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Evaluation of Anti-Bacterial Activity with Tannin Fraction from *Psidium guajava* leaves and barks

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Research Article

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ABSTRACT

Objective: The Ethnobotanical studies and folklore claiming reviewed that the leaves and bark of the plant *Psidium guajava* Linn. are used for anti-bacterial activity. From the literatures we found that poly-phenolic compounds like tannin have significant free radical scavenging activity in bacterial associated diseases.

Based on the above information of *Psidium guajava* is selected for this Research work.

- To separate the tannin fraction from the *Psidium guajava* Linn., leaves and bark.
- To study the anti-bacterial activity of tannin fraction of *Psidium guajava* separated from leaf and bark.

INTRODUCTION

The Medicinal plants are of great value in the field of treatment and cure of disease. Over the years, scientific research has expanded our knowledge of the chemical effect and composition of the active constituents which determine the medicinal properties of plants [1].

India is an ancient heritage of traditional medicine. Materia medica of India provides a lot of information on the folklore practices and traditional medicine based on various indigenous system including Ayurveda, Siddha and Unani [2].

In western medicine continues to show the influence of ancient practices. More recently, there has been interest in other products from traditional systems of medicine artemisinin is an active anti-malarial compounds isolated from *Artemisia annua*, a constituent of the Chinese anti-malarial preparation qinghaosu and forskolin was isolated from Coleus forskohlii, a species used in Ayurvedic preparations for cardiac disorders. A new standardized preparation, artemether has recently been introduced for treatment of drug resistant malaria, and new analogues of Forskolin are being tested for a variety of uses [3].

Nature has provided an excellent storehouse of remedies to cure all the ailments of mankind. In ancient days, almost all the medicine used was from natural sources, particularly from plants and plants continue to be an important source of new drugs even now. The importance of biological, chemical and pharmacological evaluation of plants derived agents used in the treatment of human ailments has been increasingly recognized in the last decades.

In the next of our series on far eastern plant we look at guava or *Psidium guajava* Linn. In folk medicine, extracts of roots, bark and leaves are used to treat gastroenteritis, vomiting, diarrhea, dysentery, wounds, ulcers, toothache, cough, sore throat, inflamed gums, and a number of other conditions. This plant seemed worthy of an in depth review [4].

Tannin

The term "tannin" by extension is widely applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyl's) to form strong complexes with proteins and other macromolecules [5]. The compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also in growth regulation [6].

Occurrence

Tannin is distributed in species throughout the plant kingdom. They are commonly found in both gymnosperms as well as angiosperms. Botanically, tannin is mainly physically located in the vacuoles or surface wax of plants.

Tannins are found in leaf, bud, seed, root, and stem tissues. An example of the location of the tannin in stem tissue is that they are often found in the growth areas of trees, such as the secondary phloem and xylem and the layer between the cortex and epidermis. Tannin may help to regulate the growth of these tissues.

Role of Tannin used in Anti-bacterial Activity [7]

The anti-inflammatory effect of tannin helps to control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders [8]. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine.

Tannin not only heals burns and stop bleeding, but they also stop infection while they continue to heal the wound internally [9]. The ability of tannin to form a protective layer over the exposed tissue keeps the wound from being infected even more. Tannins are also beneficial when applied to the mucosal lining of the mouth [10].

Tannin can also be effective in protecting the kidneys. Tannin has been used for immediate relief of sore throats, diarrhea, dysentery, hemorrhaging, fatigue, skin ulcers and as a cicatrizing on gangrenous wounds [11]. Tannin can cause regression of tumors that are already present in tissue, but if used excessively over time, they can cause tumors in healthy tissue. Tannins are used indirectly as molluscicides to interrupt the transmission cycle of schistosomiasis [12]. They have also been reported to have anti-viral effects. When incubated with red grape juice and red wines with a high content of condensed tannin, the poliovirus, herpes simplex virus, and various enteric viruses are inactivated.

Tannins are sometimes used to treat poisons from poison oak or from bee stings, causing instant relief [13]. Tannin has shown potential *anti-viral,* antibacterial and *anti-parasitic* effects.

It is believed that tannin isolated from the stem bark of *Myracrodruon urundeuva* are of neuro protective functions capable of reversing 6-hydroxy dopamine induced toxicity. The plant has shown promising futures for therapeutic use, which may be of benefit to neuro disease patients [14-16] discovered that the tannin isolated from the stem bark also has the anti-inflammatory and *anti-ulcer* potency on rodents, showing a strong *anti-oxidant* property for possible therapeutic applications Foods rich in tannin can be used in the treatment of HFE hereditary hemochromatosis, a hereditary disease characterized by excessive absorption of dietary iron resulting in a pathological increase in total body iron stores [17].

Antibacterial Activity of Natural Tannin

The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. In addition, the possible synergistic effects when associated with antibiotics were studied. Extracts from the following plants were utilized: *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa offficinalis* (lemon-balm), *Ocimun basilucum* (basil), *Psidium guajava* (guava), *Punica grantor* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygyum joabolanum* (jambolan) and *Thymus vulgaris* (thyme). The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized [18,19]. The highest antimicrobial potentials were observed for the extracts of *Caryophyllus aromaticus* and *Syzygyum joabolanum*, which inhibited 64.2 and 57.1% of the tested microorganisms, respectively, with higher activity against antibiotic-resistant bacteria (83.3%). Sage and yarrow extracts did not present any antimicrobial activity. Association of antibiotics and plant extracts showed synergistic antibacterial activity against antibiotic-resistant bacteria. The results obtained with *Pseudomonas aeruginosa* was particularly interesting, since it was inhibited by clove, jambolan, pomegranate and thyme extracts. This inhibition was observed with the individual extracts and when they were used in lower concentrations with ineffective antibiotics.

Biological Importance of Tannin

High molecular weight plant polyphenolics (tannin) as biological antioxidants [20]

Representative condensed and hydrolysable tannin and related simple phenolic were evaluated as biological anti-oxidants using cyclic voltammetry, the met myoglobin assay, and the deoxyribose assay. The redox potentials of the tannin were similar to those of structurally related simple phenolic. However, the tannins were 15-30 times more effective at quenching peroxyl radicals than simple phenolic or Trolox. One of the tannin, polygalloyl glucose, react an order of magnitude more quickly with hydroxyl radical than mannitol. These results suggest that tannin, which are found in many plant-based foods and beverages, are potentially very important biological antioxidants.

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Condensed vegetable tannin: biodiversity in structure and biological activities [21]

Proanthocyanidins, as an important class of secondary plant metabolites, are in many cases the active principles of the medicinal plants from which they are isolated. The structural complexity and conformational properties of the lower molecular weight oligomers have been investigated thoroughly, while the chemistry of the polymers still remains a difficult topic. Shikimate-derived phenolic like flavonoids and tannin are widely distributed in plant kingdom and are thus not of interest as classificatory tool; oxidation levels however are indicative in the attribution of evolutionary status among phyla and within each phylum. The main biological and pharmacological effects reported for condensed tannin can be classified into antibacterial and antiviral activities, enzyme inhibition, anti-oxidative effects, anti-mutagenic and anti-tumor properties, next to some more specific interactions e.g. with vascular and cardial systems and inflammation processes. Their anticipated interaction with biological systems originates in principle directly from the physical and chemical properties of the polyphenolic skeleton, although prominent individual differences have been observed.

PHYTOCHEMICAL STUDIES

Collection and Authentication

The plant specimen (Leaves and bark) for the proposed study was collected during the month of July 2010 from the garden of Vels university, Pallavaram, Chennai. It was identified and authenticated by Dr. P. Jayaraman, Director of Plant Anatomy research center (PARC), Tambaram, Chennai. A voucher specimen No. PARC /2010/594 has been deposited for further reference.

Extraction

The leaves and bark of *Psidium guajava* Linn were shade dried and coarsely powdered. About 300 gm of powdered drug was extracted with ethanol by cold maceration method after 72 hrs of maceration it was filtered. After complete extraction the extraction was concentrated by distilling off the solvent and then evaporated to dryness under reduced pressure using vacuum flash evaporator. Then it was extracted successively with solvents of increasing polarity such as petroleum ether, chloroform, and ethanol and aqueous it's to yield its fraction. All the fractions were evaporated under vacuum its color and consistencies were observed. Percentage yield was calculated on the air dried basis. The results were tabulated in **Tables 1-3.**

Table 1. The percentage yield of *Psidium guajava* leaves.

Tannin leaf fraction (TLF)	Percentage Yield (% w/w)	Colour	Consistency
Ethanol extract	9.8	Blackish Brown	Thick semi solid
Hexane fraction	0.90	Pale yellow	Greasy
Chloroform fraction	3.42	Green	Semisolid
Aqueous fraction	2.52	Brown	Greasy

Table 2. The percentage yield of *Psidium guajava* bark.

TANNIN BARK FRACTION	Percentage Yield (% w/w)	Colour	Consistency
ETHANOL EXTRACT	10.3	Blackish Brown	Thick semi solid
HEXANE FRACTION	0.88	Pale yellow	Greasy
CHLOROFORM FRACTION	4.42	Green	Semisolid
AQUEOUS FRACTION	3.52	Brown	Greasy

Table 3. Isolation of tannin fraction.

Extract/Fraction	Percentage Yield (% w/w)	Color	Consistency
Tannin leaf fraction	8.8	Light brown	Greasy
Tannin bark fraction	9.6	Brownish	Greasy

Phytochemical Screening

The leaf and bark tannin fractions were subjected to qualitative phytochemical test for identification of constituents. The results shown in **Tables 4-6.**

Chromatography [22-27]

Thin layer chromatography

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different components. Here the principles of separation are adsorption and partition. The stationary phase acts as an adsorbent. Depending on the particular type stationary phase, its preparation and use with different solvent can be achieved on the basis of partition or a combination of partition and adsorption.

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Table 4. Phytochemical Screening of *Psidium guajava* leaf.

Chemical Test	Petroleum ether fraction	Chloroform fraction	Aqueous fraction	Ethanol fraction
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	-	-	-	-
Flavonoid	-	-	+	+
Tannin	-	-	+	+
Terpenoids	-	+	-	+
Oil and fats	-	-	-	-
Steroids	+	+	-	+
(-) indicates absent; (+) indicates present				

Table 5. Phytochemical Screening of *Psidium guajava* bark.

Chemical Test	Petroleum ether fraction	Chloroform fraction	Aqueous fraction	Ethanol fraction
Alkaloids	-	-	-	-
Carbohydrates	-	-	-	-
Glycosides	-	-	-	-
Proteins	-	-	-	-
Amino acids	-	-	-	-
Saponins	+	-	-	+
Flavonoids	-	-	+	+
Tannin	-	-	+	+
Terpenoids	-	+	-	-
Oil and fats	-	-	-	-
Steroids	+	+	-	+
(+) indicates present; (-) indicates absent				

Table 6. Phytochemical Screening of *Psidium guajava* Leaf and Bark (Dried powder).

Chemical Test	Dried powder (Leaf)	Dried powder (Bark)
Alkaloids	-	-
Protein	-	-
Amino acids	-	-
Saponins	+	-
Flavonoids	+	+
Tannin	+	+
Terpenoids	-	-
Oil and fats	-	-

Table 7. Thin layer chromatography of leaf and bark fraction of *Psidium guajava* Linn.

S.NO	Test extract	Solvent system	Detecting agent	Number of spots	R_f value
1	Standard (Gallic acid)	Toluene: acetone :Glacial acetic acid(3:1:2)	5% $Fecl_3$	2	0.91
2	TLF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% $Fecl_3$	1	0.91
3	TBF	Toluene: acetone :Glacial acetic acid (3:1:2)	5% $Fecl_3$	1	0.89
R_f: Retardation factor, TLF: Tannin Leaf Fraction, TBF: Tannin Bark Fraction					

Preparation of the plates

The adsorbent used for thin layer chromatography was silica gel G, About 25 gm of silica gel G was taken in a glass mortar and about 35ml of distilled water was added to it. The mixture was stirred with glass rod until it become homogeneous and allowed to swell for 15 min. 15ml of distilled water was added to it with stirring. The suspension was then transferred to a 150 ml flask fitted with a stopper and was shaken vigorously for about 2 minutes. This suspension was then uniformly speeded immediately on thin layer chromatographic plates.

Drying and storage of plates

The freshly coated plates were then air dried and stacked in a drying rack and were heated in a oven for 30 min at 110˚C. Activated plates were kept in a desiccators, till required for further use.

Preparation of test sample

10 mg of test sample of TLF and TBF was dissolved in 5ml of 95% ethanol. 1µl was applied as a spot.

Preparation of standard sample

10 mg of Gallic acid (pure sample) was dissolved in 5 ml of ethanol. 1µl was applied as a spot.

Application of the sample

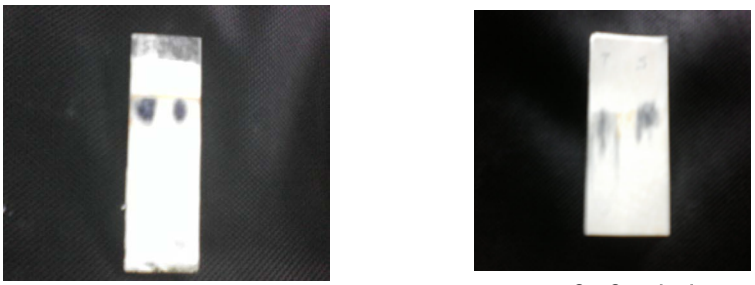
The samples was applied in the form of spot, the spot was applied with the help of fine capillaries. Spot was marked on the top of the plate for their identification.

Chromatographic chambers, conditions of saturation and the development of TLC plates

Chromatographic rectangular glass chamber was used in the experiments. To avoid insufficient chamber saturation and the undesirable edge effect, a smooth sheet of filter paper was placed in chromatographic chamber in U shape and was allowed to be soaked in the developing solvent. Having been moistened, the paper was then pressed against the walls of the chamber, so that it adhered to the wall. The experiment was carried out at room temperature in diffused day light.

Developing solvent system

A number of developing solvent systems were tried, but the satisfactory resolution was obtained in the solvent systems mentioned in the **Table 5.** After development of plates, they were air-dried and number of spots was noted and R_f Values were calculated. Spots were visualized by UV chamber after spraying the detecting agent. The results of TLC presented in **Table 7** and shown in **Figure 1.**



T -tannin leaf fraction (TLF) T- tannin bark fraction (TBF) S – Standard.

Figure 1. The results of TLC *Psidium guajava* Linn.

PHARMACOLOGICAL STUDIES

Anti-Bacterial Activity of Tannin Fraction of Leaf and Bark of Psidium guajavia

Psidium guajava leaves have been used as herbal medicine for the treatment of various human ailments such as wounds, ulcers, cholera, and coughs. They also possess antimicrobial, anti-inflammatory, analgesic, spasmolytic, antipyretic, antidiarrheal and anti-mutagenic activities [28-32].

Disc diffusion method

Staphylococcus aureus was incubated in sterile nutrient broth for 24 h at 37°C and adjusted to yield approximately 1.0 × 10^{-7} CFU/ml. A prepared inoculum was added to molten agar, mixed and poured over the surface of the nutrient agar medium in sterile petri dishes and left to solidify. A sterile paper discs 6 mm in diameter were impregnated with specified concentrations (25, 50, 75, 125 µl/disc) of *Psidium guajava* ethanolic extract and its TLF, TBF individually each with standard (Erythromycin 5 µl/disc) the discs were placed on the surface of agar plates. Following the same procedure, sterile discs were impregnated with specified concentrations (Erythromycin 5 µl/disc) were placed on the surface of agar plates. A disc without test material was used as control. The plates were left for 1hr at room temperature as a period of pre incubation diffusion to minimize the effects to variation in time between applications of the different solutions. The plates were incubated at 37°C for 24 h under aerobic conditions and observed for antibacterial activity. All disc diffusion tests were performed in four separate experiments and the antibacterial activity was expressed as the mean of inhibition diameters (mm). The results were reported in **Table 8 and Figure 2.**

Agar well diffusion method

The extract and its fractions were examined for their antimicrobial activities against the toothache bacteria named above using the micro dilution method described by Amsterdam (1996) with some modifications. Briefly, each tested compound was added into a microtiter plate containing appropriate broth to obtain the concentration ranging from 10 to 200 g/ml. The bacteria to be tested were added to the wells containing the compound to obtain a final concentration of 104 CFU/ml. A positive control (without tested compounds) and negative control (without tested bacteria) were included for each plate. After incubation at

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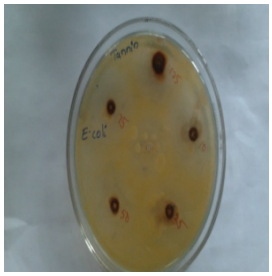
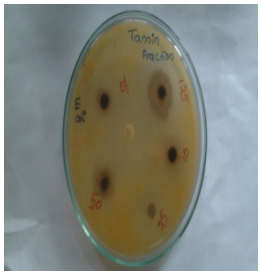
optimal temperature, bacterial growth was inspected at 24 h. The results were reported in **Table 9 and Figure 3.**

Table 8. Antibacterial activity of *Psidium guajava* and its leaf and bark tannin against *staphylococcus aureus.*

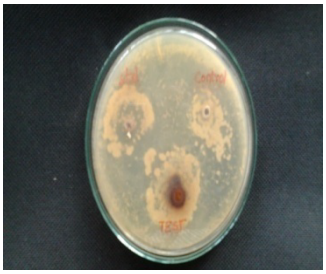
Fraction	Concentration (µg/disc)	Zone of Inhibition (in mm)
TLF	25	18
	50	20
	75	27
	125	29
TBF	25	16
	50	21
	75	23
	125	28
Standard	5	42



T -tannin leaf fraction (TLF)



T- tannin bark fraction (TBF)



Standard

Figure 2. The results of disc diffusion method.

Table 9. Antibacterial activity of *Psidium guajava* and its fractions against *staphylococcus aureus.*

Fraction	Concentration (µg/disc)	Zone of Inhibition (in mm)
TLF	25	19
	50	20
	75	23
	125	25
TBF	25	20
	50	23
	75	28
	125	32
Standard	5	42

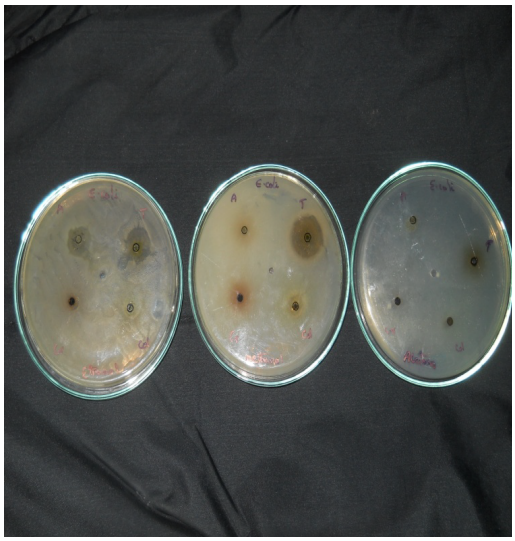


Figure 3. The results of Agar well diffusion method (1st) TLF: Tannin Leaf Fractiont; (2nd) bf: Tannin Bark Fraction; (3rd) S: Standard.

RESULTS

The present work covers study on anti-inflammatory and wound healing activity of the leaves and bark of *Psidium guajava* Linn.

Extraction

The successive extraction leaves of *Psidium guajava* Linn. was done in the order of increasing polarity i.e. Petroleum ether, Chloroform, ethanol and aqueous.

Phyto-Chemical Screening

The phytochemical test was carried out for the identification of various constituents. It answers positively for saponins, flavonoids, Phenolic compounds, tannin, terpenoids and steroids.

Chromatography (TLC) of Extract

The extracted compound identified compare with standard sample (Gallic acid) by TLC. It was performed using solvent system only glacial acetic acid (10 ml) with 5% ferric chloride ($fecl_3$) as detecting agent. The result observed in the R_f values of standard (0.93 and 0.87), tannin rich fraction R_f value (0.91 and 0.85) The results were reported in **Table 7.**

Anti-bacterial Activity

Disc diffusion method

The antibacterial effects of tannin leaf and bark of *Psidium guajava* and their formulation were evaluated by disc diffusion method against *staphylococcus aureus.* The various extracts and its showed antibacterial activity in concentration dependent manner. The results of all tested fraction and extracts were comparable with that of standard Erythromycin **(Table 8 and Figure 2)**.

Cup and Plate Method

Cup and plate method are presented in **Table 9 and Figure 3.**

DISCUSSION

The Ethnobotanical studies and folklore claiming reviewed that the leaves and bark of the plant *Psidium guajava* L., are used for wound healing, anti-inflammatory and antibacterial activities. The young leaves are used as tonic in the diseases of the digestive function and is said to be remedy for toothache. Tannin has a broad scale of biological activities among which anti-inflammatory and wound healing effects stands out. *Psidium guajava* Linn. Belongs to Myrtaceae, is a wide spread plant in India and commonly used as for antiseptic, anthelmintic, wound healing and in inflammatory conditions. It has a high content of tannin substances reviewed from literature.so the present work was focused to isolate tannin rich fraction and it was evaluated for anti-inflammatory and antibacterial activities. The tannin rich fraction of leaf and bark of the plant was formulated in the ointment form and studied for wound healing activity.

Phytochemical Study

Phytochemical screening was carried out to identify the phyto constituents present in the ethanolic extracts and its fraction. Phytochemical screening of tannin isolation shows the presence of tannin.

The total tannin content was estimated by using spectrophotometer method. The results indicates that the content of tannin was found to higher in the plant. TLC was done for the tannin fraction on support of the chemical test since it showed blue color spot with 5% ferric chloride, confirmed the presence of tannin. It was identified as gallic acid by comparing its R_f value with that of standard gallic acid.

Antibacterial Activity

Staphylococcus aureus, an aerobic organism, usually involves in superficial infections within the sebaceous unit. *Staphylococcus aureus* are the target sites of anti-acne drugs these factors provide a potential target for treatment.

The antibacterial effect of the tannin leaf and bark was evaluated by disc diffusion method against *staphylococcus aureus*. The significant activity was seen in leaf and bark was found to be in concentration dependent manner compared with control and the Zone of inhibition (mm) was found to be significant for Tannin leaf and bark.

CONCLUSION

From this study, it is concluded that *Psidium guajava* Linn. Leaf and bark tannin fraction have significant anti-bacterial models. It showed significant percentage wound protection at the tested concentration.

The anti-bacterial activity is probably due to the presence of tannin (gallic acid). Further studies need to be isolate individual tannin and explore its biological potency by various pre-clinical and clinical trials of the isolated compounds.

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