Biointerface Research in Applied Chemistry
Platinum Open Access Journal (ISSN: 2069-5837)

*Article*
**Volume 11, Issue 6, 2021, 14090 - 14102**
https://doi.org/10.33263/BRIAC116.1409014102

# Elucidation of Bioactive Potential of Two Commonly Grown North Indian *Psidium guajava viz.*, Lalit and Shweta against Pathogenic Foodborne and MDR Bacteria

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**Received: 25.01.2021; Revised: 28.02.2021; Accepted: 2.03.2021; Published: 6.03.2021**

**Abstract:** Food-borne diseases (FBD) can cause serious health hazards. Day by day, these pathogens are becoming resistant to various antibiotics. Consequently, severe outbreaks of (multidrug resistance food-borne diseases) MDR-FBD are possible. About this, there is an urgent requirement to explore new antimicrobial compounds. As *Psidium guajava* L. has been in folkloric use to treat many diseases, in this study, the phytochemical profile of two North- Indian cultivars (c.v) Lalit (Pink) and Shweta (White) was elucidated, and their bioactive potential was evaluated against antibiotic-resistant FBD. Phytochemical profile relating to good antioxidant activity was exhibited by Leaf (methane, hexane, dichloromethane, and aqueous) extracts containing a good amount of flavonoid/phenolics. Putatively, they are responsible for the antibacterial potential of the extracts. As these extracts showed inhibition of growth in all the ten bacterial strains (including *Staphylococcus aureus* (MDR), *Bacillus pumilus, Micrococcus luteus, Enterobacter epidermidis, Bacillus subtilis, Listeria monocytogens*, *Escherichia coli* (MDR), *Salmonella abony, Klebsiella pnemoneae*, and *Shigella dysenteriae)* guava leaf extracts can be formulated as a functional food for combating MDR-FBD.

**Keywords:** *Psidium guajava;* flavonoids; phenols; antioxidants, and antibacterial.



## 1. Introduction

Food-borne infections, though not well documented, can cause severe diseases [1], especially in underdeveloped and developing countries [2]. The effect of these outbreaks can worsen in case the pathogen is antibiotic-resistant and can be exponential if it becomes multidrug-resistant. Currently, indiscriminate use of antibiotics in farms coupled with horizontal gene transfer has led to the emergence and spread of bacteria, especially (MDR) multidrug resistance in food-borne pathogens (ref). CDC, USA has also classified some of these drug-resistant food-borne bacteria as *Campylobacter Shigella, Salmonella sp,* as serious threats [3]. These MDR bacteria have been reported in meat, milk, cheese, and fresh produce [4]. To prevent these outbreaks, WHO has called governments and other stakeholders to enforce policies and practices that prevent the emergence of MDR [1]. But still, the chances of contamination of fruits and vegetables and livestock due to contaminated water or human

handling are very high [5]. Thus novel and safe antimicrobials are needed, and naturally derived compounds have been an ultimate source for novel drug development. Extraintestinal clinical symptoms are also associated with these pathogens as species are capable of gaining resistance towards new medications as soon as they are implemented. The production for eliminating antibiotics that have reached the organism through MDR pumps is one of the prime means by which microbes become resistant [6,7]. Constant application of antibiotics contributes to the development of bacteria into MDR types, leading to human epidemics. (MRSA) Methicillin-resistant *Staphylococcus aureus* and (VRE) Vancomycin-Resistant Enterococci are examples of such MDR bacteria [8-11].

Therapy opportunities for these bacteria are extremely limited, and infection effects are greatly impaired. In conventional medicinal schemes for disease prevention and recovery worldwide, plants have long played a key role [12,13]. Natural agricultural sources produce various compounds, including phenolic acids, vitamins, flavonoids, tannins, terpenoids, and alkaloids that are responsible for their biological potential [14], both the antioxidants as well as antimicrobial abilities are attributed to the phenolic compounds [15,16]. The antioxidants potential of these components occurs predominantly via redox mechanisms that permit these components to act as reducing agents, metal chelators, singlet oxygen quenchers, and hydrogen donors [17,18]. Therefore, a phenolic compound may prevent the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), that involves free radicals such as hydroxyl (OH), superoxide anion ($O_2$), nitric oxide (NO ), and non-free radical species including nitrous acid ($HNO_2$) and hydrogen peroxide ($H_2O_2$) [19-21]. Previous studies have shown that the antimicrobial effects of polyphenols derived from the plant cause functional or structural damage to the cell membrane of bacteria [22]. Various research s concluded that conjugated double bond and functional hydroxyl groups may be engaged in the binding of cell wall components as the plant plant-derived substances negatively affected microbial cells by various mechanism actions that attack the cell membrane's phospholipid bilayer and interrupt enzyme system [23].

*Psidium guajava* L. (Guava), also recognized as "apple of the tropics" has a special place as it is used in traditional systems of medicine for the prevention and cure of many diseases [24,25]. There is a long history of ethnobotanical uses of guava bark and leaves in various countries [26]. Even today, its leaves are used to treat cough and cold, laxatives, and treat wounds, vomiting, dysentery, diarrhea, hyperglycemia, and gastrointestinal issues [27-29]. Guava leaves have been reported to have anti-diabetic [30], antioxidant [31,32], and antibacterial effects [33,34].

It has been reported that Guava species are rich in potentially active antioxidant compounds such as flavonoid and phenolic compounds, ascorbic acid, carotenoids, and lycopene [35-37]. It harbors many antioxidants like phenolics, flavonoids, and carotenoids [38]. Moreover, it is an excellent source of minerals like calcium, iron, phosphorus, vitamins like pantothenic acid, ascorbic acid, niacin, and vitamin A [39].

The purpose of this study is to elucidate the Bioactive compounds present in crude leaf extracts of two less explored, locally growing North Indian guava cultivar *viz,* Lalit (Pink guava) and Shweta (White guava), and evaluate the antioxidant and antibacterial activity against certain MDR and pathogenic bacteria that can cause food-borne infections.

## 2. Materials and Methods

*2.1. Preparation of plant extract.*

The leaves of *P. guajava* cv. Lalit (pink pulp guava) and Shweta (white pulp guava) have been collected from the CISH Lucknow, U.P. India. It was cleaned with water and sterilized with 0.2% mercuric chloride and washed thoroughly with sterilized distilled water and air-dried; after that, fresh leaves were dipped in liquid N2 and homogenized until power is formed which is further stored and maintained at −20ºC.

*2.2. Extraction methods used on guava.*

Leaves powdered material was extracted by Soxhlet extraction, which was continued for 15 cycles using solvents methane, hexane, (DCM) dichloromethane, and dist. water (aqueous). After filtration and concentration, the dried and measured extract was stored at -20 °C for further studies.

*2.3. Bioactive compounds analysis.*

2.3.1. Total phenol content (TPC).

Total phenol content in leaves samples was determined by the Folin–Ciocalteu method. Samples with the reaction mixture were incubated for 15 min at RT (room temperature), and then by using a spectrophotometer, absorbance was measured at 765 nm. The TPC values were expressed in terms of the (GAE) Gallic acid equivalent (mg/g) [40,41].

2.3.2. Total flavonoid content (TFC)

Determination of TFC was done by the Aluminum chloride method [42]. The reaction was incubated for 30 min at RT, and absorbance was taken at 415 nm using a spectrophotometer. TFC was expressed in terms of the (QE) quercetin equivalent (mg/g) [41].

*2.4. Antioxidant studies.*

2.4.1. DPPH radical scavenging assay.

The free radical scavenging activity assay was determined by using (DPPH) 1,1-diphenyl-2-picrylhydrazyl radical assay. Different concentrations (12.5 to 500 µg/ml) 100 µl of test samples were mixed with the reaction. Ascorbic acid was used as standard. Reactions were incubated at 37 °C for 30 min, and absorbance was taken at 517 nm by spectrophotometer [41,43]. Results are expressed as mg (AAE) ascorbic acid equivalents per g of leaf extract c.v Lalit and Shweta. The calculation of radical scavenging activity was done by using the following formula:

$$Percent\ Inhibition = [\frac{(Ac - As)}{Ac}] * 100$$

Where: Ac is the absorption of the blank sample;
As is the absorption of the extract;

2.4.2. ABTS radical scavenging activity.

Free radical scavenging activity by ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) was analyzed [44]. The reaction mixture was mixed with different concentrations (12.5 to 500 µg/ml) 100 µl with ABTS solution, and absorbance was measured at 734 nm. For blank, ethanol was used, and all sample absorbance was taken in 6 min after the reaction. Results are expressed as mg (AAE) ascorbic acid equivalents per g of leaf extract c.v Lalit and Shweta. The percentage inhibition was calculated using the formula:

$$ABTS\ Scavenging\ activity(\%) = \left[\frac{(Ac - As)}{Ac}\right] * 100$$

Where: Ac is the absorption of the blank sample;
As is the absorption of the extract;

*2.5. Antibacterial activity.*

2.5.1. Preparation of bacterial culture.

Pure cultures of ten test organisms including gram (+) bacteria (*Staphylococcus aureus* (MTCC1305), *Bacillus pumilus* (MTCC160), *Micrococcus luteus* (ATCC-4698), *Staphylococcus epidermidis* (ATCC12228), *Bacillus subtilis* (MTCC441),and *Listeria monocytogens* (ATCC19111) and gram (-) bacteria *(Escherichia coli* (ATCC25923), *Salmonella abony* (NCIM2257), *Klebsiella pnemoneae* (NCIM-2957)and *Shigella dysenteriae* (ATCC23513) (Table. 1) was purchased. Microorganisms culture was kept at -80º C in the broth containing (15%, v/v) glycerol. Before the experiment, these microorganisms with a loop were transferred to the freshly prepared aseptic nutrient broth from the stock culture to prepare new working culture, by inoculating the broth and then incubated at 37ºC for 24 hours to screen crude extracts for antibacterial activity, which was performed on agar plates by the agar well diffusion method and MIC.

**Table 1.** List of microorganisms with their accession number and culture media that are used for the elucidation of Antibacterial activity and MIC.

| Gram Strain | Microorganisms | Accession number | Culture Media |
|---|---|---|---|
| gram (+) bacteria | *Staphylococcus aureus* | NCIM2079 | Nutrient broth or agar |
| | *Bacillus pumilus* | MTCC160 | |
| | *Micrococcus luteus* | ATCC-4698 | |
| | *Staphylococcus epidermidis* | ATCC12228 | |
| | *Bacillus subtilis* | MTCC441 | |
| | *Listeria monocytogens* | ATCC19111 | |
| gram (-) bacteria | *Escherichia coli* | NCIM2571 | Nutrient broth or agar |
| | *Salmonella abony* | NCIM2257 | |
| | *Klebsiella pnemoneae* | NCIM-2957 | |
| | *Shigella dysenteriae* | ATCC23513 | |

2.5.2. Well diffusion method.

It was performed by freshly preparing the Nutrient agar (NA) medium and solidifying it in sterile Petri plates. Different bacterial strain culture was spread on the agar plate's surface by spreader. Bacterial culture was maintained at turbidity of 1×108 CFU/ml. By using a sterile 6mm diameter cork-borer, uniform wells were prepared. All well was filled with the 50µl (methanol, hexane, DCM, and aqueous) different extracts. Then these plates were incubated

for 24 hrs at 37 °C. This experiment was performed in triplicate. 5% DMSO is used as a negative control, whereas for positive control, gentamycin was used.

2.5.3. Determination of minimum inhibitory concentration (MIC).

For MIC broth, microdilution assays were evaluated as per the method prescribed by [45] with a modified method to determine MIC of each sample extract against 10 pathogenic and MDR bacteria. Thus, leaf extract (100 µl) was diluted with the broth in a 96-well microtiter plate. 100 µl of bacterial culture was added in the wells having 1 to 2 $\times 10^8$ cfu/ml (turbidity equivalent to McFarland solution) was incubated for 24hrs after this plates were incubated for 24 hrs at 37$^o$ C. The plate wells were visually examined after 24 hrs for the lowest concentration of the used extract, which have inhibited microbial growth. The least concentration at which growth was inhibited as the MIC.

*2.6. Statistical analysis.*

All experiments were carried out in triplicate. Values are presented as mean SD (n = 3). Statistical evaluation of all the means, standard deviation, and correlation was performed using one-way ANOVA analysis performed using GraphPad Prism (GraphPadIn-Stat version 8.00, GraphPad Software, San Diego, CA, USA) and excel. $P < 0.05$ was considered statistically significant.

## 3. Results and Discussion

*3.1. Determination of Bioactive compounds.*

3.1.1. Total Phenol Content (TPC).

Phenolic compounds are classified as a class of chemical constituent that contains one or more than one acidic hydroxyl residues, which are added to an aromatic ring [46]. These are the most efficient antioxidative components that confer the antioxidant potential of the plant [47]. Therefore, to evaluate its contribution to the antioxidant activity, it is necessary to estimate phenolic content. Results of TPC are shown in Table 2 (1$^{st}$ column). Results varied widely in both the cultivar of guava leaf extracts, ranging from 20.60 to 90.97 mg GAE/g of guava leaf extract. Among these methanolic extracts, of Lalit (90.97 GAE/g of guava leaf), Shweta (63.5 GAE/g of guava leaf) extracts have significantly ($p<0.05$) higher amount of the phenolic compounds compared to other extracts followed by hexane Lalit (71.5 GAE/g of guava leaf), Shweta (47.3 GAE/g of guava leaf), dichloromethane Lalit (33.8 GAE/g of guava leaf), Shweta (47.9 GAE/g of guava leaf) and then aqueous Lalit (20.6 GAE/g of guava leaf), Shweta (32.9 GAE/g of guava leaf) extract.

3.1.2. Total Flavonoid Content (TFC).

Flavonoids comprise a specific class of phenolic compounds, and they have a structure based on the carbon skeleton of diphenylpropane. Flavonoids usually have higher antioxidant activities and contain multiple hydroxyl groups than phenolic acids [48]. Flavonoid family members have many compounds, such as epicatechin, catechin, and quercetin, which are known as antioxidant compounds [49]. TFC results are shown in Table 2 (2$^{nd}$ column).TFC differs broadly in both the cultivar of guava leaf extracts, ranging from 43.4 to 114.0 mg QE/g

of guava leaf extract. Among these methanolic extracts, of Lalit (114.0 mg QE/g of guava leaf) Shweta (99.1 mg QE/g of guava leaf extract). These TFC's are the primary components that account for the antioxidant potential [50].

There are already many reports of the herbal extract being used directly or indirectly in ethnobotanical studies and subsequently preparing various modern drugs. Guava plants have been widely used to treat Mexican and African medicine for bacterial infection, diarrhea, and diabetes. It was reported that the methanolic extraction has higher constituent of the Bioactive compounds followed by hexane, dichloromethane, and then aqueous extract. Guava has abundant bioactive compounds like flavonoids, phenols, tannins, alkaloids, triterpenes, anthraquinone, saponins, and lycopene [51]. The guava leaves have a high content of flavonoids and phenols responsible for the antioxidant potential. The methanolic extract exhibited higher content of flavonoids and phenol followed by hexane, dichloromethane, and aqueous in Lalit and Shweta cv. Respectively. Lalit cultivar had a higher concentration of bioactive compounds than Shweta, as represented in (Table 2).

**Table 2.** Bioactive compounds constituents Total phenols content and Total flavonoids content with Antioxidant contents (DPPH and ABTS) in two cultivars of *Psidium guajava* leaf for four different (methane, hexane, dichloromethane, and aqueous) extracts where the results obtained were expressed as Mean ± S.D. of triplicates. Values in each row marked (*) represent significance different (P<0.05)

**Bioactive compounds and Antioxidant contents of various leaf extract**

| Guava Cultivar | Extract Standard Equivalent | Total Phenol Content (mg GAE/g leaf) | Total Flavanoid Content (mg GAE/g leaf) | DPPH radical scavenging (mg AAE/g leaf) | ABTS radical scavenging (mg AAE/g leaf) |
|---|---|---|---|---|---|
| Lalit | Methanolic Extract (mg/g) | 90.97±1.36*** | 114±1.49*** | 77.5±1.25*** | 90.3±0.81*** |
| | Hexane Extract (mg/g) | 71.5±1.25*** | 71.1±0.94*** | 60.12±0.91*** | 78.6±1.03*** |
| | Dichloro methane Extract (mg/g) | 33.8±0.83*** | 82.4±1.23*** | 68.5±1.36*** | 44.2±1.08*** |
| | Aqueous Extract (mg/g) | 20.6±1.43** | 43.4±2.84** | 47.2±1.27*** | 30.9±2.09*** |
| Shweta | Methanolic Extract (mg/g) | 63.5±0.45*** | 99.1±0.89*** | 78.5±1.17*** | 77.63±2.08*** |
| | Hexane Extract (mg/g) | 47.3±2.69** | 76.9±0.08*** | 60.61±1.50*** | 67.1±0.90*** |
| | Dichloro methane Extract (mg/g) | 47.9±0.76*** | 69.9±0.31*** | 75.3±1.17*** | 62.6±2.09*** |
| | Aqueous Extract (mg/g) | 32.9±2.82** | 52±1.79*** | 50.64±1.19*** | 24.8±1.01*** |

*3.2. Determination of antioxidant capacity.*

3.2.1. DPPH and ABTS radical scavenging activities.

Antioxidant activity by radical scavenging effects is exhibited by Phenolic and flavonoid compounds. Radical scavenging activity plays a crucial role in the deleterious effect of free radicals, which generally proceeds through a donation of electrons or hydrogen atom transfer in biological systems [52]. Two types of the assay are used to elucidate free radical scavenging activity, two guava cultivar different extracts, ABTS and DPPH. Both ABTS and DPPH assays are performed to evaluate the free radical scavenging activity of the different extracts. However, these have a significant difference in their response towards antioxidants. DPPH can easily solubilize in the organic media, not in aqueous media, which is a significant restriction when elucidating hydrophilic antioxidants' role. Whereas ABTS can be easily solubilized in multiple media, permitting its use in the elucidation of antioxidant capacity of both lipophilic and hydrophilic compounds [53].

All crude extracts of leaves (methanol, hexane, dichloromethane, and aqueous) have been examined for antioxidant activity by both methods. In the DPPH assay, all the extracts (of both cultivars) showed higher antioxidant capacity. Maximum inhibition percent was found in methanol extract (77.97% and 77.03% in Lalit and Shweta, respectively) at 250 µg/ml concentration followed by dichloromethane, hexane, and aqueous in Lalit and Shweta cv.,

respectively. If we talk about antioxidant activity by ABTS method, then best results were observed in the case of methanol extract (89.66% and 78.21% in Lalit and Shweta, respectively) followed by hexane dichloromethane and aqueous extracts for both Lalit and Shweta cv. There are moderate and significant variations in flavonoids and phenolic content among the two cultivars, as observed (Table 2).

These results specify that the concentration of flavonoids and Phenolic compounds is higher in leaves of Lalit than Shweta, which results in higher antioxidant capacity (Table 2, Figure 1). It has been reported that guava leaves contain a variable phenolic compound, including gallic acid [54]. Plant phenols exhibit (in vitro) antioxidant activities by acting as directly scavenging reactive oxygen species or chain-breaking peroxyl-radical scavengers. This can be due to the higher concentration of TPC and TFC in the leaf extract. However, there are reports that radical scavenging activity, TPC and TFC derived from different natural sources have a close correlation between them [55,56].

*3.3. Correlations among the measurements*.

To analyze relationships among TFC and TPC to assess leaf extracts' antioxidant capacity, we have calculated their correlations, and results are present in Figure 1. The Correlations between TPC and ABTS, TPC of Lalit c.v have the highest correlation value in ABTS assay ($R^2$=1.00) whereas Shweta c.v has ($R^2$=0.854). DPPH assay was correlated with TPC, but there are slightly lower correlation coefficients in Shweta and Lalit as compared to ABTS ($R^2$=0.772 and $R^2$=0.479, respectively). The Correlations between TFC and ABTS, TFC of Shweta c.v have the highest correlation coefficient value in ABTS assay ($R^2$=0.817) whereas Lalit has ($R^2$=0.599). TFCwere well correlated with DPPH assay, but their correlation value in Shweta and Lalit ($R^2$=0.812 and $R^2$=0.951, respectively). These results indicate a high correlation between TFC, TPC, DPPH, and ABTS of these four extracts. Whereas there are reports of high correlation among Foline Ciocalteu, ABTS and DPPH [57]. These correlation results suggest a relationship between the phenolic compound concentration in leaf extracts and their free radical scavenging. Thus, the existence of Phenolic and flavonoids compounds in leaf extracts significantly contributes to their antioxidant capacity. When we compared to correlation with four different extracts, methanolic extracts showed higher values followed by hexane, dichloromethane, and aqueous. On this basis, it can be said that methanolic extracts of guava leaf Shweta and Lalit contain a higher level of phenolic and flavonoids compounds, which are majorly responsible for the antioxidant potential.

*3.4. Antimicrobial potential.*

3.4.1. Agar well diffusion method.

The antibacterial efficacy of all the extracts of both cultivars was tested against the selected ten-gram (+) and gram (-) MDR and pathogenic food-born bacteria that are enteropathogenic (Table 2).The highest antibacterial activity was shown by Methanolic extract against *S. aureus* (23.7 mm), which was followed by *B. pumilus* (21.7 mm) and *B. subtilis* (19 mm) in Lalit cultivar and *S. aureus* (19.3 mm) followed by *B. pumilus* (18.3 mm) and *B. subtilis* (18 mm) in Shweta cultivar. Hexane extract showed the highest antibacterial efficacy against *M. luteus* (20.7 mm) followed by *B. pumilus* (19.7 mm) and *S. aureus* and *S. abony* (18.3 mm) in Lalit cultivar and *S. abony* (19 mm), followed by *B. pumilus* (15.7 mm) and *S. aureus* (11.7

mm) in Shweta cultivar. Dichloro methane extract showed the highest antibacterial efficacy against *B. subtilis* (20.3 mm), *M. luteus* (19.7 mm), and *B. pumilus* (18.3 mm) in Lalit cultivar, whereas *S. abony* (18 mm), *B. pumilus*,and *B. subtilis* (14.7 mm) and *K. Pnemoneae* (14.3 mm) in Shweta cultivar. The aqueous extract showed the highest antibacterial activity against *B. pumilus* (5 mm), *K. Pnemoneae* (4.33 mm), and *E. coli* (2.33 mm) ) in Lalit cultivar, whereas *K. Pnemoneae* (5.33 mm), *B. pumilus* (2.33 mm) and *B. subtilis* (1.67 mm) in Shweta cultivar (Table 3). The above result has been investigated that (MDR) *S. aureus*, (MDR) *E.coli* pathogenic *Bacillus*, and *K. Pnemoneae* is majorly inhabited by the leaf extract.

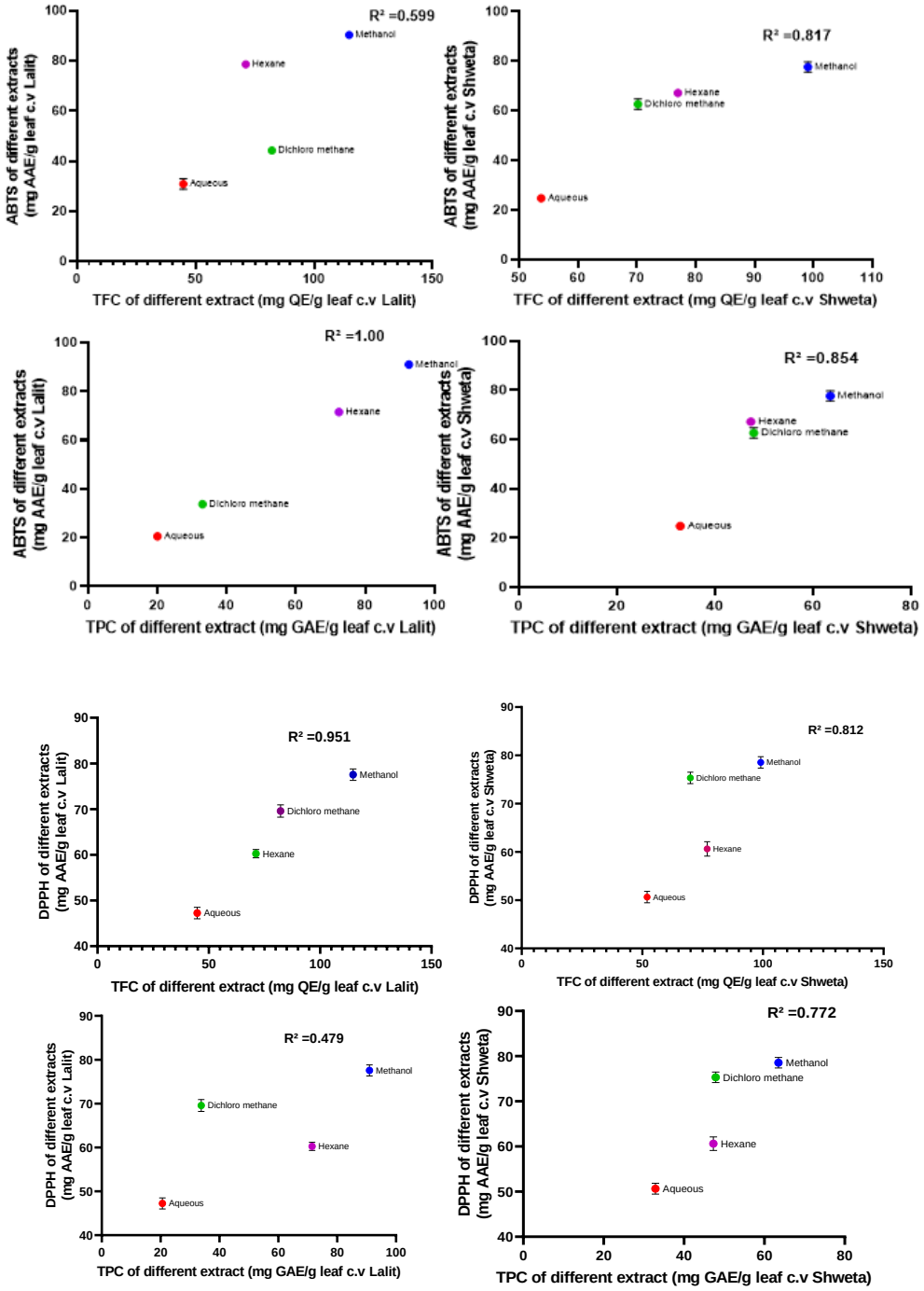


**Figure 1.** Correlations between total phenolic content (TPC) and total flavonoids content on the antioxidant capacity assays ABTS and DPPH in) in two cultivars of *P. guajava* leaf for four different (methane, hexane, dichloromethane and aqueous) extracts. Where Error bars represent the standard deviation of triplicates and $R^2$ is the Pearson's correlation coefficient for one-tailed ANOVA test.

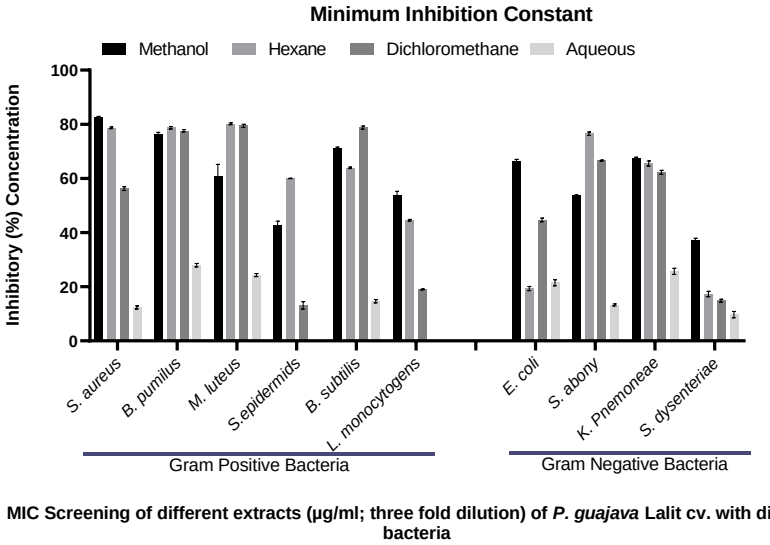


**Figure 2.** Minimum inhibitory concentration in four different (methane, hexane, dichloromethane, and aqueous) *P. guajava* Lalit leaf extracts against ten different bacteria, which was performed in triplicate. The error bars represent the standard deviation.

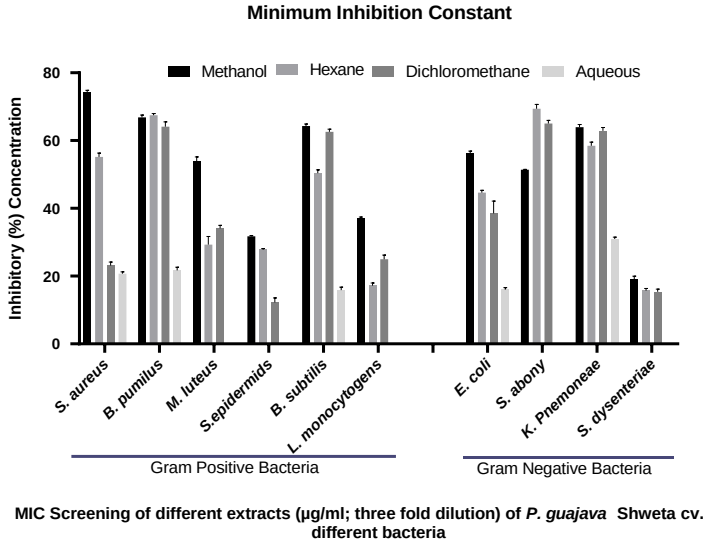


**Figure 3**. Minimum inhibitory concentration in four different (methane, hexane, dichloromethane, and aqueous) *P. guajava* Shweta leaf extracts against ten different bacteria, which was performed in triplicate. The error bars represent the standard deviation.

**Table 3.** Antibacterial activity of *Psidium guajava* leaves in four different (methane, hexane, dichloromethane, and aqueous) where the results obtained were expressed as Mean ± S.D. of triplicates. Inhibition zones are the mean, including borer (5mm) diameter ± standard deviation. Values in each row marked (*) represent significance different (P<0.05).

| Plant Cultivar | Plant Leaves Extract | Name of Bacteria and Zone of Inhibition (mm) at 10 mg/ml concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **E. coli** | ***S. aureus*** | ***S. abony*** | ***B. pumilus*** | ***M. luteus*** | ***E. epidermids*** | ***K. Pnemoneae*** | ***L. monocytogens*** | ***B. subtilis*** | ***S. dysenteriae*** |
| Lalit | **Hexane** | 1.69±0.57*** | 18.3±1.15** | 18.3±1.53** | 19.7±0.57*** | 20.7±2.08** | 8.33±1.53* | 11±1** | 5.67±0.57** | 16±2** | 1±1.73 |
| | **DCM** | 12.7±0.57*** | 14.3±2.08** | 16±1** | 18.3±2.52** | 19.7±1.53** | 2±1* | 15±1** | 8±1** | 20.3±0.57*** | 2.67±1.53* |
| | **Methanol** | 15.3±.57*** | 23.7±0.57*** | 11±1** | 21.7±1.53** | 14±1.73** | 10.3±1.53** | 15.3±2.52** | 12.3±2.52* | 19±2** | 6±2* |
| | **Aqueous** | 2.33±1.57 | 0.333±0.57 | 0.333±0.57 | 5±4.58 | 2±1* | NA | 4.33±2.08* | NA | 1.33±0.57* | 0.333±0.57 |
| (Pink Guava) | | | | | | | | | | | |
| | **Hexane** | 11.3±0.57*** | 11.7±0.57*** | 19±1*** | 15.7±0.57*** | 8±1** | 3.67±1.53* | 13±2.65* | 2±1* | 12.3±2.52* | 1±1 |
| | **DCM** | 10.3±0.57** | 10.7±1.15** | 18±2** | 14.7±0.57*** | 6.67±1.15** | 0.333±0.57 | 14.3±2.08** | 2.33±2.31 | 14.7±1.15** | 0.333±0.57 |
| | **Methanol** | 15±1** | 19.3±0.57*** | 11.3±1.53** | 18.3±1.15** | 11.7±1.53** | 5.67±2.08* | 17.7±1.15** | 9.33±1.53** | 18±2** | 3.67±2.08 |
| | **Aqueous** | 1±1 | 0.667±1.15 | NA | 2.33±0.57* | NA | NA | 5.33±1.53* | NA | 1.67±1.15 | NA |
| Gentamycin (Positive Control) | | 26±1*** | 25.3±0.57*** | 22.3±0.57*** | 23.3±1.15*** | 24.3±0.57*** | 16±1** | 24.7±1.53** | 14±1.73** | 21±1*** | 23±1*** |

3.4.2. Minimum inhibitory constant.

The MIC that is the inhibitory concentration of the different extracts was determined against ten pathogenic food-borne bacteria, and all of the extracts showed antimicrobial efficacy. The best MIC value (at 1mg/ml concentration of crude extract) with three folds of dilution was observed against gram (+) *S. aureus, B. pumilus,* and *B. subtilis* and gram (-) *K. pnemoneae*and *E.coli* in both cultivars. Lalit methanolic extract showed best MIC against *S. aureus* (82.67%), and *K. Pnemoneae* (67.33%) followed by hexane against *M. luteus* (80.20%), *S. abony* (76.50%), dichloromethane against *B. subtilis* (78.72%), *S. abony* (66.58%), and aqueous extract against *B. pumilus* (27.93%), *K. Pnemoneae* (25.68%) in gram-positive and negative bacteria, respectively. Where as in Shweta methanolic extract showed best MIC against *S. aureus* (74.21%), *K. Pnemoneae* (63.93%) followed by hexane against *B. pumilus* (67.46%), *S. abony* (69.40%), dichloromethane against *B. pumilus* (64.08%), *S. abony* (65.00%) and aqueous extract against *B. pumilus* (21.70%), *K. Pnemoneae* (30.59%) (Figure 2,3) in gram-positive and negative bacteria respectively against gentamycin was used as standard drug. It has been investigated that Guava extra has a significant inhibitory effect on *E. coli* and *Bacillus* sp. and *K. Pnemoneae* indicating that guava extracts can be used as natural ingredients against the growth of these pathogens [58,59].

## 4. Conclusions

Locally grown *Psidium guajava viz.,* Lalit, and Shweta were studied for their antioxidants and antimicrobial potential. From this study, it has been concluded that Lalit s has better activity, as all the extracts of Lalit cv showed higher concentration of antioxidants andan antimicrobial activity when compared to Shweta cv. This may be attributed to the number of bioactive compounds present in Lalit as compared to Shewta. Guava plants are now being in the limelight; the reasons for this include the emergence in the pharmaceutical pipeline of FBD MDR, emerging new pathogens, and a decline in new antibacterial medicines. On average, two to three new antibiotics from microorganisms are developed per year by the pharmaceutical industry. However, the various new antimicrobial products in the research and development process started to decrease over the past twenty years. The pharmaceutical industry is becoming increasingly more open to the future use of plant-based antimicrobials and other drugs. Moreover, all of these extracts exhibited antibacterial activity against the above selected MDR and pathogenic food-borne bacteria, implying a possible application for developing a new drug for the treatment of diseases caused by these MDR and food-borne bacteria.

## Funding

This research received no external funding.

## Acknowledgments

I would like to acknowledge Integral University, Lucknow, India (IU/R&D/2021-MCN0001042) and UP CST/D-1077.

## Conflicts of Interest

The authors declare no conflict of interest.

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