



Antioxidant and Antibacterial Activity of *Anacardium occidentale* and *Psidium guajava* Methanolic Leaf Extracts

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author OOE managed the phytochemical study and author MOA managed the antibacterial analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluates the antioxidant and antibacterial activity of *Anacardium occidentale* and *Psidium guajava* methanolic leaf extracts.

Place and Duration of Study: The study was carried out in the Biochemistry and Microbiology Laboratory, Department of Science Laboratory Technology, School of Pure and Applied Science, Lagos State Polytechnic, Ikorodu, Lagos- Nigeria for the period of three months between August and October 2015.

Methodology: Lycopene and β -carotene was assessed using the method of Nagata and Yamashita while total phenolic and total flavonoid content was assessed by the Folin-Ciocalteu assay and aluminum chloride colorimetric assay respectively. The antioxidant activity was

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evaluated using the DPPH radical scavenging activity. Antimicrobial activity was assessed by the agar well diffusion technique and mode of action was evaluated by studying the leakage of UV₂₆₀ and UV₂₈₀ absorbing materials spectrophotometrically.

Results: *A. occidentale* and *P. guajava* methanolic leaf extracts evaluated in this study possessed significant amount of antioxidant compounds lycopene, β-carotene, total phenol and flavonoids. The extracts exhibited antioxidant activity by scavenging DPPH radicals in a dose dependent pattern with IC₅₀ of 47.45, 43.49, 41.46 and 27.21 µg/mL for *A. occidentale*, *P. guajava*, vitamin C and Gallic acid respectively. Also, the plant extracts exhibited antimicrobial activity against *E. coli*, *S. aureus*, *P. auruginosa* and *C. albicans* and disrupted microbial membrane evident in the increase in absorbance values of UV₂₆₀ and UV₂₈₀ absorbing materials with time.

Conclusion: *A. occidentale* and *P. guajava* methanolic leaf extracts possess antioxidant and antimicrobial activity and serve as potential source of drugs.

Keywords: *Anacardium occidentale*; *Psidium guajava*; lycopene; β-carotene; phenol; flavonoid; drugs.

1. INTRODUCTION

Infection progresses and manifests when all physiological barriers and cells of the immune system have been compromised in an individual. Thus, the need for therapeutic intervention in the form of antibiotics used to inhibit and or halt the pathogenicity of these infectious agents. However, despite the sophistication employed in the synthesis and development of new antibiotics, evolving strains of antibiotic resistant microorganisms have undermined these efforts. Resistance is developed due to arbitrary use of antibiotics leading to alteration of their genetic and metabolic activity [1]. In recent times, researchers across the world now concentrate on the search for new bioactive compound from plant origin to combat these resistant strains of microorganism [2,3].

Oxidative stress is a condition characterized by an imbalance in free radical production and the body's innate antioxidant system to scavenge them in favor of free radicals. The connection of oxidative stress to the occurrence of numerous metabolic and degenerative diseases including stroke, diabetes, heart diseases, aging, Parkinson disease, Alzheimer's disease [4] have increased the search for dietary antioxidants compound from natural products.

Anacardium occidentale (cashew) possess great economic and vast medicinal value [5] with its plant parts-stems, leaves and bark extracts widely used for the treatment of diarrhea, colonic pain and dysentery [6] with reports validating its anti-diabetic, anti-inflammatory and anti-ulcerogenic activities [7]. Arekemase et al. [8] reported the antimicrobial activity of *A. occidentale* extracts using enterotoxin producing bacteria.

Psidium guajava (guava), belonging to the family, Myrtaceae is a plant whose parts are commonly used in the treatment and management of various infectious and non-communicable diseases including gastroenteritis, diarrhea, toothache, malaria, sore throat, vomiting, inflamed gums, dysentery, wounds, ulcers, coughs, and a number of other conditions [9,10]. Although, various reports exist on the antibacterial activity of *P. guajava* [11,12] and *A. occidentale* leaf extracts, however, none exists on their mode of action. The study aims to assess the antioxidant and antimicrobial activity of *A. occidentalis* and *P. guajava* methanolic leaf extract with reference to their mode of action.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Leaves of *A. occidentalis* and *P. guajava* used in this study were obtained from Lagos State polytechnic lawn garden. The leaves were dried under room temperature away from sunlight and heat, and were later reduced to powder form using an electric blender. The powder plant materials were stored in air tight bottles until required.

2.2 Preparation of Plant Extracts

2.2.1 Extraction

The extracts were prepared using solid-liquid extraction. Powdered *A. occidentalis* and *P. guajava* leaves (100 g) were separately extracted with 500mL of methanol each for 24hours. The extract was filtered and concentrated under reduced pressure in a rotary evaporator. The % yield of *A. occidentalis* and *P. guajava*

methanolic leaves extract was 3.18 and 5.56% respectively.

2.3 Determination of β -Carotene and Lycopene Content of the Extracts

β -Carotene and lycopene content of the extracts were evaluated using the method of Nagata and Yamashita [13]. The methanolic leaf extract of *A. occidentalis* and *P. guajava* (0.1g) were each weighed separately into a beaker. Acetone:hexane mixture (4:6, 10 mL) was added and vigorously shaken for 5 min and filtered through a disposable filter (0.45 m, Millipore). Absorbance of the filtrate was measured at 453, 505, and 663 nm and the concentration of β -Carotene and lycopene in the plants were calculated according to the following equations:

$$\beta\text{-Carotene (mg/100 mL)} = 0.216 (A_{663}) + 0.304 (A_{505}) + 0.452 (A_{453})$$

$$\text{Lycopene (mg/100 mL)} = -0.0458 (A_{663}) + 0.372 (A_{505}) + 0.0806 (A_{453})$$

The assays were carried out in triplicates, the results were mean \pm SD and expressed as mg of carotenoid/g of the extract.

2.4 Total Phenolic Content

The total phenolic contents of both extracts were determined spectrometrically using the method of Chun et al. [14]. Folin-Ciocalteu's reagent (1 mL) was added to sample (1mL, 1.0 mg/mL) and mixed thoroughly. To this mixture, 4 ml of sodium carbonate (75 g/L) and 10 mL of distilled water were added and thoroughly mixed. The mixture was allowed to stand for 90 min at room temperature. The absorbance of the reaction mixture was taken at 550 nm. The total phenolic content was extrapolated using a calibration curve ($R^2= 0.9699$) for gallic acid. The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values expressed as mean \pm SD.

2.5 Total Flavonoid Assay

Total flavonoid content of both extracts was measured by aluminum chloride colorimetric assay described by Chang et al. [15]. Each plant extract (0.5 mL) was separately mixed with methanol (1.5mL), AlCl_3 (0.1mL, 10%), sodium or potassium acetate acetate (0.1 mL, 1M) and distilled water (2.8 mL). The reaction mixture was

left for 30 min at room temperature and the absorbance was read at 415 nm. The total flavonoid content was calculated using a calibration curve ($R^2= 0.9961$) for quercetin. The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate and values expressed as mean \pm SD.

2.6 DPPH Free Radical Scavenging Activity

The free-radical scavenging activity of both extracts was measured as decrease in the absorbance of methanol solution of DPPH. A stock solution of DPPH was prepared in methanol (5 mL) was added to 1 mL of extract solution at different concentrations (25, 50, 75, 100 $\mu\text{g/mL}$). After 30 min, absorbance was measured at 517 nm and compared with standards (10-50 $\mu\text{g/ml}$). Scavenging activity was expressed as the percentage inhibition calculated using the following formula:

$$\% \text{ scavenging activity} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

2.7 Antibacterial Test Using the Agar Diffusion Method

The antibacterial activity of the methanolic leaf extract of *A. occidentalis* and *P. guajava* was determined by the agar diffusion method using the well technique. Briefly, all the extracts were dissolved in dimethyl sulfoxide (DMSO 40%, v/v) in order to obtain concentrations of 50 mg/mL. Inoculum of the bacterial strains (10^8 CFU/mL) was then plated using sterile swabs into sterilized Petri dishes containing 20 mL of Nutrient agar. Wells with diameter of 6 mm wells were cut and filled with 100 μL of extract and ciprofloxacin (100 μL , 50 mg/mL) into respective plates while DMSO was used as negative control. The Petri dishes were pre-incubated at room temperature for 3 hours in order to allow complete diffusion of the extracts [16] before incubating at 37°C for 24 h.

2.8 Minimum Inhibitory Concentration of Plant Extracts

The Broth Dilution Method of in Ibekwe et al. [17] was used to determine the MIC of the extracts. The nutrient broth was prepared according to the manufacturer's instruction (10 ml of each broth was dispensed into separate test-tube and was sterilized at 121°C for 15 min and then allowed to

cool. Two-fold serial dilutions of the extracts in the broth were made from the stock concentration of the extracts and the standardized inoculums of the microbes (0.2 mL) were then inoculated into the different concentrations of the extracts in the broth. The test tubes of the broth were then incubated at 37°C for 24 h and observed for turbidity of growth. The lowest concentration which showed no turbidity in the test tube was recorded as the MIC.

2.9 Effect of Plant Extract on Protein and Nucleic Acid Leakage from *E. coli* and *S. aureus*

The mode of action of methanolic leaf extract of *A. occidentale* and *P. guajava* was determined by following the method of Heipierper [18]. Bacterial suspension of test organisms was prepared using an overnight broth culture of test organisms. The bacterial cells were harvested by centrifugation at 6000 rpm for 15 min at 4°C, washed with 10 mM EDTA and twice with distilled water. The cells were re-suspended and the suspension was incubated at room temperature for 30 minutes before adding the plant extract at MIC. Samples were drawn at a regular interval, centrifuged and absorbance was measured at 260 nm and 280 nm to determine the presence of absorbing materials in the suspension.

3. RESULTS

Antioxidant compounds present in *A. occidentale* and *P. guajava* methanolic leaf extracts were quantified. As shown in Fig. 1, *Anacardium occidentale* contained higher concentration of β -carotene, lycopene, while higher concentration of flavonoids and total phenolics was found in *P. guajava*.

The free radical scavenging activity was evaluated by using DPPH radical as a substrate while ascorbic acid was used as reference standard. The scavenging effect of *A. occidentale* and *P. guajava* methanolic leaf extract on the DPPH radical was dose dependent that is; increased percentage radical scavenging activity was observed with increasing plant extract concentration producing IC₅₀ of 47.47 and 42.79 μ g/mL respectively.

The result of antimicrobial activity of the plant extracts is illustrated in Fig. 3. All the organisms were susceptible to the plant extracts and standard drugs (amoxyl and ciprofloxacin). *A. occidentale* produced the highest inhibition zone diameter of 32.67 \pm 0.67 mm for *S. aureus* and the least against *C. albicans* with inhibition zone diameter of 20.67 \pm 0.67 mm. *P. guajava* produced an inhibition zone diameter of

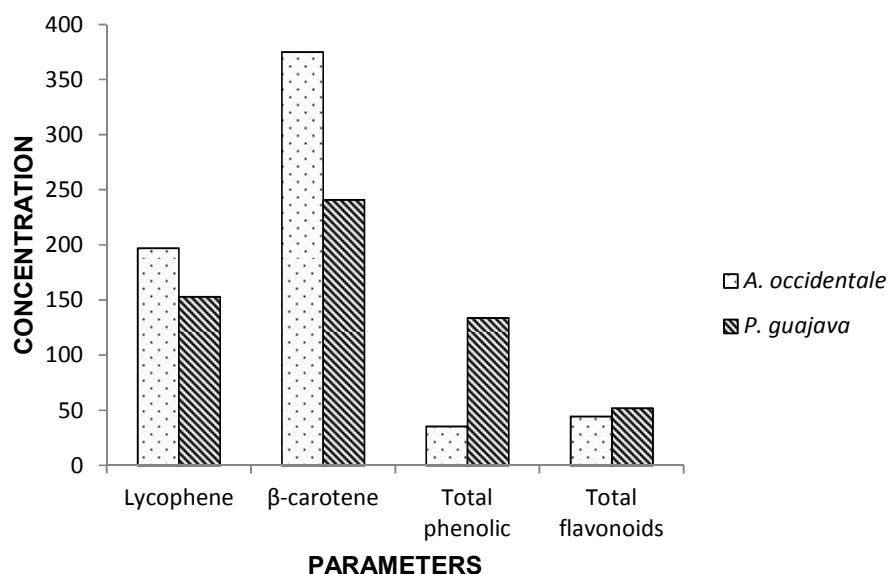


Fig. 1. β -carotene, lycopene (mg/g), flavonoids (mg/g) and total phenolic content of *A. occidentale* and *P. guajava* methanolic leaf extract
 Values are expressed as mean values of three determinants

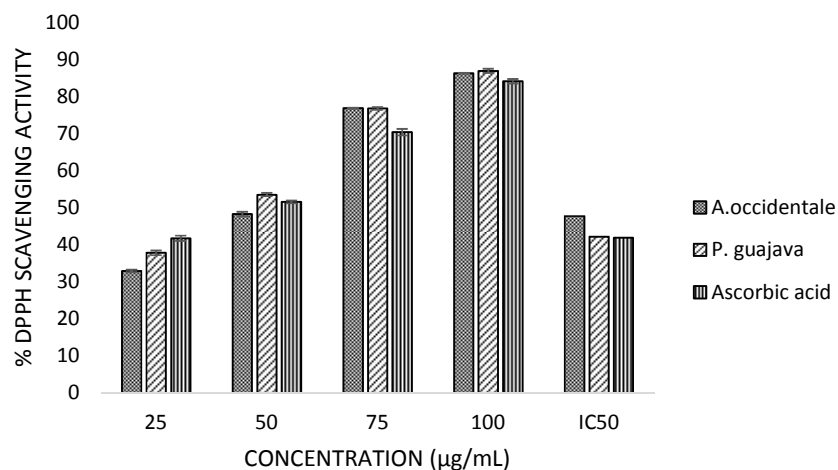


Fig. 2. Percentage DPPH radical scavenging activity of plant extracts.
 Values are expressed as mean values ± Standard deviation of three determinants

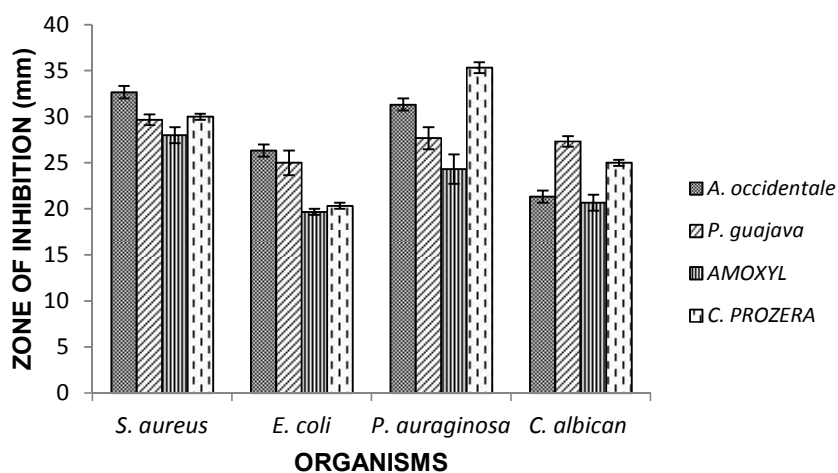


Fig. 3. Diameter zone of inhibition of plant extracts and standard drug against test organisms at a concentration of 50 mg/mL

Values are expressed as mean values ± Standard deviation of three determinants

29.67±0.58, 25.00± 0.58, 27.67±1.33 and 27.33±1.2 mm against *S. aureus*, *E. coli*, *P. auruginosa* and *C. albicans* respectively.

Methanolic leaf extract of *A. occidentalis* and *P. guajava* produced a minimum inhibitory concentration of 16 mg/mL and 8 mg/mL against *S. aureus* while against *E. coli*, 32 mg/mL and 16 mg/mL were obtained respectively.

Spectrophotometric determination of nucleic acid and protein leakage across the *E. coli* membrane was monitored for 60 minutes at 15 minutes and the result showed increase in absorbance as time progressed for both *A. occidentalis* and *P. guajava* methanolic leaf extract.

Fig. 6 illustrates the spectrophotometric determination of nucleic acid and protein leakage across *S. aureus* membrane monitored for 60 minutes at 15 minutes and the result showed increase in absorbance as time progressed for both *A. occidentalis* and *P. guajava* methanolic leaf extract.

4. DISCUSSION

Plants remain a viable source of bioactive compounds with multiple therapeutic actions for the treatment of various diseases including infectious disease caused by microorganisms. Extraction of phytochemicals from

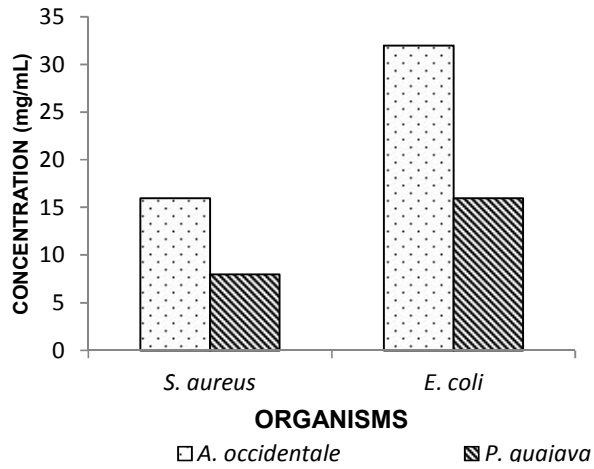


Fig. 4. Minimum inhibitory concentration of plant extracts against *S. aureus* and *E. coli*

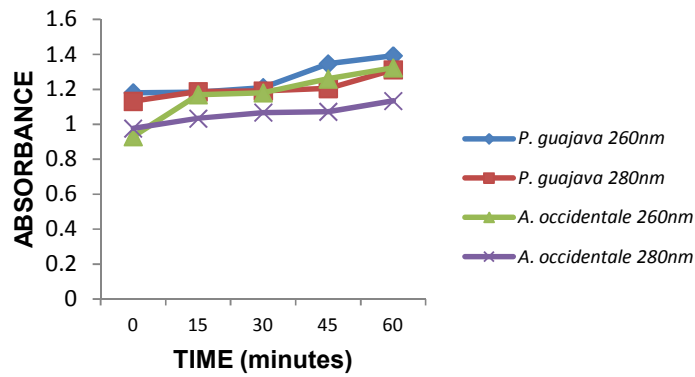


Fig. 5. Effect of *A. occidentale* and *P. guajava* methanolic leaf extract on leakage of UV₂₆₀ and UV₂₈₀ absorbing material across *E. coli* membrane

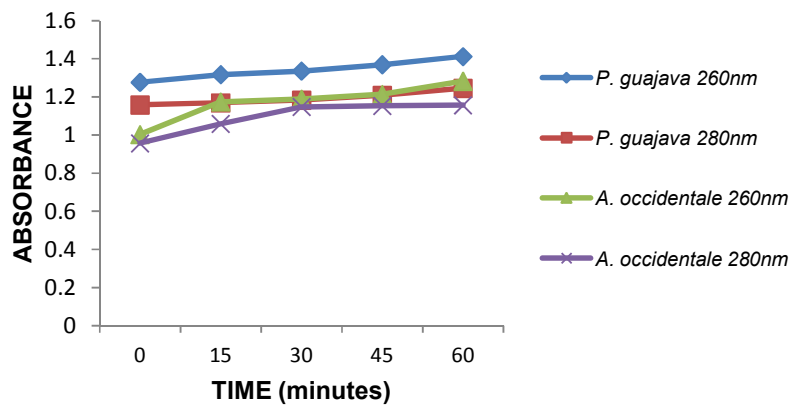


Fig. 6. Effect of *A. occidentale* and *P. guajava* methanolic leaf extract on leakage of UV₂₆₀ and UV₂₈₀ absorbing material across *S. aureus* membrane

A. occidentalis and *P. guajava* leaves using methanol as the extraction solvent produced a percentage yield of 2.36 and 1.8% respectively. Methanol was used because it will dissolve organic compounds better; hence liberates the active component patronized for antioxidant and antimicrobial activity [19].

Plant extracts contains considerable amount of antioxidant compound including phenolics, flavonoids, lycophene and β -carotene. The presence of these compounds confer the extracts with the ability to scavenge free radicals and neutralize the progression of autoxidation of biological molecules (lipids, DNA and protein) which could result to loss of cellular integrity and biological function. Occurrence of oxidative stress results into numerous metabolic and genetic disorders and diseases such as, aging, atherosclerosis, diabetes, neurodegeneration and immunosuppression [20,21,22]. Lycophene is a carotenoid but lacks Vitamin A activity. It is present in tomatoes and *Carica papaya* [23,24] while β -carotene, a pro-vitamin A, are abundantly present in fruits and vegetables [25] with various reports of being potent antioxidants [26] hence, the ability to prevent carcinogenesis and other diseases resulting from oxidative stress [27,28]. Phenolic compounds and flavonoids are extensively concentrated in plants reported to impact multiple biological effect, including antioxidant, free radical scavenging abilities, anti-inflammatory and anticarcinogenic activities [29, 30]. Flavonoids and other polyphenolic compounds possess the ability to donate electrons needed by radicals to become stable compounds [31]. It has also been reported by many researchers to possess antibacterial activity [32,33].

Antioxidant activity of plant extracts revealed that the plant extracts scavenged DPPH radicals with increase in concentration of *A. occidentalis* and *P. guajava* methanolic leaf extract. This antioxidant property is largely due to the antioxidant compounds quantified in these plant extracts [34].

The agar diffusion method used in this study for assessing antimicrobial activity is known for allowing the use of adjuvants to increase the solubility of the extracts, as well as permitting its surface diffusion [35]. Based on the parameters suggested by Alves et al. [36], the antibacterial activity was graded as very active because inhibition zone diameters (mm) produced by the test samples against the organisms was greater

than 18 mm. Considering the zone of inhibition, the test organism were susceptible to the plant extracts, however, it was observed that *S. aureus* was most susceptible than *E.coli*, a gram negative bacteria. Rajesh et al. [37] reported a lower zone of inhibition for *A. occidentalis* against *S. aureus* and *E.coli*. This can be attributed to the method of extraction employed in their study, that is, Soxhlet extraction which can destroy heat labile phytochemicals present in the plants [38]. Also, the source of the bacterial strains used in the study and concentration of the plant extracts are factors which can influence the biological activity of plant extracts [39].

In this study, introduction of plant extract at MIC concentration into microbial cell caused the leakage of nucleic acid and proteins through *S. aureus* and *E. coli* membrane, evidence in the increase of absorbance at 260 nm and 280 nm. Nucleic acids and proteins are biological molecules localized within the cell, thus, increase in absorbance as time progresses showed that those materials have passed through the membrane, a process which normally is impossible due to lipophilic nature of the membrane core. This may be possible through increase in membrane permeability or membrane disruption may be inferred and could lead to cell death, a bactericidal process [40].

5. CONCLUSION

This study has revealed the presence of bioactive compounds with antioxidant activity established by their ability to scavenge free radicals in methanolic leaf extracts of *A. occidentale* and *P. guajava*. It has further entrenched that the plants extracts could be utilized for the treatment of infections caused by the microorganisms *E. coli*, *S. aureus*, *P. auruginosa* and *C. albicans*. This shows that *A. occidentale* and *P. guajava* methanolic leaf extracts could be exploited for new potential drugs with antioxidant and antimicrobial activity.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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