava, mpera (Kiswahili), Amrud (Hindi) , mupeera (Luganda).
- **Kingdom ;** Plantae
- **Subkingdom :** Viridiplantae
- **Infrakingdom:** Streptophyta (Land plant)
- **Phylum :** Magneoliophyta
- **Superdivision :** Embrophyta

- **Division:** Magnoliopsida
- **Subdivision:** Spermatophytina
- **Class:** Dicotyledon
- **Subclass :** Rosidae
- **Superorder :** Rosanae
- **Order :** Myrtales
- **Family :** Myrtaceae
- **Subfamily :** Myrtoideae
- **Genus :** Psidium
- **Species :** **P.** guajava

Forage Management :

Guava is propagated both by seeds and vegetatively. In the wild, the seeds are spread by birds and in some places it has become a troublesome weed of pastures. Average yields of fruit from improved trees may be between 12-15 t/ha and up to 50 t/ha have been obtained. Guava trees that have been vegetatively propagated start bearing fruit 2-3 years after planting and are fully productive at 8-9 years. Guava trees propagated from seed require more time for fruit production. In India, 8-10 year old trees from seedlings may produce 400-500 fruits per year while grafted trees at the same age may produce 1000-2000 fruits. Guava can be harvested all year round. The fruit is ready to be harvested when it is yellow. In agroforestry systems, guava can be intercropped with fodders plants such as maize, sorghum and cowpea. The fruits are most commonly harvested by hand.

Microscopy of leaf :

- Shape : Leaves are dorsiventral with prominent midrib, 1.4 mm thick, broadly concave adaxial side and wavy shallow ridges with furrow and lamina being vertical in orientation
- Vascular bundle ; Wide, thin deeply bowl shaped. Xylem thin walled angular in outline occur in short parallel lines (1.9 mm in horizontal plane and 150µm thick. Phloem element occurs at the end of the xylem, as small nests
- Lateral vein: Elliptical collateral vascular bundle with parenchymatous bundle sheath.
- Mesophyll: Palisade zone is one or two layered, short, cylindrical compact cells and five layers of short vertically compact cells.

- Ground Tissue: Parenchymatous, thin walled less compact, less tanniferous. Secretory cavities are more frequent in the axial part.
- Epidermis: 230µm thick, smooth and even. The adaxial epidermis is thin with narrow tubular cells. The subdermal layers of the cells are dilated, four layered, and rectangular without tannins. Calcium oxalate druses in a dilated cell are frequently seen in the adaxial epidermis.

Chemical constituent :

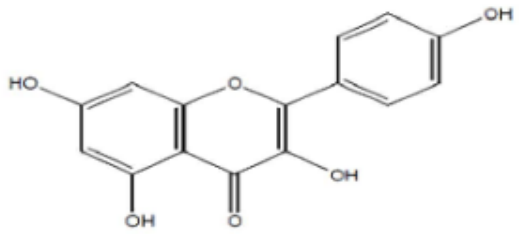
Proximate Composition :

Guava leaves (GLs) are a rich source of various health-promoting micro- and macronutrients as well as bioactive compounds. They contain 82.47% moisture, 3.64% ash, 0.62% fat, 18.53% protein, 12.74% carbohydrates, 103 mg ascorbic acid, and 1717 mg gallic acid equivalents (GAE)/g total phenolic compounds

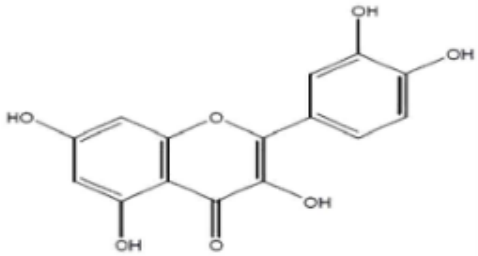
- **The guava fruit** - contains vitamin A, C, phosphorus, iron and calcium. It contains more vitamin C than that of orange. The fruit contains saponin, oleanolic acid, lyxopyranoside, arabopyranoside, guaijavarin,and flavonoids.8-10 Citric acid and acetic acid are the major ingredients of guava that play major role in antimutagenic The chemical structures of quercetin and ascorbic acid

- **Leaves** - which contains essential oils such as isopropyl alcohol, menthol, α-pinene, terpenyl acetate, limonene, β-pinene, caryophyllene, longicyclene and β-bisabolene. Oleanolic acid is also found in the guava leaves.8, 17 Leaves have high content of limonene about 42.1% and caryophyllene about 21.3%.8, 18 Leaves of guava have a lot of volatile compounds.

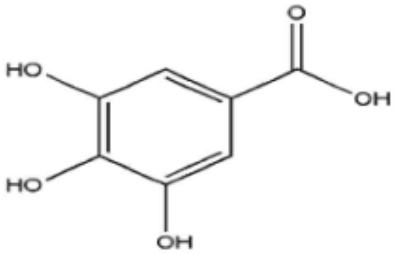
- **The bark** - 12–30% of tannin and one of the sources declares that it includes tannin 27.4%, or polyphenols, resin and the crystals of calcium oxalate. Tannin is also present in roots. Leukocyanidins, gallic acid and sterols are also present in roots. Carbohydrates with salts are present in abundance. Tannic acid is also its Parts .



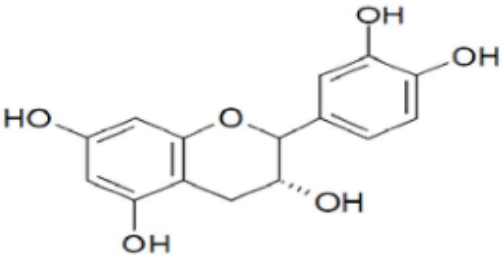
Gallic acid



(+)-catechin



Kaempferol



Quercetin

Phytochemical Analysis :

- **Preparation of Leaf Powder**

The leaves of Psidium guajava were collected, cleaned and air dried under shade for about three weeks. After drying, the leaves were then blended using a household electric blender. This fine powder was analyzed for the phytochemicals present in it.

- **Preliminary Phytochemical Analysis**:

The leaf powder of the study plant was dissolved in two solvents and the preliminary phytochemical tests were carried out on both extracts

A) Aqueous Extract.

B) Alcoholic Extract.

- Test for Alkaloids

  Mayer's Reagent: To 1ml of the extract, 2ml of Mayer's reagent was added. Appearance of dull white precipitate indicated the presence of alkaloids.

- Test for Flavonoids:

  To 1ml of extract, 1ml of neutral ferric chloride was added. The formation of brown colour confirmed the presence of flavonoids.

- Test for Steroids

Lieberman-Burchard's Test: The extracts were dissolved in 2ml of chloroform to which 10 drops of acetic acid and five drops of concentrated sulphuric acid were added and mixed. The change of red colour through blue to green indicated the presence of steroids.

- Test for Terpenoids :

  Salkowski Test: Five ml of each extract was mixed in 2ml of chloroform and concentrated sulphuric acid (3ml) was carefully added to form a layer. A reddish brown precipitate of the interface indicated the presence of terpenoids.

- Test for Quinone:

  To 1ml of extract, a few drops of concentrated hydrochloric acid were added. A yellowish brown colour was observed that showed the presence of quinone.

- Test for Phenols:

  To 1ml of extract, lead acetate solution was added and the precipitate formation indicated the presence of phenolic compounds.

- Test for Starch:

  To 1ml of extract, a few drops of iodine solution were added. Any characteristic colour change showed the presence of starch.

- Test for Anthocyanin :

  NaOH Test: A small amount of extract was treated with 2ml of NaOH and observed for the formation of blue green colour.

- Test for Carbohydrates :

  Molisch's Test: Two drops of Molisch reagent was added to an aqueous or hydrochloric acid solution of the extract and two ml of concentrated sulphuric acid was added by the side of the test tube. The formation of reddish violet ring at the junction of the liquids indicated the presence of carbohydrates.

- Test for Cellulose:

  To 1ml of extract, a few drops of iodine solution were added followed by a few drops of sulphuric acid. Dark brown (or) red colour observed, showed the presence of cellulose.

- Test for Fixed Oil and Fat:

  To 1ml of extract, a few drops of Sudan III solution were added. A shining orange colour obtained showed the presence of fixed oil and fat.

Sr no	Test	Aqueous Extract	Alcoholic Extract	Sr No	Test	Aqueous Extract	Alcoholic Extract
1	Alkaloids	-	-	8	Starch	-	+
2	Flavonoids	-	-	9	Anthocyanine	-	-
3	Terpenoids	+	+	10	Proteins	-	+
4	Quinone	-	-	11	Carbohydrates	+	+
5	Oil and Fats	+	+	12	Cellulose	+	+
6	Sterols	-	-				
7	phenols	+	+				

Table : Preliminary Phytochemical Analysis of Psidium guajava , '+': Present '-' : Absent

Health Benefits :

	Compound	Effect
Leaves	**Phenolic compounds, isoflavonoids, gallic acid, catechin, epicathechin, rutin, naringenin, kaempferol**	**Hepatoprotection, antioxidant,anti-inflammatory, anti-spasmodic, anti-cancer, antimicrobial, antihyperglycemic, analgesic**
Pulp	**Ascorbic acid, carotecoids (lycopene, β-carotene, β-cryptoxanthin**	**Antioxidant, anti-hyperglycemic, Anti-neoplastic**
Seed	**Glycosids; Carotenoids, phenolic compounds**	**Antimicrobial**
Skin	**Phenolic compounds**	**Endothelial progenitor cells and improvement of their intestinal absorption**
Bark	**Phenolic compound**	**Strong antibacterial activity (against multi-drugresistant Vibrio cholera); stomachache and diarrhea**

Table : Some compounds in guava leaves, pulp, seed, skin and bark and their pharmacological effects.

Discussion : Biological Effects of Psidium Guajava leaves extract.

he compounds from guava leaf extracts possess multidirectional biological activities, including antioxidant, hypoglycemic, anticancer, and other biological activities. It was also reported that polysaccharide fractions of sulfated GLP possess stronger biological activities, such as antioxidant, antibacterial, and antitumor effects compared to unsulfated ones. The useful bioactivities of GL extract are presented in the following subsections..

- **Antidiarrhoeal Effect :**
  The methanolic extract of *P. guajava* (leaves) showed significant inhibitory activities against the growths of two isolates *of Salmonella* and *Shigella* spp. *(Shigella flexneri, Shigella virchow* and *Shigella dysenteriae)* and two isolates of the enteropathogenic Escherichia coli. The results have confirmed the effectiveness of the medicinal plant as an antidiarrhoeal agent . It was confirmed in a study that guava sprout extracts constitute a feasible treatment option for diarrhoea caused by *E. coli* or Staphylococcus aureus-produced toxins with characteristic fast therapeutic action, easy availability in tropical countries and low cost .

- **Antimicrobial activity:**
  The extracts of **Psidium guajava** leaves were tested for antibacterial potential and found to be effective against *Staphylococcus aureus, Streptococcus mutatis,* **Pseudomonas aeruginosa**, **Salmonella enteritidis**, **Bacillus cereus**, *Proteus* spp. *Shigella* spp. and ***Escherichia coli***; the major causal agents of intestinal infections in humans . The methanolic root extract of ***Psidium guajava*** that consists of quercetin was also found to be fungicidal . Both aqueous and m**ethanolic extract**s of the leaves are found to be effective inhibitors of spore formation and enterotoxin production of *Clostridium prefringens* type A

- **Antiplasmodial and other antiparasitic activities:**
  The aqueous leaf, stem bark and fruit extracts of ***Psidium guajava*** L. were used to examine anti-plasmodial activity via ***in vitro*** parasite lactate dehydrogenase assay method . The leaves are used in Africa as an ingredient in the preparation of fever teas and are also used as part of pot herb used in steam treatment for malaria, the main ethnotherapeutic use in Africa is said to be for malaria. In addition, KwaZulu-

Natal province of South Africa, ***Psidium guajava*** was found to be effective for the treatment and/or prophylaxis of malaria as the stem-bark extract contains anthraquinones, flavonoids, seccoirridoids and terpenoids. This was confirmed by an ***in vitro*** antiplasmodial assay was carried out using a chloroquine-sensitive strain of malaria parasite

- **Antitussive activity:**

  A study showed that water infusion from *Psidium guajava* leaf extract decreases the frequency of coughing induced by capsaicin aerosol . These results suggest that guava leaf extract could be used as a cough remedy. Also in Senegal and Peru ***Psidium guajava*** leaves boiled together with lemon grass (*Cymbopogort citratus*) to make a decoction is very effective for cough and treatment of tracheobronchitis

- **Hepato-protective activity:**

  Research using a Wister rat demonstrated that aqueous leaf extract of ***Psidium guajava*** was confirmed to possess the hepatoprotective effect. The leaf extract at doses of 500 mg kg^{-1} produced significant hepatoprotection Pretreatment with asiatic acid (a triterpenoid extracted from *Psidium guajava* L. leaves and fruit at doses of 25, 50 mg kg^{-1} or l00 mg kg^{-1} significantly blocked the LPS (lipopolysaccharide) and (D-galactosamine) D-GalN-induced increases in both serum aspartate amino transferase and serum alanine amino transferase levels, showing improved nuclear condensation and ameliorated proliferation with less lipid deposition.

- **Antioxidant activities:**

  Cellular damage or oxidative injury arising from **free radical**s or Reactive Oxygen Species (ROS) now appears to be the fundamental mechanism underlying a number of infections, human neurodegenerative disorders, diabetes, inflammation, viral infections, autoimmune pathologies and digestive disorders. Free radicals are generated through normal metabolism of drugs, environmental chemicals and other Xenobiotics as well as endogenous chemicals especially stress hormones ***Psidium guajava*** L. has been used as health tea and contains copious amounts of phenolic phytochemical which inhibit peroxidation reaction in the living body and therefore, can be expected to prevent various chronic diseases such as diabetes, cancer and heart diseases The decrease of **free radical**s has antioxidising effect in the body due

to the guava leaf polyphenols that prevent arterial sclerosis, thrombosis, cataract and inhibition of senescence changes of the body and skin.

- **Anticancer effects:**

Some recent reports have indicated that *Psidium guajava* possess anticancer activity. The aqueous extract of ***Psidium guajava*** leaves inhibited the viability of cancer cell line DU-145 in a dose dependent manner. At 1.0 mg mL^{-1}, the extract reduced the viability of Pca DU-145 (the androgen independent Pca cells) to 36.1% and 3.6%, respectively after 48 and 72 h of incubations Essential oil leave extracted from ***Psidium guajava*** L. was reported to be highly effective in reducing the growth of human mouth epidermal carcinoma and murine leukemia (P388). Guava leaf oil showed the highest antiproliferative activity with an IC_{50} value of 0.0379 mg mL^{-1} (four times more potent than vincristine) on P388 cell lines Another study also demonstrated a chemo preventive activity of a methanol leaf extract on mice, in case of induced cancer inoculated with B16 melanoma cells. Anti-tumor effect was evaluated from jacoumaric acid (isolated from guava seeds). It was also reported to reduce the incidence of tumors significantly These findings suggested that ***Psidium guajava*** L. extracts have the potential to be developed as new chemotherapeutic agent to inhibit the growth of tumors and cancers.

- **Cardiovascular effects:**

Cardiovascular activities of ***Psidium guajava*** has been reported in a study of an aqueous leaf extract of ***Psidium guajava*** L. which showed cardioprotective effects against myocardial ischemia-reperfusion injury in isolated rat hearts. reported using cholinergic mechanisms of an aqueous leaf extract of ***Psidium guajava*** that caused hypotension in the experimental animal model. Significant reduction in systemic arterial **blood pressure**s and **heart rate**s of hypertensive animal were noticed after acute intravenous administrations of the leaf extract. An Aqueous leaf extract of ***Psidium guajava*** L. produce contraction of the aorta rings significantly in a dose-dependent manner (0.25-2 mg mL^{-1}). The sensitivity of the aortic rings to cumulative doses of ***Psidium guajava*** L. was significantly enhanced in the presence of phentolamine, suggesting that the effect of **Psidium guajava** L. was to a large extent mediated by activation of an alpha-adrenoceptor and to a lesser extent by activating via calcium ion channels

- **Antihyperglycemic activity:**

  The alarming increase in diabetes mellitus is becoming a serious problem to human health in all parts of the world particularly Nigeria. With the distinctive ethno medical opinions and natural medicines mainly originated in herbs, traditional medicine offers good clinical opportunities and shows a brighter future in the therapy of diabetes mellitus and its complications. During various episodes of screening of **medicinal plant** extract from *Psidium guajava*. The decoction of the leaves was screened for hypoglycemic activity in alloxan induced **diabetic rats**. In both acute and sub-acute tests, the extract showed a significant hypoglycemic activity . Another study revealed that treatment with ***Psidium guajava*** L. aqueous leaf extract (0.01-0.625 mg mL^{-1}) also showed significant inhibition on LDL glycation in a dose-dependent manner.

- **Anti-inflammatory and analgesic effect:**

  A decoction of ***Psidium guajava*** L. leaves was used worldwide for the treatment of various inflammatory diseases including rheumatism. The anti-inflammatory property of an aqueous leaf extract was investigated in rats using fresh egg albumin induced paw edema while the analgesic effect of the plant extract was evaluated by the hot plate and acetic acid test models of pain in mice Anti-inflammatory and analgesic activities of 70% ethanol extract of leaves were also investigated in rats. Extracts which exhibited an anti-inflammatory activity were screened for analgesic activity using the Randall-Selitto method in rats. *Psidium guajava* L.

- **Central nervous system related activity:**

  A study showed that the leaves of the guava tree in decoction is used for spasms, epilepsy and other cerebral affections The relaxant properties of ***Psidium guajava*** L. extract are largely due to the presence of terpenes, especially caryophylleneoxide and ß-selinene which potentiate pentobarbital sleeping time and the latency of convulsions induced by leptazol in mice .

Toxicity in Psidium guajava leaves Extract :

Phytochemical screening revealed the presence of flavonoids, quinone, triterpenoid/steroid, tannins and saponins, and the absence of alkaloids. We found that the treatment with 2000 and 5000 mg/kg b.w. of the extract did not show any differences in body weight changes, number of hepatocyte in liver, and podocyte in kidney compared with control (*p>0.05). Moreover, we noticed all mice lived and were healthy during observation.

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Physiochemical studies of psidium guajava leaves :

The extracts of the plants were standardized according to WHO guidelines and other Pharmacopoeial procedures. Physicochemical standardization which includes extractive values in different solvents, total cash value, acid insoluble ash value, loss on drying, pH values (1% and 10% solutions) were checked in triplicate according to the prescribed Standard methods in Indian Pharmacopoeia

Quantitative Analysis

- **Determination of total ash :**

2 grams of the whole plant powder of the Psidium guajava, was placed in a previously ignited (350 0C for 1 hour) and tarred crucible accurately weighed. Dried material was spread in an even layer in the crucible and the material ignited by gradually increasing the heat to 550 0C for 5 hours in a maffle furnace (Naber them) until it is white, indicating the absence of carbon. Cooled in a desiccator and weighed. Total ash content was calculated in mg per g of air-dried material.

- **Determination of moisture content** :

Accurately weighed 5 g of powdered of Psidium guajava leaves were taken in a crucible. It was kept in a hot air oven at 105 – 110 °C, until free from moisture. The percentage of moisture content was then calculated with reference to the air-dried sample.

- **Determination of methanol and water-soluble extractive value :**

20 g of air dried, coarsely powdered Psidium guajava leaves were macerated with 100 ml of petroleum ether in a closed flask for 24 hrs, shaking frequently during the first 6 hrs and wasallowed to stand for 18 hrs. Then it was filtered rapidly and precautions were taken againstloss of petroleum ether. 25 ml of the filtrate was evaporated to dryness in a Petri dish, dried at105 °C and weighed. The percentages of petroleum ether soluble extracts were calculated with reference to the air dried sample. The procedure followed as above using chloroform, methanol and water instead of petroleum ether.

- **Determination of Saponification value :**

Saponification value was determined by mixing 1.5 g sample with 25 ml of 0.1 N Ethanolic acid and KOH by gently stiring and then 3 drop of phenolphthalein indicator was added. And Titration 0.5M HCl was continued until pink color is disapperared.

Saponification value = 56.1 x N (Vb -Va) /M Where, N= normality of HCl soln., vb = volume of HCl soln. used in blank, va = volume of HCl used in sample, M= mass of the oil used.

- **Determination of acid value :**

Take 25 ml of diethyl ether or 25 ml of ethanol and add 3 drop of phenolphthalein then Titrated with 0.1 N KOH (end point dark pink color) volume of 0.1 N KOH will be noted Acid value = 56.1xNxV/M Where N= normality of KOH, M = mass of the oil used v= volume of 0.1 N KOH used for titration.

- [illegible]

Solvent Profile :

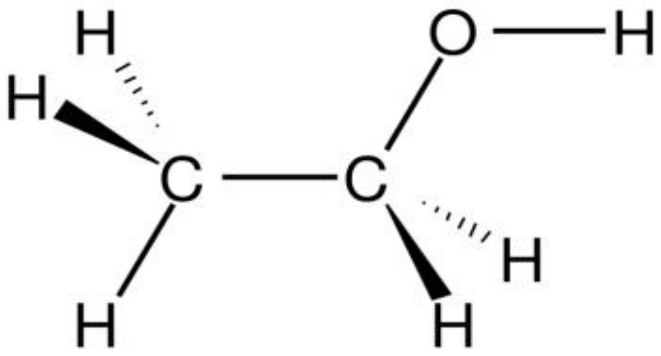
Absolute alcohol : Ethanol

Absolute alcohol is a common name for the chemical compound ethanol. To qualify as "absolute," the ethyl alcohol must contain no more than one percent water. In other words, absolute alcohol is liquid alcohol that is at least 99 percent pure alcohol by weight. Ethanol is a colorless liquid with molecular formula C_2H_5OH. It is the alcohol found in alcoholic beverages. Also Known As: ethanol, ethyl alcohol, pure alcohol, grain alcohol.

- Chemical safety : Flammable
- Molecular formulae : CH_3CH_2OH
- Molecular Weight : 46.07
-

Ethanol is a primary alcohol that is ethane in which one of the hydrogens is substituted by a hydroxy group. It has a role as an antiseptic drug, a polar solvent, a neurotoxin, a central nervous system depressant, a teratogenic agent, a NMDA receptor antagonist, a protein kinase C agonist, a disinfectant, a human metabolite, a Saccharomyces cerevisiae metabolite, an Escherichia coli metabolite and a mouse

metabolite. It is a primary alcohol, an alkyl alcohol, a volatile organic compound and a member of ethanols. It is a conjugate acid of an ethoxide.



Structure : Ethanol Molecule

Objective of these study to screen the anti-obesity effect of guava leaves .the Ethanolic extract showed greatest weight loss in obese persons ;

Methodology

The method is based on the Principle of Homeopathic Dilution and Potentiation. Details Procedure is mention below :

1. The leaves of Psidium guajava were collected, cleaned and air dried under shade for about three weeks. After drying, the leaves were then blended using a household electric blender.
2. The leaf powder was dissolved in Ethanol solvents, allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by filtration or decantation after standing.
3. Allow for evaporation process on water bath for obtaining dried extract.
4. Collect dried extract and dissolve in appropriate Quantity of Ethanol as solvent and prepare different concentrations. which are given to obese subjects in different proportion for 28 days .

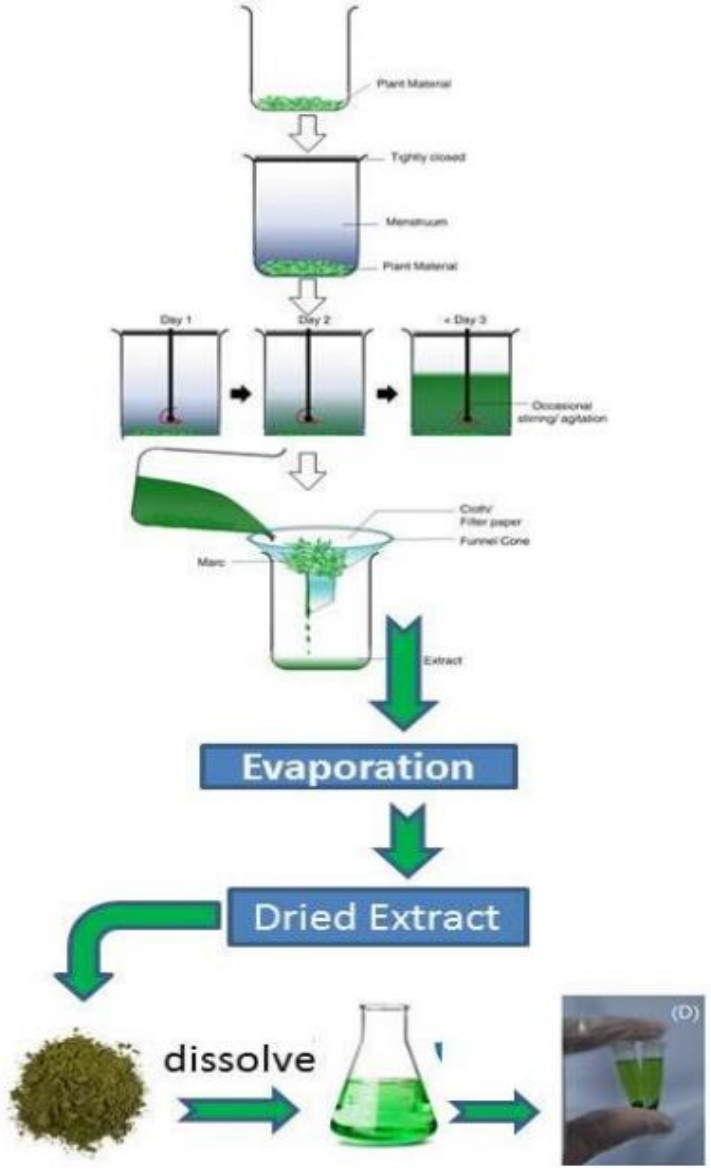


Fig : method used to prepared Guava leaves Extract

400 mg dried extract + 40 ml Ethyl Alcohol

= 10 mg /ml Concentration

1 ml = 18 drops of Ethanol = $\frac{10}{18}$ = 0.55 mg

So, **1 drop consist of 0.55 mg Guava leaf Extract (GLE)**

2 Drops = 1.1 gm GLE

4 Drops = 2.2 gm GLE

6 Drops = 3.3 gm GLE

Ave concentrations are used to check Antiobesity Effects in selected Groups for 28 days.

Observation table :

Sr no	No of volunteers in each Group	No of Drops	Weight (KG) (28 D)				Waist size (cm) (28 D)				Side effect
			1w	2w	3w	4w	1w	2w	3w	4w	
1	8	2	9.48 ±1	0.10 ±0.1	0.08 ±0.01	0.21 ±0.1	2.58 ±1	0	0	0.09 ±0.1	
2	6	4	7.88 ±1	0.19 ±0.1	0.21 ±0.1	0.29 ±0.1	3.46 ±1	0	0.17 ±0.1	0.17 ±0.1	
3	4	6	8 ±1	0.05 ±0.1	0.09 ±0.1	*0.10 ± 0.1*	0.07 ±0.01	0.07 ±0.1	0.10 ±0.1	0.10 ±0.1	
4	4	8	3.85 ±1	0.1 ±0.1	0.1 ±0.1	0.1 ±0.1	0.03 ±0.01	0.12 ±0.1	0.10 ±0.1	0.10 ±0.1	

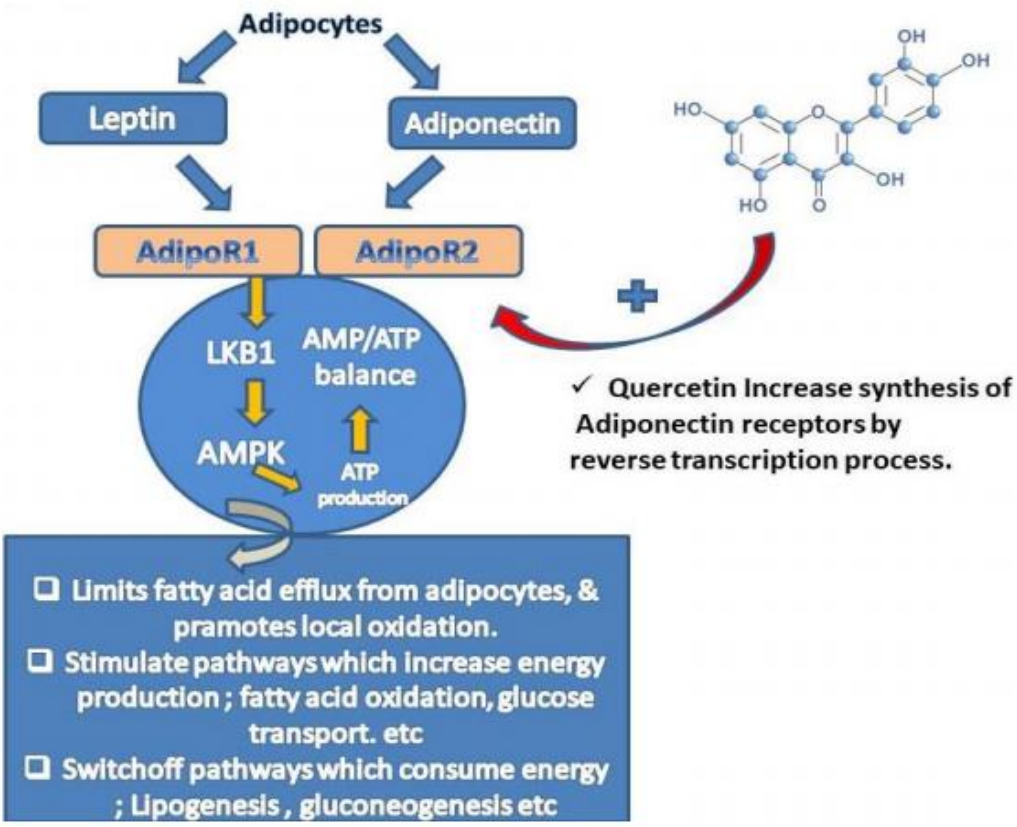
Pharmacology of Preparation :

Quercetin act by two ways :

1. By decreasing adipogenesis / Lipogenesis by activating AMPK Pathway.
2. Reducing intracellular oxidative stress.

(Both are discuss in details below.)

1. Decrease Adipogenesis by activating AMPK signal
   - I. Leptin and adipolectin are differentially expressed adipokines in obesity and cardiovascular diseases. Leptin levels are directly associated with adipose tissue mass ,while adiponectine levels are downregulated in obesity
   - II. Leptin and adipoleptin are activators of adipose tissue AMPK (protein kinase) through receptor ADIPOR1 & ADIPOR2 . Quercetin increase adipose receptor synthesis by reverse transcription process.(as shown in fig)
   - III. These activation probably involves changes in the AMP/ATP ratio through the upstream kinase LKB1. LKB1 encodes serine threonin kinase that directly phosphorelatesand activate AMPK.
   - IV. Activated AMPK involves in cellular energy homeostasis.
   - V. AMPK stimulates pathway which increase energy production ; glucose transport, fatty acid oxidation , etc and switches off pathways which consume energy ; Lipogenesis , protein synthesis, gluconeogenesis , etc
   - VI. Also AMPK limits free fatty acid efflux from adipocytes and favours local fatty acid oxidation.
   - VII. AMPK activation also reduce cytokines secretion in adipose tissue



2. **Reducing intracellular oxidative stress.**

Important Terminologies :

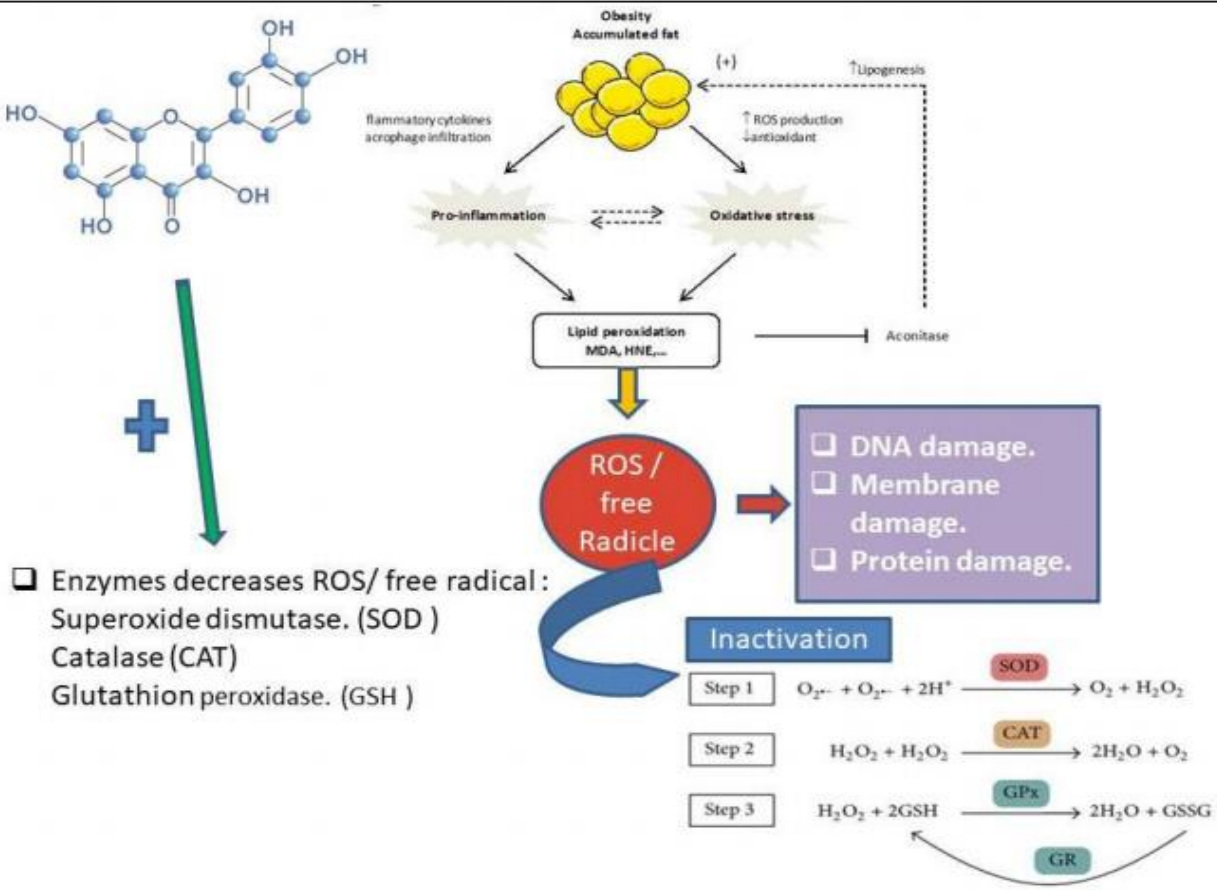
- **Free radical / Reactive oxygen species (ROS) :** when oxygen molecule split into single atoms with unpaired electron which are known as free radical. Eg oxygen radical, superoxide anion, peroxide, hydroxyl radical, hydroxyl ion.
- **Lipid peroxidation :** lipid peroxidation is the oxidative degradation of lipid .itis the process in which free radical steal electrons from the lipid in a cell membrane, resulting in cell damage. free radicals are initiators and terminators of lipid peroxidation.

I. **Reactive oxygen species (free radical) are comstantly formed in biologycal systems . They ra removed by enzymatic and non-enzymatic defence system such as**

A. Superoxide dismutase (SOD
B. Glutathione catalase (GSH)
C. Catalase (CAT)

II. Tissue injury such as lipid peroxidation result from an increase formation of free radical eg ; by the mytochondrial respiratory chain or by activating phagocytic cell .Antioxidant Quercetin could protect oxidative stress by decreasing lipid peroxidation, protein oxidation and increasing superoxide dismutase & catalase activity.

- Quercetin also induces GLUTATHIONE synthesis .which is hydrogen donor in reaction.
- Among antioxidants enzymes , the first line of defence against Reactiv eoxygen species (ROS) catalyse , the dismutation of superoxide Anion (FR) into hydrogen peroxide
- Catalase and glutathion (hydrogen donor in reaction) convert hydrogen peroxide into water and oxygen .



Conclusion :

We conclude that presence of a wide variety of bioactive compounds in the leaves of Psidium guajava that are capable of showing beneficial effects on human health. If we consider that chronic degenerative diseases have reached epidemic proportions in many countries and increase the socio-economic burden for the public health system, it is necessary to find non-allopathic alternatives that minimize risk factors of these diseases and help in the treatment. Furthermore, population consumes medicinal plants also to treat other kind or diseases because of high costs of allopathic medications.. The Guava leaf extract containing Flavonoids (Quercetin) are very active against Obesity , thus making up important potential source of new Anti-obesity compounds.

References

1)Study of best extractive solvent for use with guava leaves (Psidium guajava Linn) for high anti-oxidant efficacy . Article in Food Science nutrition March 2014. http://www.researchgate.net/publication/262113212 :Marcus L Elam , Saraha A Johnson , Bahram A Arjamandi .

2) Monograph of Psidium guajava Linn Leaves . A M Metwali , A A omar ,N. M ghazy & F M harraz, S.M. EI Sohaty. S. M. EI Sohaty , EL khartoum Square , Azarita, faculty of Pharmacy, Alexandria egypt.

3. Anti Hyperglycemic and Anti hyperlipidemic Effect of guava leaf extract ,yariko Deguchi, Kouji Miyazaki. Nutrition & metabolism.

4) Journal of nutritional Therapeutics 2012 .Guava leaf extract inhibit 3T3 –L1 Adipocytes Differentiation Via Activating AMPK. HISAE Yoshitami , Linglin Queen , Tonghua Liu and ming gao. Scool of pharmaceautical science , mukogawa womens university .

5) Review : Antioxidant aaactivity of Quercetin and its complex for medicinal Application. Dong XU Meng Jiao Hu, Research centre of traditional chinese medicine , Tianjin University of Traditional Chinise medicine Tiajin, 300193 china

6) Sukhdev Swami Handa, Suman Preet Singh Khanuja, Gennaro Longo, Dev Dutt Rakesh. 2008. Extraction technologies for medicinal and aromatic plants, International centre for science and high technology.

7) Natural Products for the Prevention and Treatment of Chronic Inflammatory Diseases: Integrating Traditional Medicine into Modern Chronic Diseases CareReview Article | Open **Access**Volume 2017 |Article ID 894**8)** Health Effects of *Psidium guajava* L. Leaves: An Overview of the Last Decade Elixabet Díaz-de-CerioVito Verardo, Ana María Gómez-Caravaca, Alberto Fernández-Gutiérrez,and Antonio Segura-Carretero Anti-hyperglycemic and anti-hyperlipidemic effects of guava leaf

10) Antimicrobial Activities of Leaf Extracts of Guava (*Psidium guajava* L.) on Two Gram-Negative and Gram-Positive BacteriaBipul Biswas,[1] Kimberly Rogers, Fredrick McLaughlin, Dwayne Daniels, and Anand Yadav

11) Kim and D. Y. C. Fung, "Antibacterial effect of crude water-soluble arrowroot (*Puerariae radix*) tea extracts on food-borne pathogens in liquid medium," *Letters in Applied Microbiology*, vol. 39, no. 4, pp. 319–325, 2004.View at: Publisher Site | Google Scholar

12)A. Ibrahim, M. M. Salameh, S. Phetsomphou, H. Yang, and C. W. Seo, "Application of caffeine, 1,3,7-trimethylxanthine, to control *Escherichia coli* O157:H7," *Food Chemistry*, vol. 99, no. 4, pp. 645–650, 2006.View at: Publisher Site | Google Schol

13)I. Abdelrahim, A. Z. Almagboul, M. E. A. Omer, and A. Elegami, "Antimicrobial activity of *Psidium guajava* L.," *Fitoterapia*, vol. 73, no. 7-8, pp. 713–715, 2002.View at: Publisher Site | Google Scholar