

# IN-VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *PSIDIUM GUAJAVA* ON SOME ENTERIC BACTERIAL ISOLATES OF PUBLIC HEALTH IMPORTANCE

## ABSTRACT

**Aim:** The study was conducted to investigate the antimicrobial activities of *Psidium guajava* and its major antimicrobial constituents (phytochemicals).

**Material and method:** The aqueous and ethanol extracts from the leaves and stem bark of the plant was tested using well Diffusion method for their antimicrobial activity against some members *Enterobacteriales* family isolated from diarrheic stool sample (*Escherichia coli*, *Shigella spp.*, *Salmonella typhi* and *Pseudomonas aeruginosa*).

**Results:** The result shows that the extracts were active against the microorganisms. The ethanolic extract of stem showed the highest zones of inhibition against tested organisms compared to aqueous extracts. Statistical analysis of the result shows that the extracts demonstrated higher antibacterial activity against the isolates tested with the average zone of inhibition of 15.44mm, 14.78mm, 12.92mm and 11.31mm for *Escherichia coli*, *Shigella spp.*, *Salmonella typhi* and *Pseudomonas aeruginosa* respectively. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranges between 6.25 – 100 mg/ml. Preliminary phytochemical analyses showed that both stem and leaf extracts contain alkaloids, tannins, terpenoid, Anthraquinones, reducing sugar, amino acid, flavonoids, saponins, glycosides and phenols.

**Conclusion:** The application of the decoction of leaf and stem of the plants in ethno medicine is justified.

**Key words:** *Psidium guajava*; Enteric bacteria; Phytochemical; well diffusion; antibacterial activity; extracts; inhibition

## 1. INTRODUCTION

Recently, there has been a lot of attention focused on producing medicines and products that are natural. Several leaves and leaves extracts have been found to have antimicrobial activity against microorganisms [1]. There is no plant that does not have medicinal value [2]. The active components are normally extracted from all plant structure, but the Concentration of these compounds varies from structure to structure. However, plants parts known to contain the highest concentration of these

30 phytochemical constituents for therapeutic purpose can either be leaves, stem barks, root, bulks,  
31 corms, rhizomes, wood, flowers, fruits or the seeds [3].

32 The presence of phytochemical constituents in medicinal plants made them useful for healing as well  
33 as for curing of human diseases [4]. Phytochemical are naturally occurring compounds in the  
34 medicinal plants, [5]. Large populations of the world, especially in developing countries depend on the  
35 traditional system of medicine to treat variety of diseases [6]. Several hundred genera of plants were  
36 utilized traditionally for medicinal purposes. The world health organization [7] reported that 80% of the  
37 world population relies chiefly on traditional medicine and a major part of the traditional therapies  
38 which involve the use of plant extract and their constituents [8].

39 Guava, *Psidium guajava* belongs to the family Myrtaceae which is considered to have originated from  
40 tropical South America, Guava tree grown in tropical and sub-tropical area of the world like Asia,  
41 Africa and Hawaii etc [9]. *Psidium guajava* common names are Guava (English), Gwaiba (Hausa),  
42 Goifa (Yoruba), Ugova (Igbo), Gutiba (Spanish), Goyave (French) and Goejaaba (Dutch) [10]. Many  
43 parts of the plant have been used in traditional medicine to manage conditions like malaria,  
44 Gastroenteritis, vomiting, diarrhea, dysentery, wound, ulcer, toothache, cough, sore throat and a  
45 number of other conditions [11]. The leaves are particularly rich in flavonoids and distinctly in  
46 quercetin [12]. The leaf methanolic extract also showed in vitro antimicrobial activities against  
47 *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and a strong antimicrobial action have been  
48 reported on Gram positive and gram negative organism [11]. Therefore, the basic phytochemical  
49 investigation of its extract for major phytoconstituents is also vital. The phytochemical analyses of  
50 Guava plant shows that its extracts contain over twenty (20) compounds [13].

51 The objective of the study was to determine the antibacterial effects of leaves and stem of *Psidium*  
52 *guajava* extracts (Both aqueous and ethanolic) against some members of Enterobacteriales namely  
53 *Escherichia coli*, *Shigella spp*, *Pseudomonas aeruginosa* and *Salmonella typhi*, to screen for the  
54 phytochemical constituents of the extracts and to evaluate the minimum inhibitory concentration (MIC)  
55 and minimum bactericidal concentration (MBC) of the extracts against the test isolates.

## 56 2. MATERIALS AND METHODS

### 57 2.1 Plant materials

58 The plant materials used in this research consisted of the leaves and stems of *Psidium guajava* which  
59 were collected from botanical garden of Government Secondary school Gundutse, Kura Local

Government Area of Kano State at about 08:30 a.m. Identification and Authentification of the plant materials has been done at Herbarium unit in the department of Biological science Ahmadu Bello University, Zaria with the following voucher numbers 3253. Voucher specimen has been deposited there for future reference. The samples were washed with water to remove dust and rinsed with distilled water. Samples were air dried for two-weeks and pulverized into powder form using sterile mortar and pestle in the laboratory as described by Mukhtar and Tukur, [14]. The powdered samples were bagged in a black polythene bag and store in air tight container for further work.

## 2.2 Test organisms

Clinical isolates of *Escherichia coli*, *Shigella*, *Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from Department of Microbiology of Murtala Muhammad Specialist hospital for further experiment. Identification and characterization of the isolates was conducted there by using three procedures namely Gram staining, cultural characterization using selective or indicative media and biochemical characterization. The pure isolates of each of the test organism were inoculated in sterile slants containing Nutrient agar and transported to the department of Microbiology KUST and refrigerated at 4 °C before use.

## 2.3 Preparation of Plant Extract

The ethanol and aqueous extract of both plant samples was carried out according to Bengum [13]. 25g of the powdered leaves was weight out and dissolved in 250ml of both solvent in a sterile beaker and allowed to stand for seven days

## 2.4 Antimicrobial Assay of Extracts

The mixture was filtered using Whatman No.1 filter paper and the extracts were evaporated to dryness using rotary evaporator and water bath. The solid residues obtained were reconstituted in DMSO and water at stock concentration, stored in the refrigerator at 4 °C until used. The agar well method was used to determine the antibacterial activity of the plant extracts. 0.1ml of the different standardized organisms were introduced separately and thoroughly mixed with Mueller Hilton Agar in a sterile Petri dish and allowed to set then labeled. A sterile cork borer 6mm was then used to punch holes (i.e. 5 wells) in the inoculated agar and the agar was then removed. Four wells that were formed were filled with different concentrations of the extract which were labeled accordingly; 200mg/ml, 150 mg/ml, 100mg/ml and 50 mg/ml while the 5th well contained the solution used for the research to serve as control, tetracycline (Chi pharmaceutical limited, Lagos Nigeria) 125mg/ml, was used as

control in this research. These were then left on the bench for 1hour for adequate diffusion of the extracts and incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each well were measured to the nearest millimeters along straight line i.e. 180° to each other and the mean of the readings were then calculated [2].

## 2.5 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Overnight Nutrient broth cultures of *E. coli*, *Shigella*, *Salmonella typhi* and *Pseudomonas aeruginosa* at 37 °C were prepared. The culture was adjusted to obtain turbidity comparable to that of the turbidity of McFarland 0.5 standard, and then further dilute in Nutrient broth. The inoculums thus prepared expected to obtain  $1.5 \times 10^8$  C.F.U/ml. The MIC and MBC were determined using procedure described by Kowser and Fatima, [15].

## 2.6 Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem of *Psidium guajava*. The presence of Alkaloid, saponin, Glycoside, Tannin, flavonoids, steroid, terpenoid, Anthraquinones, Protein and amino acid were determined using procedure described by Sofowora [16].

## 3. RESULTS AND DISCUSSION

### 3.1 Antibacterial activity

The antibacterial activity of aqueous and ethanolic leaf extract *Psidium guajava* was represented in the table below (Table 1) showing the mean diameter of zone of inhibition of extract on the test isolate with *Shigella spp* being the most susceptible isolate at 200 mg/ml concentration (23.3mm) and *Pseudomonas aeruginosa* the least susceptible (6 mm).

**Table 1: Mean diameter zones of inhibition of Aqueous and ethanolic leaf extract *P. guajava***

| CONCENTRATION<br>(mg/ml) |     | MICROORGANISMS/ ZONE OF INHIBITION (mm) |                     |                      |                         |
|--------------------------|-----|-----------------------------------------|---------------------|----------------------|-------------------------|
|                          |     | <i>E. coli</i>                          | <i>Shigella spp</i> | <i>P. aeruginosa</i> | <i>Salmonella typhi</i> |
| ALE                      | 50  | 09.6                                    | 10.6                | 06.0*                | 07.3                    |
|                          | 100 | 11.3                                    | 11.6                | 11.0                 | 10.6                    |
|                          | 150 | 11.6                                    | 12.3                | 10.3                 | 18.0                    |
|                          | 200 | 13.6                                    | 16.3                | 12.6                 | 18.3                    |
| CONTROL                  |     | 18.0                                    | 18.6                | 17.3                 | 17.6                    |
|                          | 50  | 11.6                                    | 11.3                | 07.6                 | 08.3                    |

|     |     |      |      |      |      |
|-----|-----|------|------|------|------|
| ELE | 100 | 15.3 | 18.3 | 14.3 | 10.6 |
|     | 150 | 17.3 | 20.3 | 15.6 | 13.3 |
|     | 200 | 20.0 | 23.3 | 17.0 | 18.6 |

113 ALE = Aqueous Leaf Extract. ELE = Ethanolic Leaf Extract. \* = No Inhibition

114 The antibacterial activity of aqueous and ethanolic stem extract *Psidium guajava* was represented in  
 115 the table below (Table 2) showing the mean diameter of zone of inhibition of extract on the test isolate  
 116 with *Shigella spp* being the most susceptible isolate at 200 mg/ml concentration (23.6mm) and  
 117 *Salmonella typi* the least susceptible (11.3 mm).

118 **Table 2: Mean diameter zones of inhibition of Aqueous and ethanolic stem extract *P. guajava***

| CONCENTRATION<br>(mg/ml) |     | MICROORGANISMS/ ZONE OF INHIBITION (mm) |                     |                      |                        |
|--------------------------|-----|-----------------------------------------|---------------------|----------------------|------------------------|
|                          |     | <i>E. coli</i>                          | <i>Shigella spp</i> | <i>P. aeruginosa</i> | <i>Salmonella typi</i> |
|                          | 50  | 12.3                                    | 13.3                | 11.6                 | 11.3                   |
| ASE                      | 100 | 14.0                                    | 14.6                | 12.3                 | 13.3                   |
|                          | 150 | 18.3                                    | 20.0                | 14.6                 | 17.3                   |
|                          | 200 | 22.3                                    | 23.3                | 18.3                 | 20.3                   |
| CONTROL                  |     | 18.6                                    | 18.3                | 17.3                 | 17.6                   |
|                          | 50  | 14.3                                    | 14.6                | 12.0                 | 13.3                   |
| ESE                      | 100 | 15.3                                    | 17.3                | 13.3                 | 16.0                   |
|                          | 150 | 20.3                                    | 21.3                | 17.3                 | 18.3                   |
|                          | 200 | 23.3                                    | 23.6                | 20.3                 | 21.3                   |

119 ASE = Aqueous Stem Extract. ESE = Ethanolic Stem Extract. \* = No Inhibition

### 120 3.2 Minimum inhibitory concentration (MIC) and Minimum bactericidal conc. (MBC)

121 Table below (Table 3) shows minimum inhibitory concentration (MIC) of the plant extracts. The result  
 122 shows that both aqueous and ethanolic leaf extract of the plant can inhibit the growth of the test  
 123 isolates at concentration of 6.25 - 25mg/ml. The aqueous stem extract has higher MIC of 50mg/ml in  
 124 *E. coli* and *Shigella spp* than in *S. typi* and *P. aeruginosa* but MIC of 12.5-50mg/ml for ethanolic  
 125 extract. This shows that ethanolic extract is more effective against the test isolates.

126 **Table 3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration**  
 127 **(MBC) of the *Psidium guajava* extracts against test isolates**

| EXTRACTS | ORGANISMS/ MIC/MBC (mg/ml) |                     |                      |                        |
|----------|----------------------------|---------------------|----------------------|------------------------|
|          | <i>E. coli</i>             | <i>Shigella spp</i> | <i>P. aeruginosa</i> | <i>Salmonella typi</i> |
| ALE      | 25/25                      | 25/25               | 50/50                | 50/50                  |
| ELE      | 12.5/25                    | 12.5/25             | 25/50                | 25/25                  |

|            |         |         |         |         |
|------------|---------|---------|---------|---------|
| <b>ASE</b> | 25/50   | 12.5/50 | 25/ND   | 12.5/ND |
| <b>ESE</b> | 6.25/25 | 6.25/25 | 12.5/50 | 6.25/25 |

ALE = Aqueous leaf extract ELE = Ethanolic leaf extract ASE = Aqueous stem extract

ESE = Ethanolic stem extract ND = Not detected.

### 3.3 Phytochemical screening

The Table below (Table 4) showed that the phytochemicals were present in both leaf and stem of *Psidium guajava*. Both leaf and stem contain Alkaloid, saponin, phenol, flavonoids, protein and amino acid, Anthraquinones, terpenoid and tannin except Steroid

**Table 4: Phytochemical constituents of leaves and stem back of *Psidium guajava***

| S/N | PHYTOCHEMICAL          | LEAF EXTRACT | STEM EXTRACT |
|-----|------------------------|--------------|--------------|
| 1.  | ALKALOID               | +            | +            |
| 2.  | SAPONIN                | +            | +            |
| 3.  | PHENOL                 | +            | +            |
| 4.  | FLAVONOID              | +            | +            |
| 5.  | PROTEIN AND AMINO ACID | +            | +            |
| 6.  | TANNIN                 | +            | +            |
| 7.  | REDUCING SUGAR         | +            | +            |
| 8.  | ANTHRAQUINONES         | +            | +            |
| 9.  | STEROID                | —            | —            |
| 10. | TERPENOID              | +            | +            |

+ = Presence of phytochemical, - = Absence of phytochemical.

The antimicrobial activity is higher at a concentration of 200mg/ml against *Shigella spp* and *Escherichia coli* as compared to *Pseudomonas aeruginosa* and *Salmonella typhi*. *Psidium guajava* ethanolic extract showed higher anti-bacterial activity compared to aqueous extract, this is attributed to better solubility of the active component by the ethanol than water. This shows similarities to the findings of Nwanneka *et al.* [17] which investigated the antimicrobial activity of *Psidium guajava* leaf extract, the results showed that both aqueous and ethanolic extracts of guava leaf inhibited the growth of the bacteria and fungi tested but the ethanolic extract showed stronger inhibition than the

aqueous extract against the organisms. My results was also in conformity with that of Pandey and Shweta, [18] where the results of antibacterial activity of *Psidium guajava* leaf and stem reveals that ethanolic extract showed stronger anti-bacterial activity than aqueous extract. However, this was contrary to the findings of Emmanuel, [19] he stated that the antimicrobial activity of aqueous extract of *Psidium guajava* is higher than that of ethanolic extract. The result of antimicrobial effects of *Psidium guajava* leaf extract by Elekwa *et al.*, [20] showed that aqueous extract of guava leaf had higher inhibitory effects on some organisms than ethanolic extract. This is contrary to my findings. The results of the present research on the antibacterial activity of stem and leaf of *Psidium guajava* on the tested isolates revealed that stem extract posses higher antimicrobial activity than corresponding leaf extract. This hold true with the results of Elekwa *et al.* [20] in which the antibacterial study on the effect of *Psidium guajava* showed that the stem extract is more effective than leaf extract. From the results of MIC determination (Table 3), the minimum inhibitory concentration showed that a very low concentration of 6.25 -12.5mg/ml the ethanolic stem back extract of *Psidium guajava* inhibit the growth of all tested isolates.

Preliminary Phytochemical Analysis of *Psidium Guajava* In the present study was carried out to identify the active constituents such as alkaloids, flavonoids, sterols, terpenoid, Anthraquinones, protein and amino acid, phenol, carbohydrate and cellulose present in the leaves and stem of guava plant. Preliminary phytochemical analysis of leaf and stem back extract, (ethanol and water) of *P. guajava* showed the presence of the following chemical constituents; Alkaloid, saponin, phenol, flavonoids, protein and amino acid, Anthraquinones, terpenoid and tannin (Table 4). Earlier work have revealed the presence of alkaloids, flavonoids, glycosides, poly-phenols, reducing compounds, saponins and tannins in the aqueous extract of *Psidium guajava* leaf [21]. This finding can be attested to the work of Offo, [22] who also reported similar finding on the phytochemical of guava leaf extract, which contain alkaloid, saponin, flavonoids, phenol, steroid, tannin, protein and glycoside. Pandey and Shweta, [18] reported the phytochemicals mainly present in *Psidium guajava* were reducing sugar, tannin, saponin, phlobatannin, terpenoid, alkaloid and phenols. The finding is also similar to that of Joseph and Priya, [23] where the preliminary phytochemical analysis of leaf, stem back and root back extracts of *Psidium guajava* showed the presence of carbohydrate, glycoside, saponin, Anthraquinones, flavonoids, tannin and alkaloids.

#### 4. CONCLUSION

The medicinal uses of these plants *Psidium guajava* are supported by the presence of phytochemical constituents present in them and the antimicrobial activities they exhibit. Based on these findings, the application of the decoction of leaf and stem of the plants in ethno medicine is justified. Hence, the need to exploit the potentials of these plants especially in areas of traditional medicine and pharmaceutical industries arises. It is recommended that Government, Non-governmental organizations (NGOs) and philanthropists should encourage researchers in this field so that the spread of antimicrobial resistant pathogens can be restrained.

## ETHICAL APPROVAL

Ethical approval was obtained from Kano State Hospital Management Board based on the consent of Murtala Muhammad Specialist Hospital ethical committee

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