

Phytochemical Analysis, Anti-Bacterial, Anti-Oxidant, And Cytotoxic Potential of *Psidium Guajava* Leaves Extract

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ABSTRACT

There is a growing interest in the exploration, characterization, and isolation of bioactive substances originating from ethnomedicine. Pharmacotherapy of herbal medicine has been used for hundreds of years for the prevention, management, and treatment of different diseases. *Psidium guajava* leaf preparations have traditionally been used to treat a variety of diseases and disorders. Pharmacological research, both *in vitro* and *in vivo*, has been widely used to demonstrate the potential of leaf extracts for the co-treatment of various diseases with high prevalence worldwide, upholding traditional medicine in cases such as diabetes mellitus, cardiovascular diseases, cancer, and bacterial infections. The most important isolated compounds that show antibiotic activity are guajaverin and psidium acid. Furthermore, the bioactive content of the leaves, certain phytochemical subclasses, or even individual molecules have been linked to biological activity. Guava leaf phenolic chemicals have been linked to blood glucose regulation. Thus, the goal of this study was to consolidate data from *in vivo* investigations done with guava leaves, linking the effects to clinical applications, to focus future research on discovering particular bioactive components. Some dietary uses (guava tea and aquaculture additional feed) and *in vivo* outcomes

are also included. In antibacterial activity, the methanolic, n-hexane, and aqueous extract of *Psidium guajava* leaves have 6mm, 13mm, and 10mm ZOI against *E. coli*. The same extract has 10mm, 12mm, and 15mm ZOI against *S. aureus*. The antioxidant activity was calculated by the DPPH Scavenging assay. The %DPPH activity obtained from *Psidium guajava* leaves extract was in the range of 19-70%. Maximum activity was shown by the methanolic extract, i.e., 70.219%. The phytochemical analysis showed the presence of a significant amount of total phenolic content in methanolic extract (347.81mg GAE/g), and total flavonoid content showed the highest content in the aqueous extract (365.833mgCE/g). FTIR showed that it contains alcohol, aldehyde, carboxylic acid, alkanes, and alkenes.

Keywords: *Psidium guajava*, antibacterial, antioxidant, bioactive compounds, phytochemicals.

INTRODUCTION

Ethnomedicine, or the use of conventional medical practice, is ingrained in the traditional medicine and conception of health of native cultures all over the world [Anyinam, 1995]. Traditional Chinese practice and Indian Ayurvedic medicine are two examples of the longest-lasting home medicines still in use. These systems seek to enhance health and quality of life through therapies that emphasise the use of natural remedies that can be found nearby [Patwardhan, 2005]. Because plants have historically been utilized as herbal treatments, many techniques are currently being employed to discover new bioactive compounds [Fabricant et al., 2001]. A little tree belonging to the Myrtaceae family of plants is the guava, or *Psidium guajava* L.. Guava trees have been planted in numerous additional countries with tropical and subtropical climates, enabling global production [Salazar, 2006]. Tropical locations from southern Mexico to northern South America are home to them. The leaves have historically been prepared and utilized in traditional medical practice in different nations, generally to treat diarrhea. On every continent barring Europe, numerous additional uses have also been documented [Shruthi et al., 2013, Morais-Braga et al., 2016]. According to the condition, the medication may be either topically or orally. Infusion, decoction and boiled formulations are frequently taken in Pakistan, India, Bangladesh and China to treat a number of illnesses, such as rheumatism, diarrhea, diabetes mellitus, and cough. [Sanda et al., 2011], Poultice is

administered externally for cutaneous and wound treatments in Brazil, Nigeria, the Philippines, and Mexico, whereas decoction is used as a gargle for mouth ulcers in Southeast Asia and in Nigeria as an anti-bacterial agent. Guava fruits have a light yet nice smell and are juicy and sweet. The fruit is rich in vitamins A,C, B3, B4, calcium, phosphorus, iron, fibre, protein, and other minerals. On average one guava fruit has over fourfold the vitamin C comparable to an orange and a generally wide spectrum of vital elements. [Begum et al., 2002]. However, different guava cultivars have different nutrient contents. One serving (90 mg) of the strawberry guava (*P. littorale* var. *cattleianum*) provides 100% of the Dietary Reference Intake for Adult Males, although having around 25% less vitamin C than more popular varieties. Guava has a reasonably high potential antioxidant value since it contains carotenoids and polyphenols such as (+)allocatechin. [Begum et al., 2002] guaijaverin, leucocyanidin, and amritoside. [Ghosh et al., 2010], which are the main antioxidant pigment families, among plant-based diets. Red-orange guavas have a larger concentration of pigments in the form of polyphenols, carotenoids, and provitamin A, sources of retinoids, than yellow-orange guavas since these pigments determine the colour of the fruit's skin and flesh. Traditional medicine use both the bark and the guava plant's leaves for their alleged antibacterial properties and as a treatment for diarrhoea. Traditional diabetic remedies include the use of guava leaves or bark. A prepared tea from fresh leaves of guava is utilized to treat dysentery, diarrhea, fever in Trinidad. [Chiwororo et al., 2008]. Guava contains large amounts of vitamins, phenols, tannins, flavonoids, triterpenes, saponins, fatty acids, carotenoids, fibre, lectins and essential oils. Guavas have greater content of vitamin C (80 milligram per 100 gram) and contain significant quantity of vitamin A compared to citrus fruits. Guavas are also rich in pectin, a type of dietary fibre. Guava leaves contain large amounts of the flavonoid quercetin.

The majority of guava's therapeutic effects are brought on by these flavonoids. There is evidence that flavonoids have antibacterial properties. According to some research, quercetin helps guava's antidiarrheal properties by calming intestinal smooth muscle and preventing intestinal spasms. Guava leaves also contain other flavonoids and triterpenes that have antispasmodic properties. Guava's polyphenol content in the leaves gives it antioxidant properties as well.

[Begum et al., 2002]. The study was designed for the structural comparison and the characterization of *Psidium guajava* for improve bioactives. The study aimed to assess the anti bacterial, cytotoxic activities and anti oxidant activities of *Psidium guajava* leaves extract.

MATERIAL AND METHODOLOGY

The study was designed for the structural comparison and the characterization of *Psidium guajava* for improve bioactives

Collection and Identification of Leaves:

Psidium guajava fresh leaves were collected from a mature plant available in local area of Faisalabad, Pakistan, identified and certified by the Department of Botany, Government College University Faisalabad.

Drying of Leaves

To make the leaves free from dirt and dust the leaves were rinsed by tap water. The washed leaves were shade dried for almost a week.

Grinding of Leaves

The leaves were ground and the Powder was formed in an electrical blender and leaves powder was stored in an airtight jar for further use.

Extraction Preparation

The Powder leaves were soaked with 1: 10 overnight. For the preparation of solutions three different solvents, methanol, n- hexane and water were used. The solutions were prepared by the ratio of 20 gram/ 250 ml, 20 gram/ 250 ml and 20 gram/ 200 ml of n-hexane, water and methanol respectively.

Filtration

All the three solutions prepared were filtered firstly by using the procelain cloth and then the filtrate was refiltered by using whatman No 1 filter paper.

Drying of Extract

To dry the extract they were placed in the water bath. The aqueous solution took almost 4 days for drying while the methanol and hexane solutions were dried within 24 hours. By using the sterilize blades the extracts were scratched and were stored in eppendrofs under control temperature for further use.

Characterization by FTIR

FTIR Spectroscopy

FTIR spectra were captured between 4000 and 800 cm^{-1} using a Perkin Elmer PE2000 spectrometer outfitted with thermal deuterated triglycine sulphate (DTGS) detectors and potassium bromide (KBr) beam splitters. The single beam spectrum's resolution was found to be 4 cm^{-1} . FTIR measurements were made in a subfreezing condition at 21.0 °C. We employed a water reference spectrum for the bacterial pellets and a sample reference spectrum for the biofilm monitoring tests.

To lower the spectral background, the spectrum that was taken right before the phase under study was used. Water vapour removal and baseline correction were both carried out. The spectra were saved, processed, and saved using Perkin Elmer Spectrum 5.0.1 software. The spectra of bacteria at various growth phases were gathered using a nine-reflex diamond ATR device (DurasampliIRTM, SensIR Technologies). The crystal's refractive index was 2.4, and the angle of incidence was 45°. The PE2000 spectrometer was used to obtain the data, which required 20 analyses per spectrum and a 4-minute accumulation period.

Enzymatic anti oxidant evaluation of *Psidium guajava*

Catalyse CAT and peroxidase POD Activities

Chance and Maehly methods were utilized to calculate catalase and peroxides levels. The given ingredients were added to 1 ml of reaction mixture for about 3 minutes. 100 μl of enzyme extract 50 mM of phosphate buffer (pH7), 20 mM 2-Methoxy and 40 mM hydrogen peroxide. The catalyst reaction solution contains about 50 mM phosphate buffer, 5.9 mM H_2O_2 and 100 μl extract of enzyme. One unit of POD corresponds to unit/ μg of protein.

Protease Specific Activity

The casein digestion technique was utilized to calculate the PRT specific activity. The material extracted was centrifuged for about 5 minutes at 14000 rpm and 4 degree centigrade. The supernatant (0.5 ml) and 2 ml of 1% solution were mixed after incubating for 30 minutes. After half an hour of incubation the mixture was stopped and 2 ml of 10% TCA was added in the solution. The reaction mixture was again centrifuged for 5 minutes at 10000 rpm under same temperature. The protein units per mg show the prt specific activity. For the determination of protein

concentration bradford method was used and as a reference Bovine serum albumin was used.

Superoxide Dismutase (SOD)

By using Giannopolitis and Dies method, the superoxide dismutase activity was determine by observing the photochemical reduction of nitro blue tetrazolium that was inhibited. 222 mg of methionine and 0.0375 mL of the Titan reaction mixture are used for SOD. 350 mol m⁻² s⁻¹ light was applied to the reaction mixture for 15 minutes. One unit of SOD was defined as the quantity of enzyme needed to block nitro blue tetrazolium reduction by 50% when detected at 560 nm.

Antioxidant evaluation by DPPH, TPC and TFC assay

DPPH Scavenging Activity

The antioxidant capacity of plant extracts was assessed using the DPPH experiment, which uses 2,2-diphenyl-1-picrylhydrazyl to scavenge free radicals. For this technique, 3 mL of plant extract changed into blended with 1 mL of freshly organized 0.004% DPPH in methanol answer. The mixture became then left within the dark for 30 min. Next, the absorbance at 517 nm became detected. Free radicals can be successfully scavenged by reaction combinations with limited absorption.

As a point of comparison, the antioxidant activity of BHT and ascorbic acid was also looked at. A solution devoid of plant extract was applied as a rescue. Each test came in three copies. The DPPH radical sample samples' % inhibition was calculated using the formula below:

$$\text{DPPH Inhibition (\%)} = \frac{\text{Blank absorbance (A}_0\text{)} - \text{Sample absorbance (A}_1\text{)}}{\text{Blank absorbance (A}_0\text{)}} * 100$$

Where:

A₁ = Absorbance of sample.

A₀ = Absorbance of blank.

From the percent inhibition vs sample concentration plot, a 50% inhibition (IC₅₀) was determined. The experiments were done in duplicate. The positive control used was ascorbic acid.

TPC (Total Phenolic Content)

The Folin-Ciocalteu technique was used to compute the total phenolic gratified of the methanolic plant extract. Gallic acid at a variety of concentrations turned into

used to generate a calibration curve. At values of 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, and 0.10 mg/ml, gallic acid turned into dissolved in methanol. To 5 mL, Folin-Ciocalteu reagent was diluted tenfold, and 4 mL of 20% sodium carbonate was additionally delivered. After one hour of obtaining absorbance at 765 nm, a widespread curve was plotted the use of absorbance versus attention. The equal reagent as indicated earlier is mixed with one ml of plant extract (0.001 g/ml) and after one hour, the absorbance of the ensuing blue complex at 765 nm is measured. Each end result turned into reached three times. A quantitative analysis changed into finished the usage of gallic acid as a general. Using the following method, the overall quantity of phenolic compounds present in plant extracts become determined as gallic acid equivalents (GAE).

$$T = C \times V / M$$

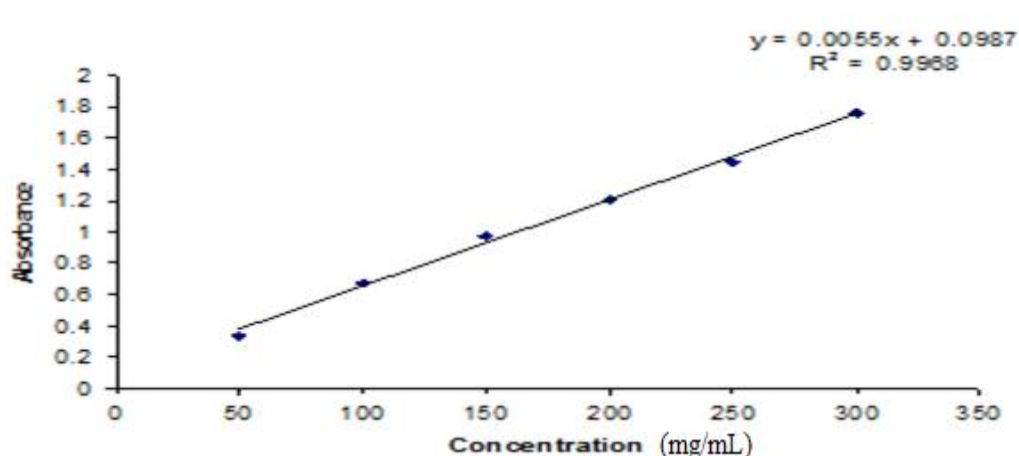
Where,

T = total contents of phenolic compound in milligram GAE/gram of *P. guajava* extract.

C = the concentration of gallic acid calculated from calibration curve in milligram/milliliter.

V = the volume of the extract in milliliter.

M = the weight of the *C.roseus* extract in grams.

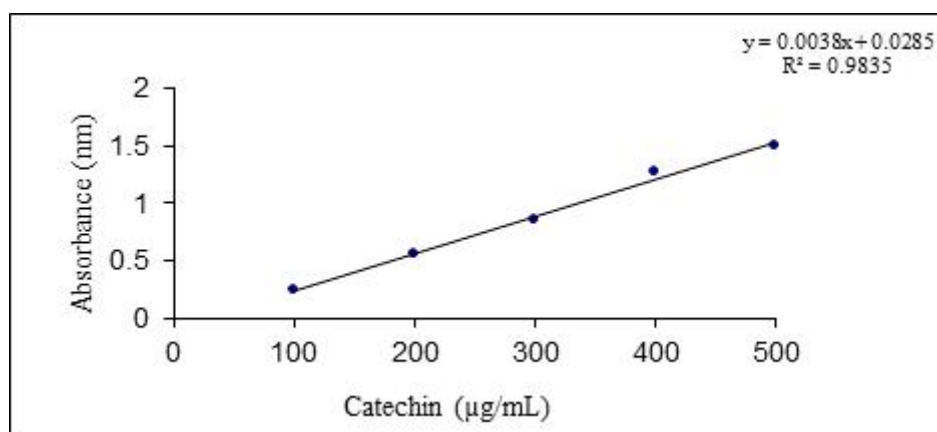


Standard curve of Gallic acid

TFC (Total Flavonoids Contents)

Using the method described in the referenced article, the total flavonoid content of the plant extract was determined. In a nutshell, the following ingredients were

mixed together: 0.5 milliliter's of plant extract, 0.15 milliliter's of 5% NaNO_2 solution, and 2 milliliter's of distilled water. The combination was then incubated for a further six minutes. After another 6 minutes of incubation, 0.15 milliliters of a 10% AlCl_3 solution and a 4% NaOH solution were added to the combination. Methanol was added to the reaction aggregate and blended nicely to increase the extent to five mL. After 15 min of incubation, the absorbance of the reaction aggregate at 510 nm changed into measured. A catechin linear regression curve turned into used to symbolize the entire flavonoid content material (TFC) of the extract as microgram equivalents of catechin according to milliliter of plant extract.



Standard Curve of Catechin

Antibacterial Activity by Agar Well Diffusion Assay

The agar-well diffusion method was used to test the anti bacterial activity of plants extract against two bacterial strains. *E. coli* and *S. aureus* were two of the strains. Bacterial isolates were initially cultivated in nutritive broth for 18 hours after being standardized to the 0.5 McFarland standard (10^6 cfu mL^{-1}) before use. The 0.5 McFarland standards were produced by mixing 0.5 mL of 0.048 M BaCl_2 with 99.5 mL of 0.36 N H_2SO_4 . In a test tube with a screw cover, the barium sulphate turbidity standard (4–6 mL) was measured.

Nutrient agar (MERCK) was dissolved in 100 ml of distilled water with a pH of 7.0 to create the nutrient agar medium. The medium was then autoclave-sterilized. Then, let it to cool to 45 $^{\circ}\text{C}$. 75 millilitres of nutritious agar with seeds were placed in Petri dishes, and they were let to settle. Wells were drilled into the agar using a sterilised cork hole punch with a 6 mm diameter. 100 microliters of the chemical

were added, which was then incubated at 37 °C after standing at room temperature for around two hours. For using several solvents to fill the wells, parallel controllers were set up. Plates have been tested for inhibition zones after 24 hours. Penicillin turned into used at a attention of 1 mg/ml to examine the consequences. Using the presented method, the relative percent inhibition of the test chemical as compared to the nice control became determined.

MIC Assay by Microtiter Plate Method

Medium: This test was conducted using iso-sensitive medium because it has a pH range. Mueller Hinton medium is advised by NCCLS for susceptibility testing. When using the iso-sensitive medium, most of the tested strains of bacteria.

Use of Standardised Bacterial Colony Numbers

Because this technique is operator-pushed and therefore subjective, it is not able to offer a fashionable quantity of CFU for all traces while evaluating turbidity to McFarland's rule, that's generally 0.5. Furthermore, considering the fact that extraordinary bacterial species have distinct optical densities, it's miles hard to evaluate them. Thus, a series of graphs representing the killing/viability curve of every bacterial stress become built to guarantee that a regular quantity of bacteria turned into usually used. For this test, a Wnal concentration of 5×10^5 cfu/mL was used. Thus, different bacterial species and different strains may be compared.

Preparation of Bacterial Culture

One colony was aseptically transferred to broth container that held 100 ml, sealed, and kept at 35°C overnight in the incubator. A clean sample of bacteria was created using a centrifuge, aseptic preparation, and 12 to 18 hours of incubation. Using proper aseptic procedures, the broth was spun for 5 minutes at 4000 rpm in a centrifuge. The supernatant was poured into the "contaminated waste" beaker. The pellet was spun once more at 4000 rpm for 5 minutes after being suspended in 20 ml of sterile normal saline. The supernatant was clarified by repeating this process until it was successful. In 20 ml of sterile normal saline, the pellet changed into then diagnosed as Bs and suspended. Using the necessary sterilization procedure, the optical density of Bs became periodically diluted until it ranged from 0.5 to at least 1.0 at 500 nm. Based on the viability graph, the actual variety of colony forming gadgets changed into calculated. To reap a cfu/mL attention, a favored dilution

factor of 5×10^6 is observed and implemented.

Preparation of Resazurin Solution

One 270 mg tablet was dissolved in 40 ml of sterile distilled water to create the resazurin solution. A vortex mixer was used to create a homogenous, thoroughly dissolved solution.

Preparation of the Plates

Aseptic techniques were used to prepare the plates. A label was on a 96-well sterile plate. Pipetting was done into the first row of the plate with 100 microliters of the test substance, Which normally has a attention of one mg/ml for pure compounds and 10 mg/ml for crude extracts. All different wells acquired 50 microliters of nutrient broth or regular saline. Serial dilutions were accomplished using a multichannel pipette. After discarding the hints after use, every nicely contained 50 microliters of the take a look at chemical in successive small quantities. Each properly obtained 10 liters of reassuring indicator answer.

A pipette turned into used to feature 30 microliters of is sensitized broth of 3.3x concentration to each well so that the final extent become the nutrient broth attention. 10 microliters of bacterial suspension (5×10^6 cfu/ml) become introduced to every properly to reap a concentration of 5×10^5 cfu/ml. To protect the microorganism from drying, hang movie changed into located gently on each plate. A column of a broad-spectrum antibiotic, typically serially diluted, such ciprofloxacin, served as a positive control and was present on each plate. There are two columns: one with all solutions but the test chemical, and the other with all solutions but the bacterial solution removed and an additional 10 microliters of nutritional broth.

Plates have been made in triplicate and incubated in an incubator at 37 °C for 18–24 h. The colour alternate turned into then visually assessed. Any trade from crimson to purple or colorless become found properly. The MIC value became detected as the lowest concentration at which a coloration alternate passed off. By averaging the three outcomes, the MIC for the check substance and bacterial stress became diagnosed.

Hemolytic Activity

The University of Agriculture, Faisalabad, Pakistan's Department of Clinical

Medicine and Surgery provided three ml of freshly collected, heparinized cow blood. The blood was centrifuged for five minutes at 1,000 x g, and then the cells were washed three times with 5 ml of cooled, sterile, isotonic phosphate-buffered saline (PBS), pH 7.4. 108 erythrocytes per millilitre were maintained for each test. Human cells (108 cells/ml) were added to 100 microliters of each chemical separately. The samples were shook after 10 minutes of incubation at 37 degrees Celsius. After being incubated for 5 minutes, samples were immediately put on ice before being centrifuged for 5 minutes at 1000 x g. Each tube's supernatant was taken out, and 100 litres of it were diluted ten times with cooled (40°C) PBS. Phosphate-buffered saline (PBS) was used as a negative control, while Triton X-100 (0.1% v/v) was utilized as a positive control. Using micro Quant, absorbance at 576 nanometers was measured. Each sample's percentage of red blood cell lysis was calculated.

Statistical Analysis

By using one way ANOVA the data will be arranged.

RESULTS

Present study has been designed to determine the various properties of *Psidium guajava*'s leaves extract. Leaves contain a variety of secondary metabolites including flavonoids, phenols, indole alkaloids but majority of secondary metabolites are Phenolic and flavonoids. To rule out organic substances, Fourier transform infrared spectroscopy (FTIR) was performed. This plant has important constituents such phenols and flavonoids, which make it an antioxidant and antibacterial agent. The DPPH radical scavenging assay and estimates of the total phenolic and total flavonoid content in the extract were used to assess the plant's antioxidant activity. Other anti oxidant activity include fresh that leaves had also be determined by CAT (Catalyse and Peroxidase activity), PRT (Protease Specific Activity) and SOD (Superoxidase Dismutase). Anti-bacterial activity had been determined by agar well diffusion method. Furthermore, hemolytic activity of this plant was also evaluated. Methanolic, n-hexane and aquoes extracts of *Psidium guajava* was used for study. All the results have been mentioned below:

Percentage Yield of the Extract

The percentage yield of the extract was determined by using the formula

$$\% \text{ yield} = \text{net weight} / \text{weight of sample} * 100$$

Net weight of *n*-hexane extract was 1.46g and that of methanol was 2.34g while 0.67g of aqueous extract was obtained.

%age yield of Extracts

Solvent	% yield
<i>n</i> -hexane	7.3%
Methanol	11.7%
Water	3.35%

The highest percentage yield was obtained in methanolic extract.

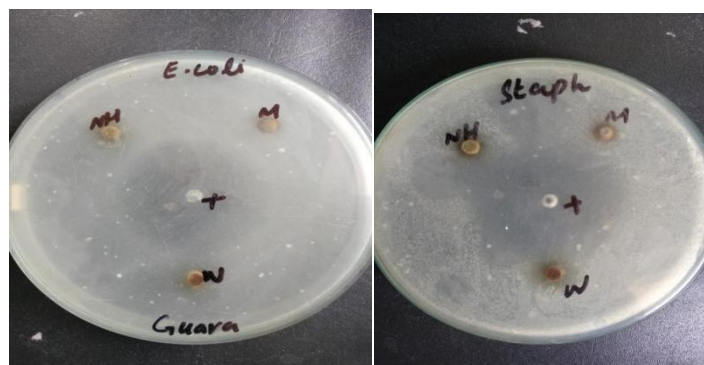
Antibacterial Activity

The antibacterial activity of *Psidium guajava* become estimated by the presence and diameter of the zone of inhibition by the usage of the agar well diffusion technique. The activity was performed by using Gram-positive and Gram-negative stains of *E. Coli* and *S.Aureus*. Ciprofloxacin was used as a control. The zone of inhibition of different extracts of *Psidium guajava* against gram +ve and gram -ve bacteria was observed that shown in table.

Measurement of Zone of Inhibition

Extracts	Zone of inhibition against <i>E. coli</i>	Zone of inhibition against <i>S. aureus</i>
Methanolic	6mm	10mm
N-hexane	13mm	12mm
Aquoes	10mm	15mm

The n-hexane extract shows the highest antibacterial activity against *E.coli* while the aquoes extract shows highest activity against *S.aureus*.



Zone of inhibition against E. coli & S. aureus

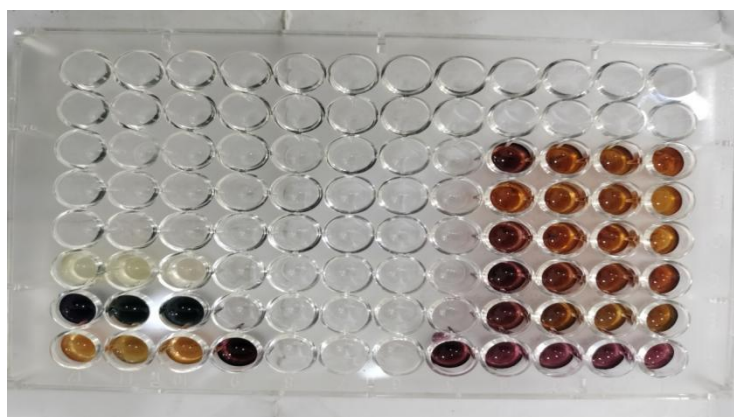
Antioxidant Activity

The substances or agents known as antioxidants have the power to scavenge ROS or free radicals. Natural antioxidants found in medicinal plants contribute to a live body's increased antioxidant capacity. Antioxidants can also be found in plants. Antioxidants derived from medicinal plants have been shown to be helpful for treating wounds brought on by the formation of free radicals and oxidative stress. When the amount of reactive oxygen species rises, they speed up the ageing process and harm human cells, which are the root causes of many diseases including cancer, arthritis, and neurological degeneration.

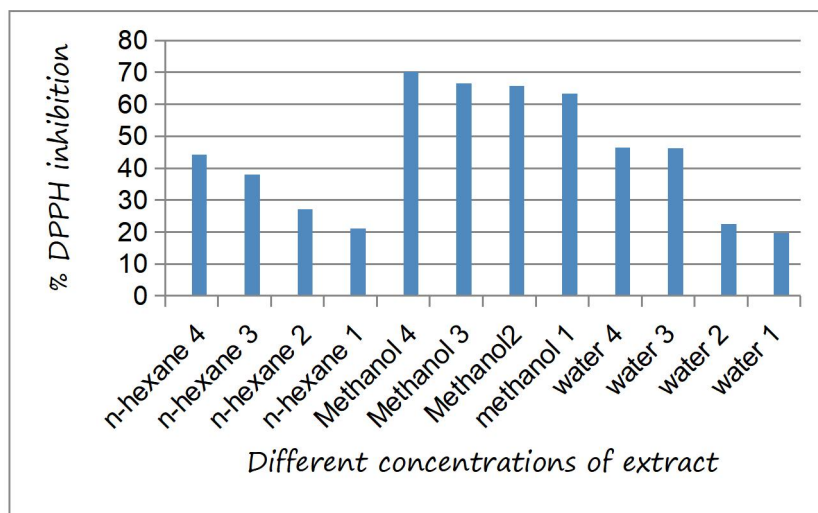
These elements aggressively combat the negative effects brought on by excessive reactive oxygen species. In the current study, we assessed the antioxidant activity of examined plant extracts made in ethanolic solvent at different concentrations due to the significance of natural antioxidants.

DPPH Assay

The antioxidant properties of the plant samples were examined using the DPPH technique, and the percentage-based scavenging activity of the DPPH in the plant sample was compared. The outcome of this test demonstrates that *Psidium guajava* leaves extract has the ability to neutralize free radicals in the body. Because components present in leaves extract react with the DPPH free radicals. This activity is shown by the plant due to presence of alkaloids, phenols and flavonoids. Graph displays the outcomes of DPPH inhibition. These findings demonstrate how powerful an antioxidant this plant is:



DPPH Scavenging Assay



%age DPPH Inhibition

The %DPPH activity obtained from *Psidium guajava* leaves extract was in the range of 19-70%. maximum activity was shown by methanolic extract i.e. , 70.219%.

Anti-Oxidant Enzymes

Catalase Activity (CAT)

Catalase (CAT) was used to assess the amount of H_2O_2 that the enzyme digested and turned into H_2O and O_2 . The outcomes of this activity were expressed as M H_2O_2 digested per minute/mg protein. Catalase has been proposed as an essential mechanism for the breakdown of H_2O_2 , which must be eliminated quickly due to its toxicity [Halliwell *et al.*, 2015].

Superoxidase Dismutase (SOD) Activity

The enzyme superoxide dismutase (SOD) catalyses the transformation of single electron reduced molecular oxygen species into oxygen and hydrogen peroxide [R. Noor *et al.*, 2002]. The first line of defense against oxidative damage and a defense against the toxicity of dioxygen is superoxide dismutase [Fridovich, 1989].

Peroxidase (POD) Activity

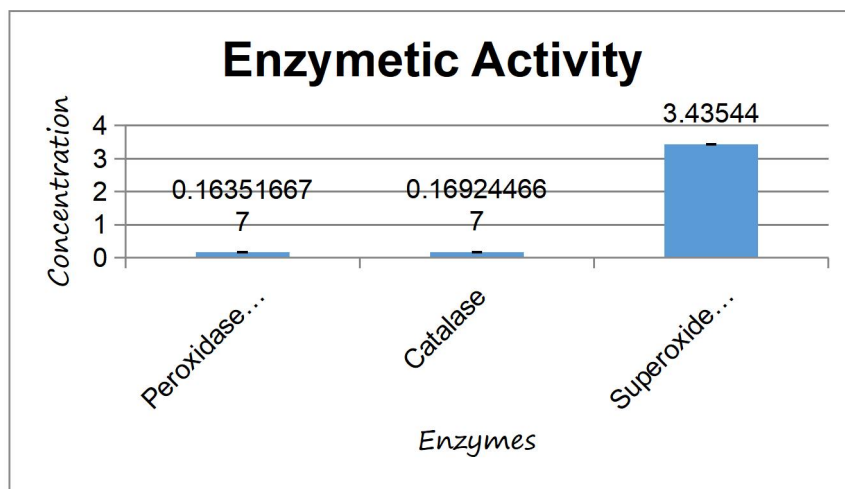
Plants and certain animal cells produce peroxidase (POD). Numerous organic compounds, such as amines (0-phenylenediamineD, /?-phenylenediamine2), benzidine3), phenols (pyrogallol4), guaiacol5), 0-cresol(7), hydroquinone(8), and leucodyes (leuco-malachite green(1)), are oxidised by the enzyme using hydrogen peroxide. Along with other oxidizable substances, ascorbic acid can also function as a hydrogen donor.

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Graphical Representation Anti Oxidant Enzymetic Activity

The superoxide dismutase showed the highest activity.

Total Phenolic Content (TPC)

The roughly 8000 naturally occurring phenols all have the same common structure, which is made up of an aromatic hydroxyl nucleus. Plant phenolics have traditionally been one of the key chemical classes that operate as significant antioxidants or free radical scavengers. As reducing agents, hydrogen atom donors, and singlet oxygen scavengers, plant polyphenols perform well.

Therefore, it makes sense to determine their overall presence in the different extracts of *Psidium guajava*. Numerous techniques for determining total phenolic have been developed as a result of the potential for interference from other easily oxidized compounds in plant materials and the heterogeneity of natural phenolic. Folin-Ciocalteu approach has been shown to be preferred to the others in the majority of instances [Hatamiet al., 2014].

Psidium guajava leaves showed antioxidant activity. The antioxidant effect of phenolic compounds may be without delay encouraged by way of the extent of phenols found. The outcome indicates that methanol extract has demonstrated greater antioxidant activity than *n*-hexane and distilled water.

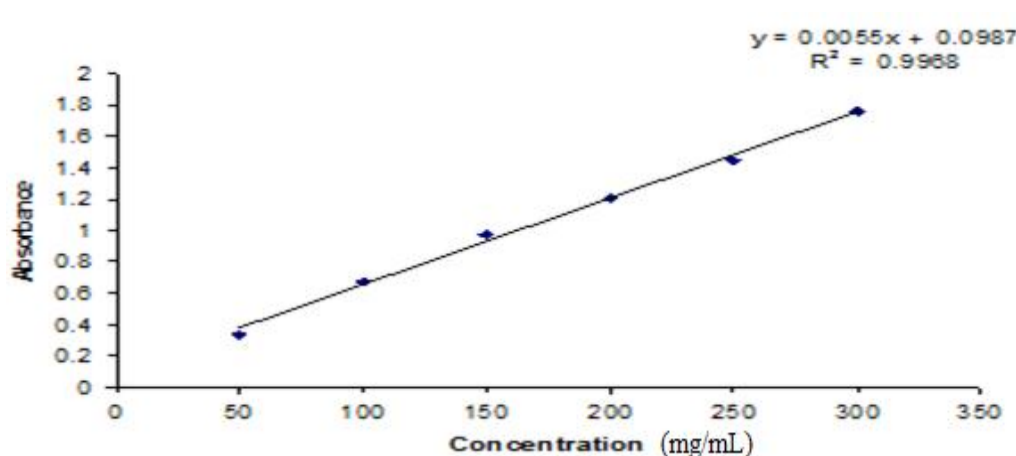
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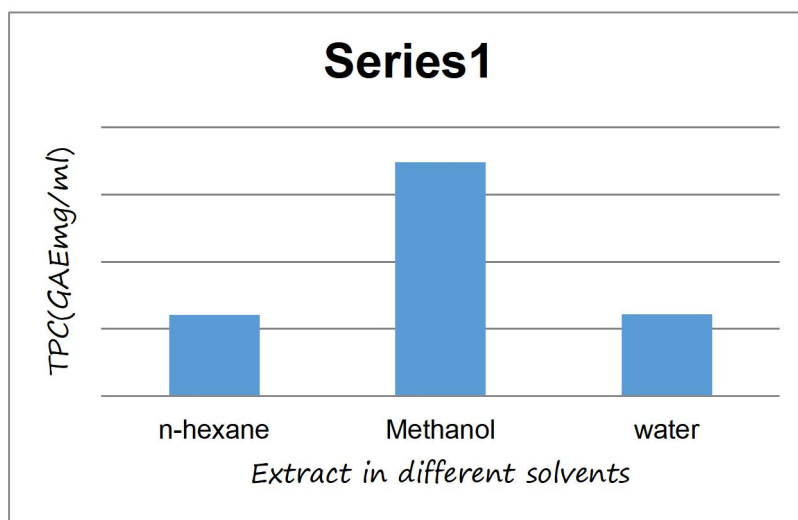
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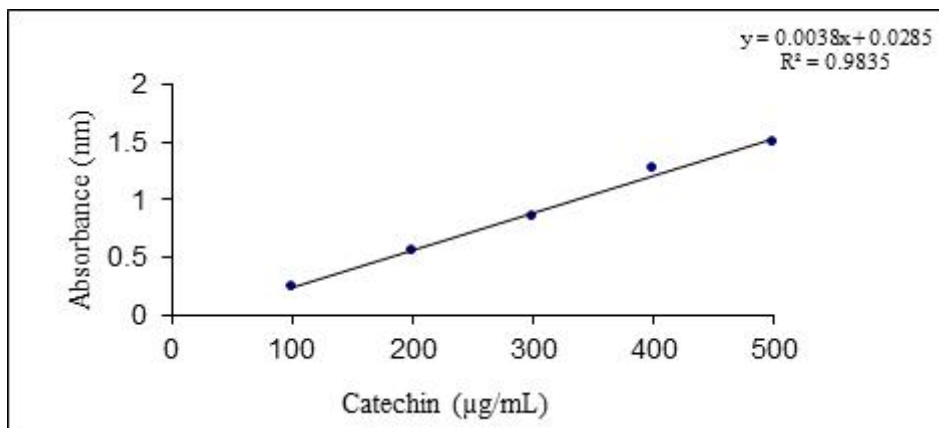
Standard Curve Of Gallic Acid



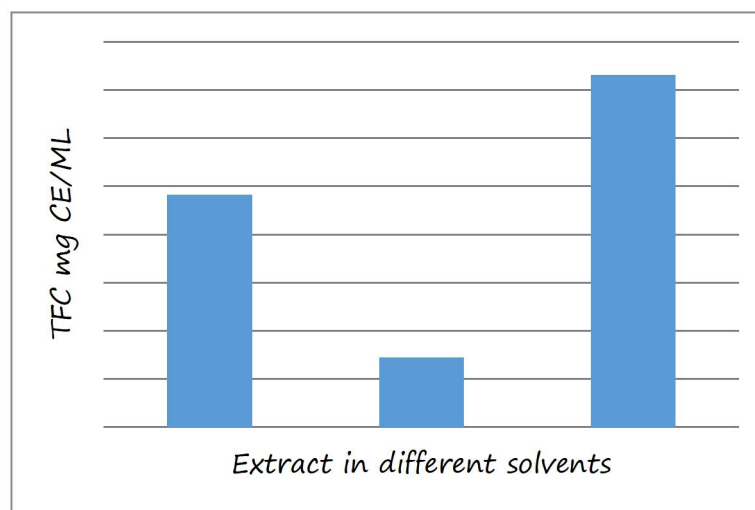
Graphical Representation of TPC

Total Flavonoids Content (TFC)

The total flavonoids content of the *Psidium guajava* extraction was examined. Comparing methanol to *n*-hexane and distilled water, the outcome demonstrates that the aqueous extract is a better antioxidant agent. During this research, *Psidium guajava* leaves have shown exceptional antioxidant activity. As a result, the pharmaceutical industry has been using *P. guajava* in the treatment of various reactive oxygen species oxidation activity.



Standard Curve Of Catechin



Graphical Representation of TFC

Cytotoxicity through Hemolytic Activity

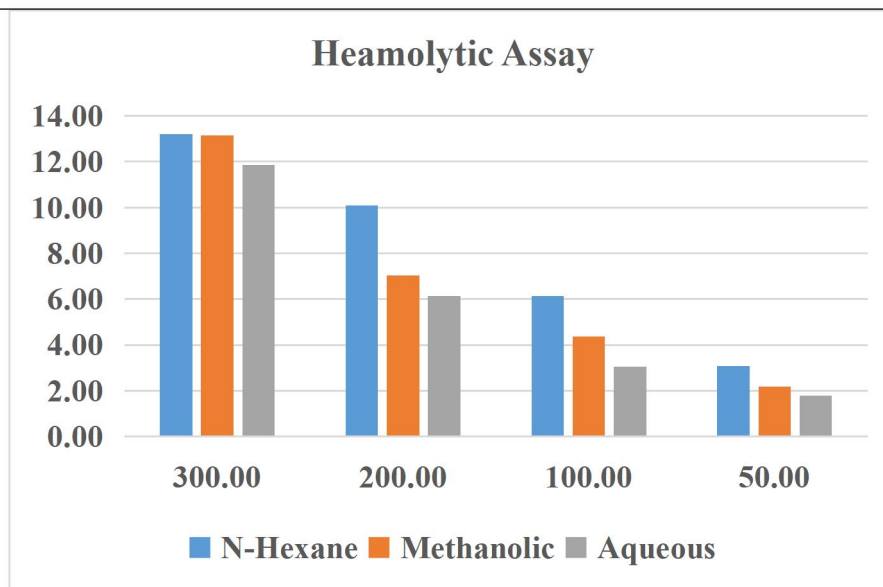
A bacterium or molecules hemolytic activity refers to its capacity to break down red blood cells and release hemoglobin as a result. The hemolytic interest of the plant is presented because the suggest trendy deviation of three replicates and expressed as percent hemolysis. The n-hexane, methanolic and aquoes extract of *Psidium guajava* at different concentrations were tested for their hemolytic activity against healthy human erythrocytes. Human erythrocytes were slightly to hardly hemolysed by extracts. The total hemolysis of *Psidium guajava* leaves extract wad obtained using Triton X-100 (positive control) and phosphate buffer saline (negative control).

The results revealed that the hemolytic activity of n-hexane extract according to the dilutions 50µl, 100µl, 200µl, 300µl were 3.08, 6.15, 10.09, 13.20. The hemolytic

activity of methanol extract according to the dilutions 50µl, 100µl, 200µl, 300µl were 2.17, 4.38, 7.02, 13.16. The hemolytic activity of aqueous extract with the dilutions 50µl, 100µl, 200µl, 300µl were 11.86, 6.14, 3.96, and 1.35.

Hemolytic Activity

Plant extracts	Concentra- tions µl	Negative Control	Positive Control Absorbance	Final cytotoxic %
<i>Psidium</i>	50	0.009	0.228	3.08
<i>guajavan-</i>	100	0.009	0.228	6.15
hexane extract	200	0.009	0.228	10.09
	300	0.009	0.228	13.20
<i>Psidium</i>	50	0.009	0.228	2.17
<i>guajava</i>	100	0.009	0.228	4.38
methanolic	200	0.009	0.228	7.02
extract	300	0.009	0.228	13.16
<i>Psidium</i>	50	0.009	0.228	11.86
<i>guajava</i> aqueous	100	0.009	0.228	6.14
extract	200	0.009	0.228	3.96
	300	0.009	0.228	1.35



Hemolytic Activity

Fourier-Transform Infrared Spectroscopy (FTIR)

Fourier rework infrared spectroscopy is usually used to discover diverse functional groups in chemical compounds that may be responsible for their pharmacological activities. Stretching vibrations of single bonds fashioned by using hydrogen and different atoms along with O-H, N-H, and C-H have been accountable for the absorption peaks in the range of four,000 to 2,500/cm. The peaks at 2500 to 2000/cm probed the absorption of light because of triple bonds, which include CC, CN. Peaks from 2000 to 1500/cm suggest the presence of compounds with double bonds, including C=C, C=O; And the absorption peaks consisted of many complicated bands from 1500 to 400/cm and become known as the fingerprint region due to the fact the spectrum of every compound became specific to it.

FTIR analyses of selected medicinal plant revealed absorption peaks at 2,500–4,000/cm, which indicated the presence of both intra- and intermolecular hydrogen bonding. The asymmetrically stretched vibration of C-H was thought to be responsible for the absorption peak at 2929.4/cm. The symmetrically stretched vibration of C=O was identified as the source of the absorption peak at 1155/cm, 1183/cm, 1731/cm, 1716/cm, 1684/cm. The stretching vibration of C=N and C=C may be connected to the absorption peaks at 1604./cm. Strong to medium vibration of the C=C and N=O functional groups may be responsible for the peaks at 1400 to 1500/cm. The asymmetrically stretched vibration of C-O was thought to be responsible for the peaks at 1155/cm, 1183/cm, 1101/cm, 1198/cm.

The findings of our research represented that selected medicinal plants contained wide range of function groups which ultimately represented the presence of various active phytochemicals in the selected herbs. The major purpose of FTIR was to explore the active functional groups.

Different functional groups, possible phytochemical constituents identified using FTIR and spectrograph representing possible functional groups and potential phytochemical components in n-hexane, methanolic and aqueous extract of studied medicinal plants are described below:

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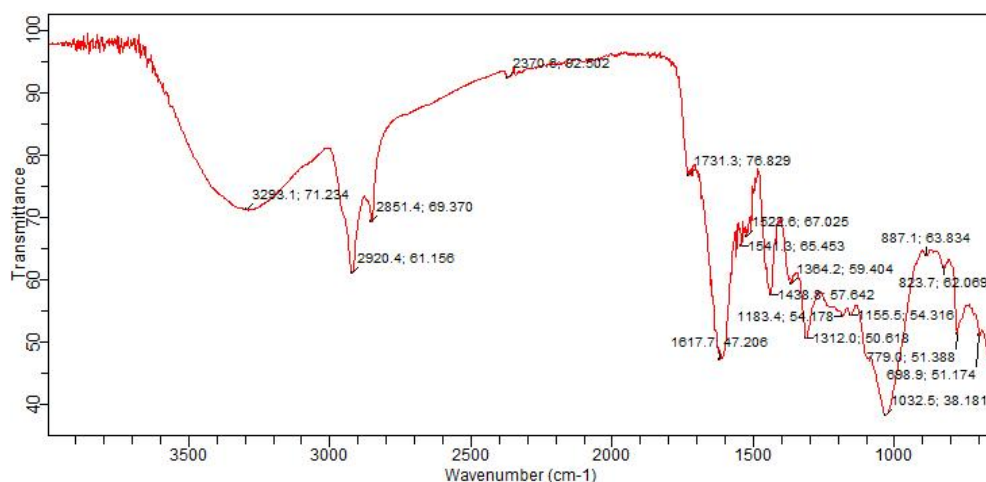
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Peak Frequency of N-hexane Extract

Peak Frequency Obtained from n-Hexane

Peak Frequency	Group	Compound Class
3293.1	O-H stretching	Alcohol
2929.4	C-H stretching	Alkane
2051.4	N=C=S stretching	Isothiocyanate
2370.6	O-H stretching	Carboxylic Acid
1731.3	C=O stretching	Aldehyde
1617.7	C=C stretching	Alpha, beta unsaturated ketone
1541.3	N-O stretching	Nitro Compound
1522.6	N-O stretching	Nitro Compound
1438.8	O-H Bending	Carboxylic Acid
1312.0	O-H Bending	Phenol
1183.4	C-O Stretching	Ester
1155.5	C-O Stretching	Tertiary Alcohol
1032.5	S=O Stretching	Sulfoxide
887.1	C=C Bending	Alkene
823.7	C=C Bending	Alkene
779.0	C=C Bending	Alkene
698.99	C=C Bending	Alkene



Peak Frequency of N-Hexane Extract

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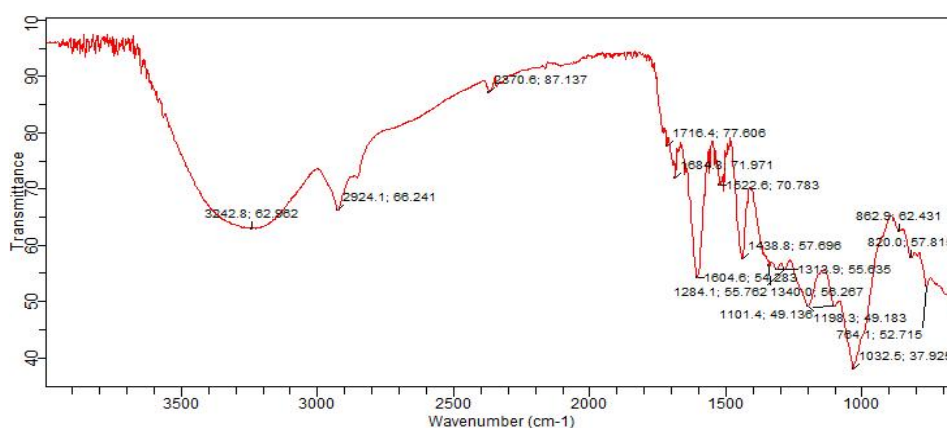
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Peak Frequency of Methanolic Extract

Peak Frequency Obtained from Methanolic Extract

Peak frequency	Group	Compound Class
3242.8	O-H stretching	Carboxylic Acid
2924.1	O-H stretching	Alcohol
2370.6	O=C=C stretching	Carbon Dioxide
1716.4	C=O stretching	Aliphatic Ketone
1684.8	C=O stretching	Conjugated aldehyde
1604.6	C=C stretching	Alpha, beta unsaturated ketones
1522.6	N-O stretching	Nitro Compound
1438.8	O-H bending	Carboxylic Acid
1313.9	O-H bending	Phenol
1284.1	C-N stretching	Aromatic Ester
1101.4	C-O stretching	Secondary Alcohol
1198.3	C-O stretching	Ester
1032.5	S=O stretching	Sulfoxide
862.9	C-CL stretching	Halo Compound
764.1	C=C bending	Alkene

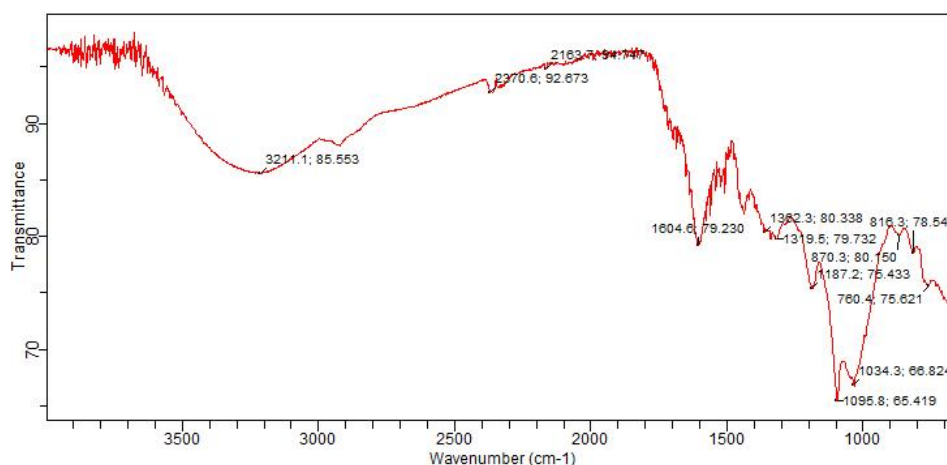


Peak Frequency of Methanolic Extract

Peak Frequency of Aquoes Extract

Peak frequency obtained from Aquoes Extract

Peak Frequency	Group	Compound Class
3211.1	O-H stretching	Carboxylic acid
2370.6	O=C=O stretching	Carbon Dioxide
1604.6	C=C	Conjugated Alkene
1362.3	S=O stretching	Sulfonate
1319.5	S=O stretching	Sulfone
870.3	C-CL stretching	Halo compound
1187.2	C-O strertching	Tertiary alcohol
760.4	C=C bending	Alkene
1034.3	S=O stretching	Sulfoxide
1095.8	C=O stretching	Secondary Alcohol
1034.3	S=O stretching	Sulfoxide
816.3	C-CL stretching	Halo Compound



Peak Frequency of Aquoes Extract

DISCUSSION, SUMMARY, CONCLUSION AND RECOMMENDATION

In this research work n-hexane, methanolic and aquoes extracts of selected indigenous medicinal plant *Psidium guajava* were used for their antibacterial, antioxidant activity and cytotoxic activity. Same extracts of same plant were also used for their phytochemical analysis. The n-hexane, methanolic and aquoes extracts of *Psidium guajava* have 13mm, 6mm and 10mm ZOI respectively against *E.*

coli Likewise the same extracts show 12mm, 10mm and 15mm ZOI against *S. aureus* respectively. While standard drug ciprofloxacin at 10µg concentration show 50mm and 55mm ZOI against *E. coli* and *S. aureus* respectively. Alkaloids, flavonoids, steroids, glycosides, insecticides, and other related bioactive, which are abundant in medicinal plants and have high medical potential, have been widely exploited in the pharmaceutical and medicine industries.. Many plants have recently been discovered to possess antibacterial properties worldwide .In the current study, the antibacterial potential of *Psidium guajava* n-hexane , methanolic and aqueous extracts, an important medicinal plant from Pakistan, has been examined. The current findings support the bio-efficacy research on plants and plant extracts and the antibacterial activity of *Psidium guajava*. It has been demonstrated that most plants may have an inhibitory effect on both gramme positive and gramme negative bacteria when they are extracted using solvents such ethanol, hexane, and methanol [BushraBeegum *et al.*, 2003]. In comparison to methanol and water, ethanol extraction generated a higher yield [Idris *et al.*, 2017]. Research from the past supports the findings of [Rabe and Van Staden 1997], who found that a methanol extract had higher overall antibacterial activity than an essential oil and other extracts.

Although using aqueous plant extracts like infusions, decoctions, and tea is a frequent practice in traditional medicine, it is unlikely that these extracts' active ingredients are the same as those that give methanol its therapeutic properties. It takes a sophisticated chemistry to chew, boil, soak, massage, and extract therapeutic herbs. According to the study, each type of extract has several, distinctive antimicrobial compounds that interact with various bacterial strains in various ways. Guava leaf extracts were discovered to have an antibacterial action and to stop *S. aureus* from growing by [Vieira *et al.*, 2001]. When [Gnan and Demello 1999] tested guava leaf extract against nine distinct strains of *Staphylococcus aureus*, they discovered that it had good antibacterial action. When testing the antibacterial activity of *P. guajava* aqueous, methanolic, and chloroform extracts on *S. aureus* strains derived from clinical patients, [Jaiarj *et al.*, 1999] found that the aqueous extracts performed better than the methanolic extracts. The antibiotic activity of the aqueous medium showed that the guajaverine and psidiolic acid are two chemicals

that are produced when the dried leaves of *Psidium guajava* are extracted [Cáceres et al., 1993]. Aqueous extract demonstrated the most activity in the current investigation against *S. aureus*, while n-hexane extract demonstrated the highest activity against *E. coli*. Our research shown that aqueous extract had the highest efficacy against *S aureus*, supporting the findings of [Cáceres et al., 1993]. The IC-50 value was determined for each sample in order to determine which sample has the capacity to eliminate 50% of DPPH with a constrained amount of sample. Among all samples, it was discovered that the methanolic extract had the highest levels of antioxidant activity, whilst the n-hexane extract had the lowest values. According to the take a look at cited above, chloroform (forty%) and n-butanol extract (35.9%) had the bottom radical scavenging quotes, whilst acetone extract (41%) had the greatest. The least effective radical scavengers have been the petroleum ether and n-hexane extract. Leaves include ascorbic acid, a large antioxidant aspect. Protocatechuic acid, quercetin, ferulic acid, gallic acid, and caffeic acid are a few greater phytochemicals observed in guava which can be also extensive antioxidants. The highest value of antioxidant activity of methanol and aqueous guava leaf extract were similarly located in research demonstrating the antioxidant study using water and methanol as the solvent. At diverse doses, the entire phenolic content of *Psidium guajava* leaf extract ranged extensively, from 120.3 to 347.8 mg GAE/g, respectively. With 347.8 mg GAE/g, the methanolic extract had the highest value. At diverse doses, the full flavonoid content in *Psidium guajava* leaf extract varied greatly, with TPC values starting from 72.2 to 365.8 mg EC/g, respectively. The aqueous extract's 365.83 mg CE/g TFC price was the highest. The lowest became within the methanolic extract. According to the aforementioned examine, n-butanol extract had the best TFC value while acetone extract had the highest TPC value. Numerous researches have also measured the concentrations of TPC and TFC in various solvents. However, TPC and TFC in solvents with various polarity may differ. This can be accounted for by differences in the polarity of the extraction solvent, extraction process, maturity at harvest, and chemical composition of the solvents. The current study concluded by demonstrating the antibacterial and antioxidant capabilities of guava leaves.

SUMMARY

The majority of the traditional usage of medicinal plants for a variety of diseases is based on indigenous knowledge passed down from generation to generation without scientific support. *Psidium guajava* has medicinal properties.

Psidium guajava belonging to Myrtaceae family is widely used to treat different diseases in traditional medicine. There are rumors that certain *Psidium guajava* components are employed in traditional medicine. The plant's many parts, including the root, bark, leaves, and fruits, have a wide range of pharmacological qualities and are used to treat a wide range of illnesses. According to several assets, the leaves and bark of the *P. Guajava* tree have a long history of medicinal use. Aqueous extract of guava leaves has been proven to be beneficial in the remedy of gastrointestinal (GIT) problems in addition to diarrhea, peristaltic reflex inhibition, and gastroenteritis. The entire plant is likewise used as a skin tonic and to treat illnesses that affect ladies, which include dysmenorrhoea, spontaneous abortion, uterine hemorrhage, and preeclampsia. The bark, fruit and leaves of this plant had been shown in latest studies to have pharmacological properties which includes antibacterial, hypoglycemic, anti-inflammatory, antipyretic and antispasmodic. The study had been designed to calculate the antibacterial and antioxidant properties of guava leaves and consistent with multiple property, leaves and bark of *P. Guajava* tree have large facts of medicinal uses. Aqueous extract of guava leaves has been proven to be useful in the remedy of gastrointestinal (GIT) troubles, together with diarrhea, peristaltic reflex inhibition, and gastroenteritis. The entire plant is also used as a pore and skin tonic and is likewise used to deal with ailments that have an effect on females. The study was developed to calculate the antibacterial and antioxidant activities of guava leaves and to assess the presence of various phytochemicals in them. Depending at the location and growing season. The vitamins range of one guava, the strawberry guava (*P. Littoralevar. Cattleianum*), is said to include 90 mg of vitamin C in keeping with serving. This way that one serving of strawberry guava provides a 100% of the day by day intake of vitamin C. Guava has a exceptionally high antioxidant value amongst plant because it also consists of carotenoids and polyphenols, which are crucial households of antioxidant

pigments. To evaluate the presence of numerous phytochemicals in them. An exceptional supply of antioxidants and antioxidant dietary fiber (AODF) is guava pulp and peel. Guava is a fruit that is rich in nutrients, fiber, fatty acids, triterpenes, triterpenoids, flavonoids, vital oils, saponins, carotenoids and lectins. Guava culmination are a very good source of the nutritional fiber pectin. However, because flavonoids, leutin, zeaxanthin, and lycopene are present, a significant amount of pharmacological activity is attributed to it. It has been demonstrated that flavonoids have antimicrobial properties. It has been suggested that the active flavonoid quercetin-3-O-alpha-l-arabinopyranoside possesses antibacterial properties. In this research plant extracts in different solvents were used. It is observed by various *in-vitro* methodologies; the plant had antibacterial and antioxidant potential. In antibacterial activity *Psidium guajava* showed 15mm ZOI against *S.aureus* and 13mm ZOI against *E. coli*. In DPPH scavenging activity with an increase in sample concentration, the DPPH scavenging percentage increased. In total phenolic content higher extract yield is obtained from the methanolic extract(347.8mg GAE/g dry weight) and In total flavonoid content higher extract yield is obtained from the aqueous extract (365.8 mgCE/g dry weight).

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