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Research Article

Antibacteria Activities of Guava (*Psidium guajava*) and Orange (*Citrus sinensis*) Leaves Extracts on *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT
This study aimed at determining the antibacterial activities of *Psidium guajava* and *Citrus sinensis* leaves extracts of aqueous, ethanol, and methanol on *Staphylococcus aureus and Escherichia coli*. Dimethyl sulfoxide was used for dissolving the leaves extracts and the minimum inhibitory concentration (MIC) of the extracts was determined using broth dilution technique to observe their antibacterial activities against *S. aureus* and *E. coli*. The phytochemical screening revealed the presence of metabolites (flavonoids, tannins, steroid saponins, saponins and alkaloids). The inhibition zone of the aqueous, ethanol and methanol extracts of *P. guajava* and *C. sinensis* against *Staphylococcus aureus and Escherichia coli* was highest at 200mg/ml (18mm and 17mm), while the least inhibition zone was at 12.5mg/ml (7mm and 0mm) respectively. The aqueous extracts of *P. guajava* and *C. sinensis* against *E. coli* and *S. aureus* had its MIC at 50g/ml and 100mg/ml respectively, while ethanol extracts of *P. guajava* and *C. sinensis* against the bacterial (*E. coli* and *S. aureus*) had its MIC at 50g/ml. The methanol extracts of *P. guajava* and *C. sinensis* showed the growth of *S. aureus* in all concentrations (12.5g/ml, 25g/ml, 50g/ml, 100mg/ml and 200mg/ml) while *E. coli* had no growth from 100mg/ml above concentrations and 50g/ml (MIC) of *P. guajava* leaf extracts. These findings, therefore support the traditional use of *Psidium guajava and Citrus sinensis* for its curative potential to synthetic drugs which are expensive, not easily accessible with potential side effects.

Keywords: Antibacterial; *Psidium guajava; Citrus sinensis;* Phytochemical; Extract

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NTRODUCTION

Plant existed on the face of this earth since time immemorial and from ancient times human being and animals derive several benefits from them. The world health organization (WHO, 2023) estimates that about 88% of the population of the world still depends on herbal medicine for treatment of various disease (Rani *et al.,* 2010). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources (Kim *et al.,* 2004*)*. 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 and antioxidant activities (Kim *et al.,* 2004*)*. Over 40% of all modern clinical drugs are of natural product and natural products play important roles in drug development in the pharmaceutical industry (WHO, 2023). Guava plant (*Psidium guajava* L.) belonging to family *Myrtaceae* and Orange (*Citrus sinensis*) belonging to the family Rutaceae are traditionally used plant because of its food and nutritional value. Guava and orange is widely grown in tropical and many areas like Nigeria, India, Bangladesh and Florida (Sushmita *et al.,* 2020).

Different parts of the *Psidium guajava* and *Citrus sinensis* are reported to be used in folk medicine (Kim *et al.,* 2004*)*. Various parts of the plant like root, bark, leaves and fruits are found to possess many pharmacological properties as it is used in the treatment of various disorders (Sushmita *et al.,* 2020). Various evidences depict that the leaves and bark of *P. guajava* tree possess a long history of medicinal uses. The aqueous extract of guava and orange leaves has been reported to be efficacious in the treatment of various types of gastrointestinal disturbances such as diarrhoea, inhibition of the peristaltic reflex and gastroenteritis (Adegboye *et al.,* 2008). Moreover the whole plant is used as skin tonic and is employed in the treatment of female related disease like dysmenorrhoea, miscarriages, uterine bleeding and premature labour. Recent studies on the pharmacological properties of the bark, fruit and leaves depicts antibacterial, hypoglycaemic, anti-inflammatory, antipyretic, spasmolytic and central nervous system depressant activities (Akinola *et al.,* 2007)

Staphylococcus aureus is a gram positive bacteria of bacillota and part of the microbiota of the body frequently found in respiratory tract, human skin, in the nose, armpit, and groin. *Staphylococcus aureus* infection can affect anyone and spread from person to person, although certain groups of people are at greater risk, including people with chronic conditions such as diabetes, cancer, vascular disease, eczema, lung disease (Faturi *et al.,* 2010). *Escherichia coli* is predominantly gram-negative bacteria commonly found in the intestine of humans causing various illness such as food poisoning, urinary tract infection, abdominal and pelvic infection, pneumonia, bacteremia, meningitis, among others (Ibe and Maduagwu, 2013). Therefore, analysis of antibacterial activities of *Psidium guajava and Citrus sinensis* will play a major role in curbing infection and spread of diseases resulting from *Escherichia coli* and *Staphylococus aureus.* This will also provide important information on the ethnomedicinal and ethno pharmacological uses of these plants.

MATERIALS AND METHODS

Area of study

This research was carried out at Biological Science Laboratory of Federal University Dutsin-Ma, Botanical Garden and ASAD Pharmaceutical laboratory, Kano.

Sample Collection and Identification of Plant Materials

Leaves of Guava (*P. guajava*) and orange (*Citrus sinensis*) were used in this study. The plant materials (Leaves) were obtained in June, 2023 from Dutsin-Ma metropolis Katsina state, Nigeria. Identification and authentication (comparison with properly identified herbarium specimen and published plant descriptions) of the plant materials were carried out at the herbarium of the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, Katsina State, Nigeria. A voucher number FUDMA *P. guajava* 0078 and *C. sinensis* 00121 were assigned. Voucher specimen was deposited in the herbarium of the Department of Plant Science and Biotechnology, Federal University Dutsin-Ma, Katsina State for future reference.

Preparation of Leaves Extracts

The fresh leaves of *Psidium guajava* and *C. sinensis* were washed and air dried for two weeks. After drying, the samples were grounded to fine powder using sterile pestle and mortar under laboratory condition. Aqueous, ethanol and methanol extracts of *Psidium guava*, *citrus sinensis* leaves were prepared separately. Fifty grams (50g) powder of the leaves was soaked in 500 ml each of distilled water, ethanol and methanol separately in a flask for three days at room temperature with intermittent shaking using a centrifuge after which filtration was done using whatman filter paper. The methanol and ethanol extracts were evaporated separately at 50°C using rotary evaporator while the aqueous extract was evaporated at 60°C in water bath until dried extract samples were obtained. All the dried extract samples were dissolved in 30% Dimethylsulfoxide (DMSO) separately to the final concentration of 200 mg/ml as a stock concentration. The extracted solutions were stored at 4°C before use as described by Usman *et al.* (2005).

Qualitative Phytochemical Screening

The phytochemical screening of the plant materials for various phytochemical constituents such as flavonoids, alkaloids, glycoside, steroids, saponin and tannin was conducted using standard methods as described by Usman *et al.* (2009)

Test for Alkaloids: Five milliliter of the extract was added to 2ml of Hydrochloric acid (HCL). One milliliter of dragendroff's reagent was added to the whole mixture, a red or orange color with precipitate indicates the presence of alkaloids.

Test for Flavonoids: One milliliter of the extract and a few drops of dilute sodium hydroxide were used. An intense yellow color produced in the mixture after five minutes becomes colorless on another addition of few drops of dilute HCL that indicate the presence of flavonoids.

Test for Steroids: Two milliliter of dissolved extract was added to 10ml of chloroform and an equal volume of concentrated Sulphuric acid was added by the side of the test tube. The presence of steroids is indicated by the appearance of a red color at the upper layer and the sulphuric acid layer.

Test for Saponins: The leaves extract was diluted in 20ml of distilled water and it was agitated in a graduated cylinder for 15mins. The formation of l cm layer foam indicates the presence of Saponins.

Test for Tannins: Five milliliter of the stem extracts and few drops of 1% (0.01ml) lead acetate were mixed. The formation of yellow precipitate indicates the presence of tannins.

Media preparation: The media used for the bacteria culture was according to the manufacturer instruction (Sigma-Aldrich).

Assessing the Antibacterial Activities: Mueller Hinton agar was poured aseptically into petri dishes and was allowed to set. The surface of the plates was streaked with 0.1 ml standardized inoculum (Mcfarland standard) of the test bacteria. Thereafter, a sterilized 6mm cork borer was used to create holes on the agar plates and the holes were filled with 100µl of 0.5g of the plants extracts dissolved in 1ml dimethyl sulfoxide (DMSO). The plates were allowed to stand for about 30 minutes for pre-diffusion of the plants extracts, and the plates were incubated at 37ºC for about 24 hours. The plates were observed and the presence of zones of inhibition around the wells were measured and taken as an indication of antimicrobial activity (Ramesh *et al.,* 2001). The experiment was carried out in two replicates and the mean for each bacterium was determined using the antibiotic (ciprofloxacin) to observe the highest minimum inhibitory concentration of bacteria growth as described by Chikezie, (2017).

Determination of Minimum Inhibitory (MIC)**:** The minimum inhibitory concentration (MIC) of the extracts was determined using broth dilution technique. Two fold serial dilutions of the extracts were prepared by adding 2ml of 100mg/ml of the extract into a test tube containing 2ml of Nutrient broth, thus producing solution containing 50mg/ml of the extract. The process continue serially up to test tube No. 5, hence producing the following concentrations; 100, 50, 25, 12.5, 6.25 mg/ml. Test tube No. 6 do not contain extracts and serve as negative control. Exactly 0.5 ml of 0.5 McFarland equivalent standards of test bacteria were introduced into the test tubes and incubated at 37^0C for 18-72 hours. After the first 24 hours of incubation the test tubes were observed for growth by checking for turbidity as describe by Chikezie, (2017).

RESULTS

The findings of this study on phytochemical analysis of *P. guajava* and *C. senensis* leaf as presented in Tables 1 and 2 revealed the presence of certain phytochemicals using aqueous, methanol and ethanol extracts. Tannin was highly detected in all extraction solution, saponin and alkaloid was detected mildly in aqueous and methanol extraction while alkaloid was highly detected using ethanol extraction. Flavonoid was highly detected during aqueous and methanol extraction and mildly detected in ethanol extraction. Glycoside was not detected in all leaves extractions of *P. guajava* and *C. senensis.*

The findings of the antibacterial activities of aqueous, ethanol and methanol extracts of *P. guajava* and *C. sinensis* leaves on *Staphylococcus aureus and Escherichia coli* as presented in Table 3 showed that the highest bacterial activity of the leaves extract on *Staphylococcus aureus and Escherichia coli* was at 200mg/ml (18mm and 17mm) while the least was at 12.5mg/ml (7mm and 0mm) respectively. The results for the minimum inhibitory concentrations (MIC) of aqueous, ethanol and methanol extracts of *P. guajava* and *C. sinensis* as presented in table 4, 5 and 6. The bacterial (*S. aureus* and *E. coli*) subjected to *P. guajava* and *C. sinensis* leaves extracts on the agar plate that showed no growth after 24 hours of incubation but showed growth on agar plates after further 24 hours with the addition of equal quantity of nutrient broth was said to have minimum

inhibitory concentration (MIC). The aqueous extracts of *P. guajava* and *C. sinensis* at 12.5g/ml, 25g/ml and 50g/ml showed bacteria (*E. coli*) growth after incubation in nutrient broth but at 100mg/ml and 200mg/ml no bacteria growth was detected, while bacterial growth (*S. aureus*) was detected only at 12.5g/ml and 25g/ml and no growth at higher concentrations. This was indicative that 50g/ml and 100mg/ml was the MIC for *E. coli* and *S. aureus* respectively (Table 4). The ethanol extracts of *P. guajava* and *C. sinensis* at 12.5g/ml and 25g/ml showed bacterial (*E. coli* and *S. aureus*) growth but at 50g/ml, 100mg/ml and 200mg/ml no bacteria growth was detected. This was indicative that 50g/ml was the MIC for *E. coli* and *S. aureus* (Table 5). The methanol extracts of *P. guajava* and *C. sinensis* showed the growth of *S. aureus* in all concentrations (12.5g/ml, 25g/ml, 50g/ml, 100mg/ml and 200mg/ml) while *E. coli* had growth at 12.5g/ml, 25g/ml, 50g/ml and no growth from 100mg/ml above concentrations and 50g/ml (MIC) of *P. guajava* leaf extracts (Table 5).

Table 1. Qualitative phytochemical screening of the aqueous, ethanol and methanol leaves extracts of *Pisdium guajava*

Phytochemicals	Aqueous	Ethanol	Methanol
Tannins	++	++	++
Saponins	+	+	+
Alkaloids	+	++	+
Flavanoids	++	+	++
Steroids	+	+	+
Glycosides	-	-	-

Keys: (-) = not detected, (+) = mildly detected, (++) = Highly detected

Table2. Qualitative phytochemical screening of the aqueous, ethanol and methanol leaves extracts of *Citrus sinensis*

Phytochemicals	Aqueous	Ethanol	Methanol
Tannins	+	++	++
Saponins	+	+	+
Alkaloids	+	+	+
Flavanoids	+	+	+
Steroids	+	+	+
Glycosides	-	-	-

Keys: (-) = not detected, (+) = midly detected, (++) = Highly detected

Table 3. Antibacterial activities of *Psidium guajava* and *Citrus sinensis* aqueous, ethanol and methanol leaf extracts on *Staphylococcus aureus and Escherichia coli*

Concentrations	**Leaf sample**	**200mg/ml**	**100mg/ml**	**50mg/ml**	**25mg/ml**	**12.5mg/ml**
Aqueous leaf extracts						
S. aureus	*P. guajava*	15mm	13mm	11mm	7mm	0mm
E. coli	*P. guajava*	13mm	11mm	7mm	0mm	0mm
S. aureus	*C. sinensis*	18mm	13mm	11mm	9mm	7mm
E. coli	*C. sinensis*	17mm	13mm	11mm	7mm	0mm
Ethanol leaf extracts						
S. aureus	*P. guajava*	18mm	13mm	11mm	7mm	0mm
E. coli	*P. guajava*	18mm	11mm	7mm	0mm	0mm
S. aureus	*C. sinensis*	17mm	11mm	11mm	7mm	7mm
E. coli	*C. sinensis*	17mm	11mm	11mm	7mm	0mm
Methanol leaf extracts						
S. aureus	*P. guajava*	18mm	15mm	11mm	7mm	0mm
E. coli	*P. guajava*	18mm	11mm	7mm	0mm	0mm
S. aureus	*C. sinensis*	18mm	13mm	11mm	7mm	7mm
E. coli	*C. sinensis*	17mm	13mm	11mm	7mm	0mm

Table 4. Minimum Inhibitory Concentration (MIC) of *P. guajava* and *C. sinensis* aqueous extracts

Concentration	**Leaf sample**	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. aureus	*P. guajava*	-	-	-	+	+
E. coli	*P. guajava*	-	-	+	+	+
S. aureus	*C. sinensis*	-	-	-	+	+
E. coli	*C. sinensis*	-	-	+	+	+

Keys: (-) = No bacterial growth, (+) = bacterial growth

Table 5. Minimum inhibitory Concentration (MIC) of *P. guajava* and *C. sinensis* ethanol extracts

Concentration	Leaf sample	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. aureus	*P. guajava*	-	-	-	+	+
E. coli	*P. guajava*	-	-	-	+	+
S. aureus	*C. sinensis*	-	-	-	+	+
E. coli	*C. sinensis*	-	-	-	+	+

Keys (-) = No bacterial growth, (+) = bacterial growth

Table 6. Minimum inhibitory Concentration (MIC) of *P. guajava* and *C. sinensis* methanol extracts

Concentration	Leaf sample	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml
S. aureus	*P. guajava*	+	+	+	+	+
E. coli	*P. guajava*	-	-	-	+	+
S. aureus	*C. sinensis*	+	+	+	+	+
E. coli	*C. sinensis*	-	-	+	+	+

Keys (-) = No bacterial growth, (+) = bacterial growth

DISCUSSION

The qualitative phytochemical screening of the aqueous, ethanol and methanol extracts of *Pisdium guajava* plan and *Citrus sinensis*. Results presented in Tables 1 and 2 show the presence of flavonoid, tannins, saponins, steroid and alkaloid. Glycosides were found to be absent. The chemical constituents present in the extracts have some therapeutic values. This agree with Tona *et al.* (2011) who reported that tannins are plant metabolites well known for their antibacterial properties, flavonoids have both antifungal and antibacterial activities, and they possess anti-inflammatory activity. Similarly Usman *et al.* (2009) reported that flavonoid, and alkaloids are known to have antimicrobial and bactericidal properties against several infections. In the antimicrobial studies, the majority of the organisms were more sensitive to methanolic extract (Usman *et al.*, 2005). The anti-bacterial activity and inhibitory effect of plant extracts can be attributed to the presence of secondary metabolites. The methanol extract of leaves of *P. guajava* and *C. sinensis* was more active against the entire microorganisms than the ethanol and aqueous extract, this agrees with the report of Adegboye *et al.* (2008).

The findings on the antibacterial activities of aqueous, ethanol and methanol leaves extracts of *P. guajava* and *C. sinensis* on *Staphylococcus aureus and Escherichia coli* showed inhibitory activities against the bacterial. The highest bactericidal activity of the leaves extract on *Staphylococcus aureus and Escherichia coli* was at 200mg/ml (18mm and 17mm) while the least was at 12.5mg/ml (7mm and 0mm) respectively (Table 3). Although the antibacterial activity of these leaves extract was observed to be more bacteriostatic than bactericidal. This findings is in agreement with the report of Balakrishnan *et al.* (2011) who reported the antibacterial scavenging activities of the stem bark of *Psidium guajava*

The results for the minimum inhibitory concentrations (MIC) of aqueous, ethanol and methanol extracts of *P. guajava* and *C. sinensis* showed that the bacterial (*S. aureus* and *E. coli*) subjected to *P. guajava* and *C. sinensis* leaves extracts on the agar plate showed no growth after 24 hours of incubation but showed growth on agar plates after further 24 hours with the addition of equal quantity of nutrient broth was said to have minimum inhibitory concentration (MIC). This finding is similar with the report of Chikezie, (2017) who reported that tubes containing antibiotics showed no bacterial growth on agar plate until after 24 hours of incubation. The aqueous extracts of *P. guajava* and *C. sinensis* at 12.5g/ml, 25g/ml and 50g/ml showed bacteria (*E. coli*) growth after incubation in nutrient broth but at 100mg/ml and 200mg/ml no bacteria growth was detected, while bacterial growth (*S. aureus*) was observed only at 12.5g/ml and 25g/ml and no growth at higher concentrations. This was indicative that 50g/ml and 100mg/ml was the MIC for *E. coli* and *S. aureus* respectively (Table 4). Biswas *et al.* (2022) reported similar findings on the ethanol extract of stem bark of *Psidium guajava* with minimum inhibition on bacterial activities. The ethanol extracts of *P. guajava* and *C. sinensis* at 12.5g/ml and 25g/ml showed bacterial (*E. coli* and *S. aureus*) growth but at 50g/ml, 100mg/ml and 200mg/ml no bacteria growth was observed. This was indicative that 50g/ml was the MIC for *E. coli* and *S. aureus* (Table 5). Similarly (Adegboye *et al.,* 2008 and Chikezie, 2017) reported that *E. coli* and *K. pneumonia* showed turbidity after incubation in nutrient broth with different concentrations but not at higher concentrations. The extracts of *Ficus thonningii* inhibited the growth of *E. coli, Pseudomonas aeruginosa* and *S. aureus* at varying concentrations (Usman *et al.,* 2009). The methanol extracts of *P. guajava* and *C. sinensis* showed the growth of *S. aureus* in all concentrations (12.5g/ml, 25g/ml, 50g/ml, 100mg/ml and 200mg/ml) while *E. coli* had growth at 12.5g/ml, 25g/ml, 50g/ml and no growth from 100mg/ml above concentrations and 50g/ml (MIC) of *P. guajava* leaf extracts (Table 5). The difference in the antibacterial effect of the different leaves extracts could be the content level of their different active compounds of *P. guajava* and *C. sinensis*. This difference in the antibacterial effect of the leaf extracts on the bacterial agrees with the report of Adegboye *et al.* (2008) and Biswas *et al.* (2022) also reported same.

CONCLUSION

The leaves extracts (aqueous, ethanol and methanol) of *P. guajava* and *C. sinensis* exhibited varying antibacterial activities against *E. coli* and *S. aureus* in this study suggest that the active compounds detected had potency that are detrimental to the growth and activities of *Staphylococcus aureus and Escherichia coli*. The phytochemicals (flavonoid, tannins, saponins, steroid and alkaloid) detected suggest curative potential of *P. guajava* and *C. sinensis* leaves and their use for ethnomedicinal purpose against the aforementioned diseases and for further pharmaceutical studies.

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