

## A Comprehensive Study On Different Methods Of Extraction From Guajava Leaves For Curing Various Health Problem

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### ABSTRACT

Psidium guajava leaf is an important part of guava tree which is useful in curing many health problems. Guava leaves having properties like antibacterial, anti-oxidant, anti-cancer, anti ulcer etc. used in many diseases. In this article different extraction methods are discussed and yield of these methods represented. Extraction processes with different solvent such as ethanol, methanol, ethyl acetate and water are discussed in this article. The purpose of this article is to introduce the different extraction processes for different compounds for curing different health problems. In this study, aqueous extract shows it's widely use in medical as compare to other solvents extract. Aqueous extract of guajava leaf have antiglycation activity and prevent neurodegenerative and cardiovascular disease and also shows antiprostata cancer activity

**Keywords** – Psidium guajava, extracts, flavonoids, tannis, quercetin, antibacterial, antimicrobial

### INTRODUCTION

Guava tree (psidium guajava) is basically from the Meso Americam area. It can also find in tropical and subtropical areas. Guava tree is member of myrtaceae family, all the parts of this tree widely use in curing many health problems. A lot of work on Pharmalogical researches has been done to demonstrate the use of extract from guajava leaves which proved that guajava leaves extracts are such a useful medicine, widely using by doctors and pharmacist. WHO (world health organization) also says that plants would be the best source for obtaining different types of medicines and drugs. These natural products are widely used by human with its effective results. Extraction from guajava leaves mostly essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C, Flavonoids particularly rich in quercetin, saponins, alkaloids, cardiac glycosides, phlobatannis and anthraquinones. Guava leaf extracts introduces many biological activities i.e. Antibacterial, antioxidant, and analgesic, anti inflammatory, antimicrobial,

phytotoxic, hepatoprotection, and anti hyperglycaemic and anti cancer activities [3]. For extraction process, first guava Leaves collected and then dried in open air circulation for some time but in shade. Now this material stored in refrigerator in plastic. There are several techniques used in the extraction explained in next section.

### BASIC EXTRACTION METHODS

#### A. Super critical fluid extraction

Extraction process is basically for separation of one component from other component by using extracting solvent. In SFE, we use supercritical fluid as extracting solvent with some co-solvent to increase its capacity to separate. For this type of extraction, we take sample of guava leaves 1 mg in a cell column. In SFE CO<sub>2</sub> is mostly taken as super critical fluid with ethanol and methanol as co-solvent. Fig.1 showing different components: a pressure cell, pressure controller, collecting vessel, heating and cooling system and pump. First liquid is converted into supercritical condition then pass it to the extraction vessel where it could easily diffuse into solid matrix of sample and dissolve the material which we have to extract. The dissolved material will swept away from cell column at lower pressure and extracted material settle out.CO<sub>2</sub> can be recycled. Temperature and pressure conditions should be 45-55 C and 200-300 bar.

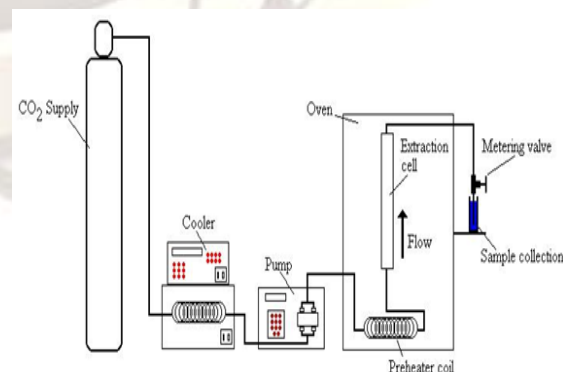


Fig.1 super critical fluid extraction

### B. Soxhlet extraction

Different components which used in Soxhlet extraction like thimble, water cooling system, and reservoir, by pass tube, siphon tube and condenser can be seen in figure 2. We will take 10 mg of solid material of leaves keep in thimble which is loaded into soxhlet vessel having flask containing extractor solvent. Solvent vapor moves up to the column and floods into the chamber housing the thimble of solid. Some part of non volatile compounds dissolves in solvent. Process repeats many times until we get desired concentrated compounds in flask. Process has been done at boiling temperature of solvent and extraction has been done in 100 ml ethanol for 3.5 hours.

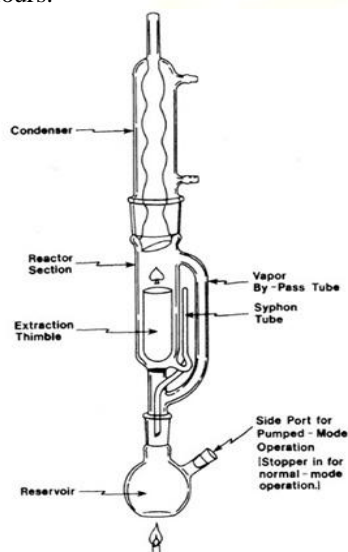


Fig-2 Soxhlet extractor

### C. Steam distillation

This process is basically for natural aromatic compounds used for temperature sensitive compounds which decompose at high temperature i.e. 25 mg of dried leaf material distilled in 300 ml water. Process carried out for 3-4 hours at boiling temperature of solvent and after distillation, volatile compounds and water separated by methyl chlorate.

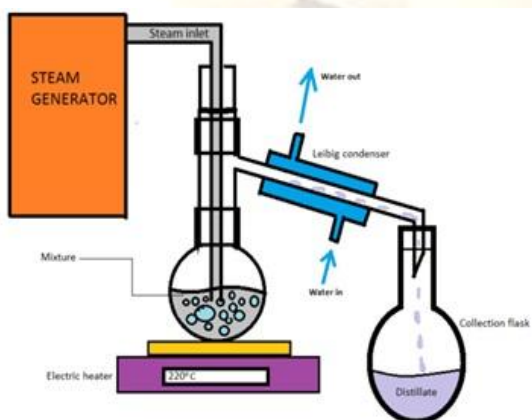


Fig.3 Steam distillation extraction apparatus

In the above figure grey represents steam, which lowers the boiling point of the compounds in the mixture so that they can be distilled before the heat destroy them.

### D. Ultrasound extraction

We will take 1.5 mg of solid sample in ultrasound apparatus. Ultrasound apparatus consist of water tank, ultrasound generator, digital timer, rotary gear and others (shown in fig.5).process temperature should be 40-50 C and process time should be 1-2 hours. After this process, material was filtered using a vacuum evaporator.

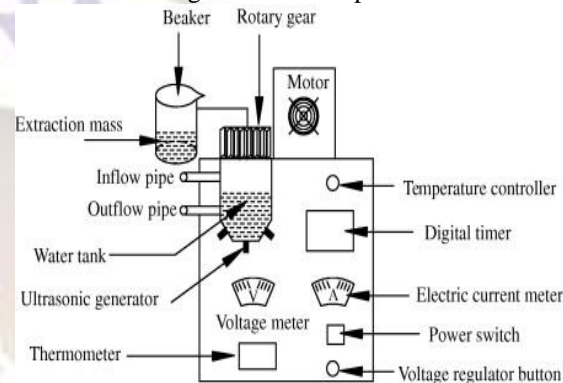


Fig.5 Ultrasound extraction apparatus

### METHODS USED IN MEDICAL APPLICATIONS

Particular method is used to extract particular compound for treatment of certain illness. Guava tree full fill all the requirements of this types of applications. We will discuss here some methods that are useful for various deceases.

#### a. Reduction of growth of swine flu viruses

Swine flu  $H_1N_1$  viruses are widely circulating in humans and after many years of this decease [4], the viruses are still functioning with high impact. Many treatment processes has been done for the reduction of growth of such viruses but these methods have some disadvantages or side effects. Guava leaf extracts due to being a natural product do not have any side effect. Guava leaf tea is widely using for health problem solutions. Guava leaf tea is showing very good result in reduction of growth of viruses. This Tea has very important component tannin that has properties like anti-influenza, anti-inflammatory, antioxidant and antimicrobial activities. Guajava leaves, which we have dried in air, taken in amount of 25 g in 1 liter water. Temperature should adjust at  $85^{\circ}C$  and process should be for 8-10 minutes with stirring softly. Now this extract filtered through PTFE (Poly Tetra Flouro Ethylene) to remove remaining leaves. Now dilute this mixture with cold water to adjust tannin content which is 40 mg/100 ml and dilute with sodium bi carbonate to maintain  $P^H$  at 6-7.

**b. Antibacterial properties**

For getting Antibacterial property [5] [11] [12] extraction should be done against methanol, ethanol, ethyl acetate and water. Agar well diffusion method used to identify its antibacterial properties. For the process, wash the guajava leaves with distilled water and kept in incubator at 40<sup>o</sup> C for 4-5 days and grinded into powder form. Now this material dissolved in 70% ethanol, 80% methanol, ethyl acetate and hot water. Quantity of plant material should be 1g in 10ml of solvent. Now kept this mixture in sterilized beaker and covered with aluminum foil and put this beaker in a dark room for 3 days. Now this mixture filtered by Whatman no. 1 and kept it in incubator at 40<sup>o</sup> C until all solvent evaporated from mixture. After this all mixture dissolve in di methyl sulfoxide and different test tried for anti bacterial properties against bacterial culture 1g positive culture staphylococcus aureus and 2g negative cultures pseudomonas aeruginosa and E. coli. Phytochemic research has proved that the antibacterial properties of guajava leave depend on Saponins, tannins and flavonoids. This study shows that extraction with ethanol have greater MIC (minimum inhibitory concentration) value. Now this ethanol extract of guajava leaves use in treatment of inflamed gum ant stomach pain caused by E. coli and S. Aureus.

**c. Treatment of diabetes**

The number of diabetes patients has been increasing continuously due to urbanization and life style changes [1] [8]. Now most of the persons take food which is high in fat and this fat increase glucose level in body and diabetes occurs. WHO research estimated 171 million people suffering from diabetes in 2000 and possibility to increase this value to 366 million in 2030. In type1DM and type2DM, case of type2DM (diabetes mellitus) has been increases comparatively. Guajava leaves tea and aqueous extracts of guajava leaf has been recommended for pre-diabetes by FOSHU (foods for specified health uses) in Japan. Guajava extracts with water and guajava tea inhibited the invitro activities of maltase, sucrose and alpha amylase according to dose given.

**d. Treatment of gastric mucosal injury**

Gastric mucosal injury [9] occurs due to ischemia and reperfusion [I-R] and found very commonly in humans. Many traditional methods has been using for the treatment of this problem but guajava leaves extract shows a natural and efficient way for the treatment of this problem. Aqueous extract of guajava leaves gives flavonoids which show anti-ulcer activities by preventing gastric mucosal lesions in ulcer models. Guajava leaves collected and dried for 5 days in open air and grinded into fine powder. This powder dissolved in ethanol (250 ml) for four days. After this put into

evaporator and then filtered with Whatman no.3 and now sealed this mixture in a container at 4<sup>o</sup> C. Now when we have to use it, at that morning, we dissolved it in physiological saline. At first this experiment has been done on rats.

**e. Treatment of human gastric cancer cell**

In the search for the new anti-cancer drug [10], this study demonstrated its anti-cancer ability. Cancer is most common cause of increasing death rate. Guava species is strawberry guava distinguished from other guava. It is cultivated in various parts of the world due to its medical applications and good quality. Guajava leaves collected from the source and air dried and extracted with 80% methanol. Amount of leaves should be 1-2 kg. Extraction should be done for 3 days at room temperature. Now this mixture filtered and extract evaporated in rotary vacuum evaporator at 40<sup>o</sup> C. Now this dried extract suspended in saline water and extracted with n-hexane, chloroform, ethyl acetate and n-butanol step by step. Now chloroform fraction powder dissolved in DMSO and diluted with saline to obtain desired concentration for anti-cancer activity.

**f. Antimicrobial properties**

Antmicrobial properties [11] are quite same as antibacterial property. Agar well diffusion method is also use for identifying this property against bacterial culture. In this property, we have to extract air dried guava leaf with acetone, methanol and hexane. Here we use 20 gm of sample for each extraction. Now this extract kept in rotary shaker for 1 day and filtered. This extract centrifuged at 5000 rpm. Further this dried compound further for antimicrobial test against bacterial culture in agar well diffusion method. The percentage yield of acetone, methanol and hexane extracts are 11-12%, 13-14% and 6-7% respectively.

**g. Treatment of blood coagulation in diabetes patient**

Patients of DM type 2 (diabetes mellitus type 2) [1] are increasing in world due to their fatty diet. 80% of diabetes patients die due to thrombotic complication and 75% of this death are due to cardiovascular events and cerebrovascular events and complications. In diabetes, hyper coagulable state is related to hyperglycemia. Rearch said that if we maintain good control on glycemic then it will help for treatment of hypercoagulable state. Hyperglycemia is a factor in diabetes vascular complications. Guava leaves tea and extract showed very effective and powerful effect on gyration in glucose and glyoxal induced model. Guava tea is now being popular because of its healthy and effective reasons. In this method, guava leaves boiled in 250 ml water for 30-40 min. And

extract filtered through a Whatman no.2 paper. Tea is ready. Now this mixture pulverized and extract yield we get is 9-10%. This guajava tea exhibits excellent effect on antiglycemic. The inhibitory effect of guajava leaf extracts against coagulant effects is evaluation of the capacity of extracts as anticoagulant in alleviating the development of cardiovascular complications in diabetes were examined.

#### h. Labeling of blood constituents with technetium-99m (Tc-99m)

For labeling of blood constituents [16] this experiment uses antioxidant properties of guajava leaves. Nuclear drugs and medicines use blood constituents labelled with Tc-99m. Guajava leaf extracts can modify the labelling of BC of Tc-99m. For this experiment, we use rat's blood for experiment and incubated with different concentration of guajava leaf and percentage of incorporated activity (%API) in BC was examined Guajava leaves collected from the source and 20 mg of dried leaves were grind in 0.9% NaCl (2 ml) at 100° C for 5 min. Now extract filtered and centrifuged (1500 rpm) for 5 min. Final extract concentration was 10mg/ml of guava leaf and now this extract examined on the blood of waster rats at different concentration and analyze it. Results show that guava extract decreases the fixation of radioactivity in blood cell in dose dependent manner. Guava extract modify labeling of Tc-99m in plasma only at high concentration because the binding sites in these proteins could be the same binding sites of Tc-99m and due to presence of guava extract compounds, plasma protein labeling could be reduced.

#### i. Treatment of liver diseases: heptoprotective activity

Guava extract of leaves posses' a good hepatoprotective activity [17]. We use this activity in liver injury induced by CCl<sub>4</sub>, peracetamol and chronic liver damage induced by CCl<sub>4</sub>. In liver disease problem, CCl<sub>4</sub> is most commonly used hepatotoxins, hepatotoxic effect of CCl<sub>4</sub> is due to generation of free radicals. Guava leaf drugs having antioxidant properties are very effective in treatment of CCl<sub>4</sub> induced liver injury. Guava leaf extract shows very good hepatoprotective activity at higher concentration of 250 and 500 mg/kg at dose dependent manner. Lower dose does not show this effect in chronic hepatic damage induced by CCl<sub>4</sub>. This effect due to higher dose is similar to that produced by Silymarin (a widely used hepatoprotective agent). To get guava leaf extract, dried guava leaves ground in distilled water as 100g leaf per 300 ml of water. Now this leaves boiled and filtered through a cloth. Now this extract evaporated until get a powdery form, yield was 4% (W/W). The powder subjected to phytochemical

analysis to detect chemical constituents in extract. Now this powdery extract suspended in water and examined it in hepatic injury model for hepatoprotective activity.

#### j. Anti diarrhoeal activity

For anti diarrhoeal activities [2] [3], we collect Guava leaves and ground into fine powder and stored in cool place. Now 500g of dried leaves powder dissolved in 3l ethanol in absence of air for 15 days with stirring after 2-3 days interval continuously. Now this extract filtered with cloth and again filtrate evaporated to dry by RVE (rotary vacuum evaporator). Now this extract has been tested against albino rats for anti diarrhoeal activity. This plant leaf extract is used for treatment of diarrhoeal, stomach ache and hepatic problems. The result shows that leaf extract protect diarrhoea up to level of 55.6%. In Bangladesh, guava leaves are chewed to check diarrhoea.

### RESULTS AND DISCUSSION

Many extraction methods are discussed in this article but global yield of these methods are different. Global yield of these methods explain into fig 6. This yield shows the ability or efficiency of extraction process having most extraction process is higher than SFE with exception of steam distillation. Soxhlet process gives highest global yield but separation of compounds is difficult in this method but in SFE gives better recovery of functional compounds. Soxhlet process needs additional separation process for the recovery of compounds. SFE process due to being selective shows the best result to obtain bioactive compounds as compare to other process. SFE gives higher no. of functional compounds [6][7]. After analyzing all this extraction methods for curing various problems, we would say that guajava leaf extract is such a explored area that is very useful as natural drugs and medicines. All the compounds of guajava extract are useful in curing health problems. By analyzing all these methods, we can easily explain that: Quercetin used to decrease mortality from heart disease and it has antioxidant activity

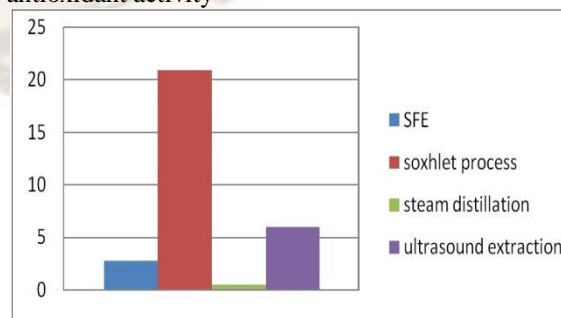


Fig-6 Global yield of different extraction methods  
One research shows that guajava leaf extracts also have Terpenoids such as Betulinic and lupeol and

shows antimicrobial and Phytoxic activities. Guajava leaf extract also have compounds named Rutin and Kaempferol that decrease HMG-CoA reeducates activity in hepatic tissue and also improve lipid profile

Extracts	Compounds	Properties	Diseases
Aqueous extract: tea	Tannins,	Antiviral effects, anti influenza activity	Growth of clinical isolated H1N1 viruses: swine & Spanish flu
Aqueous extract: tea	Tannins, flavonoids	Anti hyperglycaemic, anti hyperlipidemic	Diabetes mellitus type2
Ethanol extract	Flavonoids, polyphenolic compounds	Anti-ulcer, anti-oxidant	Gastric mucosal injury
Methanol and chloroform extract	Quercetin, flavonoids	Antiproliferative activity, anti-cancer activity	Gastric cancer cell
Acetone, hexane and methanol extract	Essential oil and functional compounds	Antimicrobial, antibacterial activity	Infectious diseases
Aqueous and ethanol extract	Aromatic oil and functional compounds	Antibacterial, antimicrobial	Gastroenteritis
Hexane and ethyl acetate extraction	Essential oil and other compounds	Antibacterial, antimicrobial	Antidiarrhoeal, antibacterial
Aqueous extract	Ferulic acid, gallic acid, quercetin	Anti coagulant, antiglycative agent	Blood coagulation, diabetes mellitus
Aqueous extract	Quercetin, flavonoids and	Anti-inflammatory, hemostatic	Labelling of blood constituents with

	functional compounds	agent	Technitium-99m
Aqueous extract	Quercetin, flavonoids,	Hepatoprotective activity, anti-oxidant	Jaundice, cirrhosis, fatty liver and liver damage
Methanol extract	Caryophyllene oxide, caryophyllene, tannins	Anti-oxidant	Tumour
Aqueous and alcoholic extract	Flavonoids	Anti-inflammatory activity	Acne lesions, effect on dental plaque

Table 1

Extract of guajava leaf having compounds used in diabetes treatment. This inhibits the glycation of protein and prevents diabetes. Ethanol extract of guajava leaf shows antiproliferative activity, anti-cancer, chemopreventive anti allergic effects. Leaf extract compounds having glycosids, carotenoids, phenolic compounds shows antimicrobial property Methane extract shows reduction of elevated serum levels of enzymes, wound healing effect due to presence of tannins and flavonoids. This also be useful in treatment of gastric ulcer disorders due to presence of volatile compounds, flavonoids and saponins. Guajava leaf has high capacity of reducing polymerization and for membrane stability effect due to compounds having flavonoids and tannins. Guajava leaf extract having compounds ascorbic acid, carotocoids shows anti oxidant, antihyperglycemic and anti-neoplastic. Methanol and ethanol extract of guajava leaf shows anti diarrhoeal activity. Aqueous and alcoholic extracts of guajava leaf inhibit the growth of staphylococcus aureus, E. Coli and shows anti bacterial and antimicrobial properties. Water infusion from guava leaves decreased cough problem as compare to control, within 10 min after the injection of this extract. Aqueous extract of guajava leaf shows hepatoprotective effect when tested on rats. Hot water extract of dried leaves gives phenolic compounds that describe its anti-oxidant and radioprotective activities. 70% extract compounds such as phenolic compounds triterpenoids and other compounds show anti-inflammatory and analgesic activities. All extraction methods and their compounds and properties and solved diseases are given in table 1. All the above discussion shows the ability of guajava leaves in curing health problems. So we have solvent for extraction is ethanol, methanol,

ethyl acetate and water. In all these extraction, methanol extract shows the best anti bacterial activity. Table 2 shows the the best inhibition against bacteria exhibits by methanolic extract as compare to other extracts. Zone of inhibition is dia of zones of inhibition by leaf extract in mm. This value of zone of inhibition represents MIC (minimum inhibitory concentration) values for all extracts

Extracts	Zone of inhibition (mm)
Aqueous extract	8-16nm, 22-25nm
Ethanolic extract	14-21nm,25-27nm
Methanolic extract	27-30nm
Ethyl acetate	20-25nm

Table 2

Till now we have discussed many extraction processes in which aqueous extract is most widely using process because aqueous extract is safe as compare to alcohol and acids. Here present a fig 7 which shows the percentage uses of different solvent extracts. Solvent taken in chart are water, methanol, ethanol and ethyl acetate.

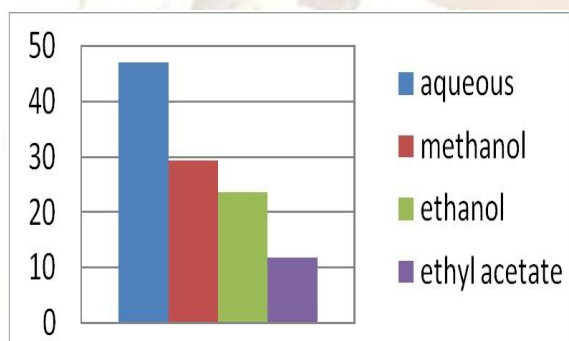


Fig- 7 Different solvent used in extraction

## CONCLUSION

Here we are concluding guajava leaves as very useful drugs or medicines. Many researches has been done and will be continue in future on this. Natural products are widely using by humans worldwide because these products does not have any side effect. Guajava leaf extracts are such a wide spread area in field of medical and science. Guajava leaf extracts contain flavonoids, tannins,  $\alpha$ -pinene, limonene, phenolic compounds, saponins and many other important compounds and these compounds show very useful and important properties such as anti-diarrhoeal, anti bacterial, anti cancer, anti microbial, anti malarial, hepatoprotective effect, anti-oxidant, anti allergic, anti glycemic, anti inflammatory, wound healing, analgesic and many more. Guajava tea due to being a herbal tea is very healthy drink. Guajava tree prove to be a very useful for natural drugs. Aqueous extract of guajava leaf is most widely used as compare to other solvents but methanol

extract shows highest yield because aqueous extracts is much safer than acid and alcohol.

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