

IN-VITRO ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *PSIDIUMGUAJAVA* ON SOME ENTERIC BACTERIAL ISOLATES OF PULIC HEALTH IMPORTANCE

ABSTRACT

Aim: The study was conducted to investigate the antibacterial activity of *Psidiumguajava* and its major antibacterial 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 antibacterial activity against some members Enterobacteriaceae 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 bark showed the highest zones of inhibition against tested organisms compared to aqueous extract. 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 bark and leaf extracts contain alkaloids, tannins, terpenoid, Anthraquinone, reducing sugar, amino acid, flavonoid, saponins, glycosides and phenols.

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

Key words: *Psidiumguajava*; Enteric bacteria; Phytochemical; well diffusion; antibacterial activity; extracts;

1. INTRODUCTION

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 antibacterial 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

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The extracts showed adverse effect

30 phytochemical constituents for therapeutic purpose can either be leaves, stem bark 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 bark of medicine to treat variety of diseases [6]. Several hundred genera of plants
36 were utilized traditionally for medicinal purposes. The world health organization [7] reported that 80%
37 of the world population relies chiefly on traditional medicine and a major part of the traditional
38 therapies which involve the use of plant extract and their constituents [8].

39 Guava, *Psidiumguajava*, 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]. *Psidiumguajava* 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 antibacterial activity against
47 *Escherichiacoli*, *Salmonellatyphi*, *Staphylococcusaureus* and a strong antibacterial 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 compounds[13].

51 The objective of the study was to determine the antibacterial activity of leaves and stem bark of
52 *Psidiumguajava* extracts (Both aqueous and ethanolic) against some members of Enterobacteriales
53 namely *Escherichia coli*, *Shigellaspp*, *Pseudomonas aeruginosa* and *Salmonella typhi*, to screen for
54 the phytochemical constituents of the extracts and to evaluate the minimum inhibitory concentration
55 (MIC) 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 stem bark of
59 *Psidiumguajava* which were collected from botanical garden of Government Secondary school

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

67 2.2 Test organisms

68 Clinical isolates of *Escherichia coli*, *Shigella*, *Pseudomonas aeruginosa* and *Salmonella typhi* were
69 obtained from Department of Microbiology of Murtala Muhammad Specialist hospital for further
70 experiment. Identification and characterization of the isolates was conducted there by using three
71 procedures namely Gram staining, cultural characterization using selective or indicative media and
72 biochemical characterization with reference to Cheesbrough [24]. The pure isolates of each of the test
73 organism were inoculated in sterile slants containing Nutrient agar and transported to the department
74 of Microbiology KUST and refrigerated at 4 °C before use.

75 2.3 Preparation of Plant Extract

76 The ethanol and aqueous extract of both plant samples was carried out according to Bengum [13]. 25g
77 of the powdered leaves and stem bark was weighed out and dissolved in 250ml of both solvent in a
78 sterile beaker and allowed to stand for a week.

79 2.4 Antibacterial Assay of Extracts

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

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control in this research. These were then left on the bench for 1 hour 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].

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

Overnight Nutrient broth cultures of *E. coli*, *Shigella*, *S. typhi* and *P. 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 10⁵ cfu/ml. The MIC and MBC were determined using procedure described by Kowser and Fatima [15].

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2.6 Phytochemical Screening

This was done on different extract to ascertain the presence of bioactive component present in the leaves and stem bark 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 (23.3 mm) and *Pseudomonas aeruginosa* the least susceptible (6 mm).

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

CONCENTRATION (mg/ml)			MICROORGANISMS/ ZONE OF INHIBITION (mm)			
			<i>E. coli</i>	<i>Shigella</i> spp	<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
ELE	50	11.6	11.3	07.6	08.3	
	100		15.3	18.3	14.3	10.6
	150		17.3	20.3	15.6	13.3

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E. coli, *Escherichia coli*; *P. aeruginosa*, *Pseudomonas aeruginosa*; *S. typhi*, *Salmonella typhi*

200	20.0	23.3	17.0	18.6
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ALE = Aqueous Leaf Extract. ELE = Ethanolic Leaf Extract. * = No Inhibition

The antibacterial activity of aqueous and ethanolic stem bark extract *Psidium guajava* was represented in the table below (Table 2) 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 (23.6mm) and *Salmonella typhi* the least susceptible (11.3 mm).

Table2: Mean diameter zones of inhibition of Aqueous and ethanolic stem bark extract *P. guajava*

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

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ASE = Aqueous Stem bark Extract. ESE = Ethanolic Stem bark Extract. * = No Inhibition

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

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

Table3: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the *Psidium guajava* extracts against test isolates

EXTRACTS	ORGANISMS/ MIC/MBC (mg/ml)			
	<i>E. coli</i>	<i>Shigella</i> spp	<i>P. aeruginosa</i>	<i>Salmonella typhi</i>
ALE	25/25	25/25	50/50	50/50

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ELE	12.5/25	12.5/25	25/50	25/25
ASE	25/50	12.5/50	25/ND	12.5/ND
ESE	6.25/25	6.25/25	12.5/50	6.25/25

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

129 ESE = Ethanolic stem bark extract ND = Not detected.

130 3.3 Phytochemical screening

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

134 **Table 4: Phytochemical constituents of leaves and stem bark of *Psidiumguajava***

S/N	PHYTOCHEMICAL	LEAF EXTRACT	STEM BARK EXTRACT
136	1. Alkaloid	+	+
137	2. Saponin	+	+
138	3. Phenol	+	+
139	4. Flavonoid	+	+
140	5. Protein and amino acid	+	+
141	6. Tannin	+	+
142	7. Reducing sugar	+	+
143	8. Anthraquinone	+	+
144	9. Steroid	-	-
145	10. Terpenoid	+	+

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

148 The antibacterial activity is higher at a concentration of 200mg/ml against *Shigella* spp. and
 149 *Escherichia coli* as compared to *Pseudomonas aeruginosa* and *Salmonella typhi*.
 150 *Psidiumguajava* ethanolic extract showed higher antibacterial activity compared to aqueous extract,
 151 this is attributed to better solubility of the active component by the ethanol than water. This shows
 152 similarities to the findings of Nwanneka *et al.* [17] which investigated the antibacterial activity of
 153 *Psidiumguajava* leaf extract, the results showed that both aqueous and ethanolic extracts of guava

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154 leaf inhibited the growth of the bacteria and fungi tested but the ethanolic extract showed stronger
155 inhibition than the aqueous extract against the organisms. This result was also in conformity with that
156 of Pandey and Shweta, [18] where the results of antibacterial activity of *Psidium guajava* leaf and stem
157 bark reveals that ethanolic extract showed stronger anti-bacterial activity than aqueous extract.
158 However, this was contrary to the findings of Emmanuel, [19] who investigate the antibacterial effects
159 of some Ghanaian aromatic plants including *Psidium guajava*, he stated that the antibacterial activity of
160 aqueous extract of *Psidium guajava* is higher than that of ethanolic extract. This is due to differences
161 in geographical location of the plant he used compared to the one of the present study. The result of
162 antibacterial effects of *Psidium guajava* leaf extract by Elekwaeta, [20] showed that aqueous extract of
163 guava leaf had higher inhibitory effects on some organisms than ethanolic extract. The results of the
164 present research on the antibacterial activity of stem bark and leaf of *Psidium guajava* on the tested
165 isolates revealed that stem bark extract possesses higher antibacterial activity than corresponding leaf
166 extract. This holds true with the results of Elekwaeta, [20] in which the antibacterial study on the effect
167 of *Psidium guajava* showed that the stem bark extract is more effective than leaf extract. The higher
168 antibacterial activity shown by stem bark extract is as a result of having higher quantity of the
169 phytochemicals in stem bark extract compared to leaves extract.

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170 From the results of MIC determination (Table 3), the minimum inhibitory concentration showed that a
171 very low concentration of 6.25 -12.5mg/ml the ethanolic stem bark extract of *Psidium guajava* inhibit the
172 growth of all tested isolates.

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173 Preliminary Phytochemical Analysis of *Psidium Guajava* In the present study was carried out to identify
174 the active constituents such as alkaloids, flavonoids, sterols, terpenoid, Anthraquinones, protein and
175 amino acid, phenol, carbohydrate and cellulose present in the leaves and stem bark of guava plant.
176 Preliminary phytochemical analysis of leaf and stem bark extract, (ethanol and water) of *P.*
177 *guajava* showed the presence of the following chemical constituents; Alkaloid, saponin, phenol,
178 flavonoids, protein and amino acid, Anthraquinones, terpenoid and tannin (Table 4). Earlier work have
179 revealed the presence of alkaloids, flavonoids, glycosides, poly-phenols, reducing compounds,
180 saponins and tannins in the aqueous extract of *Psidium guajava* leaf [21]. This finding can be attested
181 to the work of Offo, [22] who also reported similar finding on the phytochemical of guava leaf extract,
182 which contain alkaloid, saponin, flavonoids, phenol, steroid, tannin, protein and glycoside.
183 Pandey and Shweta, [18] reported the phytochemicals mainly present in *Psidium guajava* were

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184 reducing sugar, tannin, saponin, phlobatannin, terpenoid, alkaloid and phenols. The finding is also
185 similar to that of Joseph and Priya, [23] where the preliminary phytochemical analysis of leaf, stem bark
186 and root extracts of *Psidium guajava* showed the presence of carbohydrate, glycoside, saponin,
187 Anthraquinone, Flavonoid, tannin and alkaloids at high concentration.

188 4. CONCLUSION

189 The antibacterial activity of the plant showed that the plant part extracts demonstrated
190 antimicrobial effect against the test isolates with higher activity in stem bark extract compared to
191 leaves extract. Higher antibacterial activity was recorded in ethanol extract compared to aqueous
192 extract. The antibacterial activity of the plant parts is due to the presence of the Phytochemicals
193 identified in this study. The minimum inhibitory concentration (MIC) and minimum bactericidal
194 concentration (MBC) of the extracts ranges from 6.25 – 50mg/ml of the extracts. The
195 Phytochemical screening of the plant parts revealed the presence of Alkaloid, Tannin, Saponin, and
196 Cardiac glycoside, protein and amino acid, Flavonoid, Terpenoid, Phenols and Anthraquinone.
197 Findings from this work support the use of extracts from *P. guajava* stem bark and leaves as
198 medicinal plant. It is recommended that Government, Non-governmental organizations (NGOs)
199 and philanthropists should encourage researchers in this field so that the spread of antibacterial
200 resistant pathogens can be restrained.

201 ETHICAL APPROVAL

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

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