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Determination of Antibacterial Activity of *Psidium guajava* Leaf Extract against Bacteria Isolated from Mobile Phones of Umaru Musa Yar'adua University, Katsina Community

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Abstract

Mobile phones are increasingly becoming one of the indispensable accessories of professional and social daily life, although constant handling and usage in various environmental conditions makes it fomite. Thus, antibacterial activity of *Psidium guajava* leaf extract was evaluated against bacteria isolated from mobile phones within Umar Musa Yar'adua University, Katsina community. A total of sixty (60) mobile phones were randomly swabbed, twenty (20) each, from hawkers, students, and staff of the University community. These were used as candidates for isolation of bacterial contaminants using standard protocols. Variable concentrations (500mg/ml, 300mg/ml and 100mg/ml) of aqueous extract of the *Psidium guajava* were prepared and tested against mobile phones bacterial isolates using agar well diffusion method. Preliminary phytochemical screening revealed the presence of flavonoids, cardiac glycosides, saponins, tannins and terpenoids. *Staphylococcus aureus* (39.6%), *Escherichia coli* (29.7%), *Klebsiella* sp. (18.8%), *Proteus* sp. (8.3%) and *Pseudomonas aeruginosa* (4.2%) were the bacterial contaminants isolated and identified from the mobile phones. Aqueous leaf extract of the plant displayed promising antibacterial activity at 500mg/ml against all the isolates, with average zones of inhibition of 25.0 mm for *S. aureus*, *Proteus* sp., *P. aeroginosa* and 24mm, 6.0mm for *E. coli* and *Klebsiella* sp. respectively. The use of *Psidium guajava* leaf extract as candidate for production of antibacterial agent which can be used to disinfect mobile handsets is suggested.

Keywords: Mobile phones, contaminants, Antibacterial activity, *Psidium guajava*, antibacterial hand wash

INTRODUCTION

According to Al-Abdalall (2010) mobile phones are long range, portable electronic devices for personal telecommunication and have become integral and indispensable accessories of professional and social daily life. They are increasingly becoming an important means of communication worldwide being easily accessible, economical and user friendly (Gondar, 2015). Due to the benefits of mobile phones, their hazard to health is often overlooked (Suganya and Sympathy, 2012). The constant handling of mobile phones by different users exposes it to an array of microorganisms and the heat generated from mobile phones makes a good breeding ground for transmission of microbes (Brady *et al.*, 2006).

The phenomenon of drug resistance against commonly used antibiotics cannot be overemphasized. Guava extracts have been previously demonstrated as broad spectrum antibacterial agents (Farhana *et al.*, 2016). Previous studies revealed that bacteria are unable to easily develop resistance to the multiple and/or chemically complex phytochemicals present in plant extracts without known side effects (Gupta and Birdi 2017). This research evaluated antibacterial activity of *Psidium guajava* leaf extracts against bacteria associated with mobile phones in UMYU community environment. The objective of the study was to isolate and identify bacterial contaminants associated with mobile phones of staff, students, hawkers and bacterial load of mobile phones in UMYU community, to screen for phytochemical profile of *Psidium guajava* aqueous leaf and it's antibacterial activity against bacteria isolated from the mobile phones.

MATERIALS AND METHODS

Sample Collection and Processing

The study was conducted at UMYU campus. A total of 60 mobile phones were swabbed randomly, 20 each from staff, students, and hawkers. Sterile swab sticks were soaked in sterile peptone water and used to swab the target phone over its surface. The cotton end of each swabbed sample was aseptically cut off and soaked in 10ml of peptone water. These were incubated at 37° C for 24 hours and then used as stock cultures (Kawo and Musa, 2013).

Psidium guajava leaves were obtained from Sauri ba Gurin Zuwa, Katsina. Authentication and confirmation of taxonomic identity of the plant material was done at herbarium unit of Biology Department, UMYU and assigned a Voucher number **UMYU 1927**. The *Psidium guajava* leaves were washed with water; air dried under shade and pulverized into powder using clean mortar and pestle, ultimately sieved and fine powder was obtained according to the methods of (Bukar *et al.*, 2009).

Enumeration of Aerobic Mesophilic Bacterial Count

Enumeration of aerobic mesophilic bacterial count was carried out using-ten-fold serial dilution. An aliquot of 0.1ml of suitable dilutions was pour plated in duplicate using sterile Nutrient agar plates from all the dilutions. The prepared plates were labeled and aerobically incubated at 37° C for 24 hours. Mean counts of colonies were recorded cfu/ml (Cheesebrough, 2001).

Isolation and Identification of Bacterial Contaminants of Mobile phones

Loopfuls of discrete colonies on Nutrient agar were selected and sub-cultured on selective and differential media: Eosin methylene blue agar, MacConkey Agar and Mannitol salt agar media. Inoculated plates were incubated at 37°C for 24 hours. Discrete colonies were subjected to Gram's staining and biochemical tests: catalase, citrate, indole, urease, MR-VP and motility (Cheesbrough, 2001; Akintobi *et al.*, 2013).

Extract Preparation

The method of Olayemi and Opaleye (1999) was adapted for the extraction of plant. Water solvent was used for extraction using the maceration procedure. This was carried out by measuring 50g of fine powder of *Psidium guajava* (leaves) on an electronic weighing balance. This was dispensed into flat bottle flask containing 500ml of water. The flask was placed on a flat form shaker. After 3 days of soaking in the solvent, the mixture was filtered with muslin cloth and then Whatman No. 1 filter paper, which was then, evaporated using rotary evaporator. Then, transferred to an evaporating dish and placed on a water bath till the dried crude extract was obtained. The extract was stored in screw cap bottles at 4°C until needed.

Phytochemical Screening

The aqueous *P. guajava* leaves extract was subjected to phytochemical screening to test for the presence or absence of secondary metabolites using the method described by Sofowara (1993). Briefly mention the phytochemicals and the methods.

Preparation of Varied Concentrations of Plant Extracts

Stock solutions of plant extracts were prepared by dissolving 1g of the extract in 1ml of Dimethyl sulfoxide (DMSO). From the stock solution, 0.1ml was transferred in to a sterile bijou bottle and 0.9ml of DMSO was added to give 100mg/ml. Subsequently, 0.3ml of the stock solution was transferred into another bottle containing 0.7ml DMSO, to give 300mg/ml. Similarly, 0.5ml of the stock solution was transferred in to bijou bottle containing 0.5ml DMSO to give 500mg /ml (Deeni and Hussein, 1991).

Preparation of McFarland Standard and Standardization of Inocula

0.5 McFarland standard was prepared by adding 0.6ml of 1% Barium Chloride ($BaCl_2$) solution onto 99.4ml of 1% Sulphuric acid (H_2SO_4) solution. Isolated and identified Bacterial colonies were sub-cultured onto Nutrient agar and incubated for 24 hours at 37ºC. Obtained colonies were placed into a test tube containing 2ml normal saline continuously, until the turbidity of inoculum test tube matched with the turbidity of McFarland standard test tube (Cockeril *et al.*, 2012).

Antibacterial Activity of the Extract

Antibacterial activity of the *P. guajava* aqueous extracts was tested using agar well diffusion method on Mueller Hinton agar (MHA) (Saulawa and Muhammad 2017). MHA was prepared and poured into sterile petri dishes (25ml) in triplicates and allowed to solidify at room temperature. The standardized inoculum of the bacterial isolates was streak onto the MHA plates using carpet streaking. A sterile cork borer (6mm) was used to bore three wells in the inoculated medium. Thereafter, 0.1ml of different concentrations (500mg/ml, 300mg/ml and 100mg/ml) of the extracts was dispensed into separately labeled wells using sterile 1ml syringe. The preparations were incubated at 37ºC for 24 hours. The mean zones of inhibitions were measured using centimeter rule and recorded in millimeters. Triplicate readings were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

The different concentrations of the extracts (500mg/ml, 300mg/ml and 100mg/ml) were prepared using sterile 10ml test tubes. From each concentration, 1ml was dispensed into test tubes containing 4ml Nutrient broth. Furthermore, 0.1ml of each standardized bacterial inoculum was inoculated into each of the prepared test tubes. The tubes were incubated at 37ºC for 24 hours. The tubes were examined for absence of growth by checking absence of turbidity. The test tube containing the lowest dilution that showed no sign of growth gives the MIC (Saulawa and Muhammad 2017).

Determination of Minimum Bactericidal Concentration (MBC)

The test tubes that showed no visible growth from MIC were sub cultured on sterile Nutrient agar plates. The plates were incubated at 37ºC for 24 hours. The plate containing the lowest concentration that showed no growth was the MBC (Saulawa and Muhammad 2017).

Statistical Analysis

All statistical analyses were conducted using Microsoft Excel Data Analysis ToolPak (2013 version) (Berk & Carey, 2010). The comparisons were computed using One-way ANOVA to test whether a statistically significant difference exists between: the occurrence positive and negative samples amongst the mobile phones involved in the study; the distributions and frequencies of the bacteria isolated from

Hawkers, Staff and Students; and the zones of inhibition elicited by the *Psidium guajva* aqueous leaf extracts verses the positive control. Likewise, the zones generated by the extract and the positive control were compared using two-tailed t-test. In all calculations, p values ≤ 0.05 are considered significant.

RESULTS

The results of the present study (table 1) showed that mobile phones of hawkers had the highest contamination of bacteria, with 18 out of 20 (37.5% of all positive samples in the study area) followed by students with 16 (33.3%); with the staff having the lowest proportion of mobile phones from which bacteria were isolated i.e. 14(29.1%).

Table 2 revealed that the overall mean aerobic mesophillic bacterial counts of 2.12×10^6,2.54×10^5 and 2.14×10^3CFU/ml were recovered from hawkers, students and staff respectively. The findings (table 3) showed that the frequency of occurrence of identified bacterial isolates of mobile phones from members of the UMYU community, that there is no significant difference in the frequency of the various bacteria (P = 1, F_{cal} = 0, F_{crit} = 3.84); and also there is no significant difference between the three groups; Hawkers, Staff and Students (P = 0.41, F_{cal} = 1.01, F_{crit} = 4.46). This shows that the frequency of distribution of the species of bacteria contaminating mobile phones shows equal distribution; likewise, the composition of the bacterial contaminants on the mobile phones of hawkers, staff and students is homogeneous, because there is no statistically significant difference between the composition of the bacteria from hawkers, staff and students' mobile phones. Phytochemical analysis of *Psidium guajava* leaves in the present study revealed the presence of flavanoids, glycosides, saponins, tannins and terpenoids. Study showed that guava leaves aqueous extract exhibited marked antibacterial activity as evidenced by zones of inhibition, ranging from 18mm to 25mmfor *S. aureus*, 18mm to 24mm for *E. coli*, 6mm for *Klebsiella* spp, 20mm to 25mm for *Proteus* spp, 18mm to 25mm for *P. auroginosa* which were comparable to that of oxytetracycline, which exhibited zones of 23mm, 22mm, 21mm, 26mm, 7.0mm for *S. aureus*, *E. coli*, *Klebsiella* spp, *Proteus* spp, *P. auroginosa* respectively, to the highest concentration tested.

Table 1: Prevalence of Bacteria Isolated from Mobile Phones of UMYU Community

Source of samples	Number-of samples	Bacterial Isolates			
		Positive		Negative	
		No	%	No	%
Hawkers	20	18	37.5	2	16.67
Students	20	16	33.3	4	33.3
Staff	20	14	29.2	6	50
Total	**60**	**48**	100	**12**	100

P (one-way ANOVA statistically significant difference between positive and negative samples) = 0.0018, F_{cal} = 54.00 F_{crit} = 7.71.

Table 2: Bacterial Load of Mobile Phones of UMYU Community

Sample source	Aerobic Mesophilic Bacterial (cfu/ml)
Hawkers	2.12×10^6
Students	2.54×10^5
Staff	2.14×10^3

Table 3: Frequency of Isolation of Bacteria from Mobile Phones of UMYU Community

Organisms	Source of sample			
	Hawkers No (%)	Students No (%)	Staff No (%)	Total No (%)
Staphyloccus aureus	7 (36.8)	6 (31.6)	6 (31.6)	19 (100)
Escherichia coli	4 (28.6)	5 (35.7)	5 (35.7)	14 (100)
Pseudomonas species	1 (50.0)	0 (00.0)	1 (50.0)	2 (100)
Proteus species	3 (17.0)	1 (6.25)	0 (00.0)	4 (9.0)
Klebsiella species	3 (33.4)	4 (44.4)	2 (22.2)	9 (100)

NB: P (one-way ANOVA statistically significant difference in distribution of the bacteria) = 1, F_{cal} = 0, F_{crit} = 3.84; P (one-way ANOVA statistically significant difference between Hawkers, Staff and Students) = 0.41, F_{cal} = 1.01, F_{crit} = 4.46.

Table 4: Phytochemical Screening of *Psidium guajava* Leaf Extract

Extract	Phytochemical profile				
	Flavanoids	Glycosides	Saponins	Tannins	Terpenoids
Psidium guajava	+	+	+	+	+

Key: + indicates positive

Table 5: Antibacterial Activity of *Psidium guajava* Aqueous Leaf Extract against Bacterial Isolates of Mobile Phones

Bacterial isolates	*P. guajava* aqueous leaf extract concentrations(mg/ml)			Commercial (mg/ml)		Oxytetracycline
	100	300	500	100	300	500
	Zones of inhibition (mm/SD)					
S. aureus	18.0 ±1.73	20.0 ±1.50	25.0 ±1.45	14.0±1.41	22.0 ±2.51	23.0 ±0.36
E. coli	18.0 ±0.87	20.0 ±1.32	24.0 ±0.66	22.0±2.31	19.0 ±1.41	22.0 ±0.70
Klebsiella spp	6.0 ± 0.00	6.0 ± 0.00	6.0 ± 0.00	12.9±2.80	14.0 ±0.90	21.0 ±0.26
Proteus spp	20.0 ±0.46	22.0 ±1.00	25.0 ±2.65	22.0±2.12	20.0 ±1.47	26.0 ±1.31
P. auroginosa	18.0 ±0.96	22.0 ±1.25	25.0 ±1.61	6.0 ± 0.00	6.0 ± 0.00	7.0 ±0.26

Key: Zones of inhibition were presented as average ± standard deviation of three replicates. A reading of 6.00, i.e. the diameter of the well, indicates no activity.

NB: P (statistically significant difference of zones of inhibition of the extracts vs positive control, using t-test) = 0.79, T_{cal} =0.27, $T_{crit\ two\ tail}$ = 2.05; and for one-way ANOVA, (p = 0.79, F_{cal} = 0.07, F_{crit} = 4.20).

Table 6: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of *Psidium guajava* Aqueous Leaf Extract

Bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
S. aureus	300	500
E. coli	100	300
Klebsiella spp.	ND	ND
Proteus spp.	100	300
P. auroginosa	100	100

Keys: ND indicates not detected

DISCUSSION

Among all the studied participants *Staphyloccus aureus* was the most frequently isolated organism followed by *Escherichia coli* and *Klebsiella species*. In a similar study Kawo and Musa (2013) reported that the most frequently isolated bacteria were: *E. coli* 7 (25.0%) and *S. aureus* 15 (53.6%). The study also corresponds to the findings of Abdalall (2014), from Saudi Arabia, whereby *S. aureus* (56.58%) was the most abundant bacteria isolated from mobile phones of students and staff of Biological Sciences Department, King AbdulAziz University, followed by *Staphylococcus epidermidis* (13.57%). However in their study, *Pseudomonas aeruginosa* (8.01%) was the third most commonly isolated bacterium, which is contrary to the current study, as it was the least isolated bacterium. They also identified *Neissera sicca* (7.73%), *Micrococcus luteus* (6.51%), *Proteus mirabilis* (3.66), *Bacillus subtilis* (2.85%) and *Enterobacter aerogenes* (1.09%).

Likewise, another study that contradicts the current study was the study carried out by Koscova *et al*. (2018), wherein they reported that the common skin commensal bacteria, coagulase negative *Staphylococci* were diagnosed most frequently. Results of this study were consistent with results recorded by Gondar (2015), where the most commonly isolated bacteria were *S. aureus* (35.30%), *E. coli* (23.53%), *Streptococcus* sp. (17.65%) and *Enterobacter aerogenes* (23.53%), respectively.

This The study was closely in agreement with the findings of Enass (2015) whereby a total of 25 samples were collected from the mobile phones of students of college of science Biology Department, Baghdad University, and the bacteria encountered include: *S. aureus*, coagulase negative *Staphylococci* (*S. epidermidis*), *S. pyogenes*, *E. coli* and *P. aeruginosa*.

The counts from this study are within WHO limits. This corresponds to the findings of Kawo and Musa (2013), where all the sampled mobile phones groups had viable counts lower than 10^6 CFU/ml. The observation that hawkers have aerobic mesophilic bacterial counts exceeding the WHO limit, are similar to the findings of Yusha'u *et al*. (2010), who reported heavy bacterial contamination of both commercial and personal mobile phones in some location within Kano metropolis.

The phytochemicals reported in the current study were reported previously in a study by Uboh *et al*. (2010) revealed the presence of alkaloids, flavonoids, glycosides, poly-phenols reducing compound, saponins, and tannins in the aqueous extract of *P. guajava* leaf. The result of this study is closely similar to the findings of Pandey and Shweta (2015), who reported the presence of reducing sugar, tannin, saponin, terpenoid, alkaloid, phenol and phlobatannin. Offor (2015) reported that the phytochemicals present in the in *P guajava* mostly include alkaloids, saponins, flavonoids, phenol, steroid, tannin, protein and glycosides.

The zones of inhibition obtained in the study vary significantly with increasing concentration of the leaf extract (P=0.0027) and that it also varies significantly with the type of bacteria isolated (P=0.0000). The study also showed that there is no significant difference in the antibacterial activity of the leaf extract and the positive control (oxytetracycline) on the bacterial isolates (P=0.79).

The findings of this study are in agreement with those obtained by Vieira *et al*. (2001). In their study, they observed that the growth of *S. aureus* was inhibited by aqueous leaf extract of *P. guajava*. Additionally, Demello and Gnan (1999) reported that *P. guajava* extracts inhibited the growth of *S. aureus*. This also corresponds to the finding of Kenneth *et al*. (2017), who reported significant levels of activity by the extract against isolates similar to those used in the current study, at lower concentrations.

In the current study, *Klebsiella* sp. isolates showed resistance to the extract. This might be caused by the concentration of the extract used (Uthman *et al.*, 2019), i.e. the isolates might be susceptible at concentrations higher than those tested in the current study. The absence of a statistically significant difference between the zones of inhibition elicited by the *P. guajava* extract in comparison to those produced by Oxytetracycline shows that the extract compares favorably against the antibiotic, and thus potential antibacterial properties.

CONCLUSION

The study identified mobile phones as potential agents in the transmission bacteria of public health importance. *Psidium guajava* aqueous leaf extract have demonstrated antibacterial activity on the bacteria isolated which is not significantly different from that of the standard antibiotic used as a control. The study indicated that *Psidium guajava* aqueous leaf extract could be exploited as candidate material for the production of antibacterial agent which can be used to disinfect mobile handsets.

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