Available: https://doi.org/10.54117/ijnfs.v3i3.63 *Research article*

IPS Journal of Nutrition and Food Science
IPS J Nutr Food Sci, 3(3): 234-241 (2024)
DOI: https://doi.org/10.54117/ijnfs.v3i3.63

Antibacterial Effects of *Piliostigma thonningii* and *Anacardium occidentale* against Some Food Spoilage Pathogens

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Abstract

This study investigated the antibacterial activities of *Piliostigma thonningii* and *Anacardium occidentale* against food spoilage bacteria, specifically *Staphylococcus aureus* and *Escherichia coli*. Additionally, the phytochemical contents of these plants were analyzed. Bacterial isolates were obtained from the Microbiology Laboratory at Federal University Birnin Kebbi and re-identified through various biochemical tests. Leaf samples of both plants were collected from the university premises, authenticated by the Botany Unit of the Department of Biological Sciences, and then subjected to drying and extraction. The antibacterial activities of the plant extracts were determined using the agar well diffusion method. Biochemical tests confirmed the identities of *E. coli* and *S. aureus* as the test bacteria. Phytochemical analysis revealed the presence of tannins, terpenoids, flavonoids, steroids, alkaloids, cardiac glycosides, glycosides, saponins, and volatile oils in the plant extracts. The antibacterial activity results indicated inhibition zones of 14 mm for methanolic extracts and 26 mm for aqueous extracts of *P. thonningii* against *E. coli*. Similarly, *P. thonningii* showed inhibition zones of 16 mm for methanolic extracts and 22 mm for aqueous extracts against *S. aureus*. For *A. occidentale*, inhibition zones of 18 mm for methanolic extracts and 25 mm for aqueous extracts were observed against *E. coli*, while zones of 20 mm for methanolic extracts and 26 mm for aqueous extracts were recorded against *S. aureus*. The minimum inhibitory concentration (MIC) values for *P. thonningii* against *E. coli* were 50 mg/ml for methanolic extracts and 25 mg/ml for aqueous extracts. For *S. aureus*, the MIC values were similarly 50 mg/ml for methanolic extracts and 25 mg/ml for aqueous extracts. For *A. occidentale*, the MIC values against *E. coli* were 100 mg/ml for both methanolic and aqueous extracts, while against *S. aureus*, the values were 50 mg/ml for methanolic extracts and 25 mg/ml for aqueous extracts. The minimum bactericidal concentration (MBC) values for *P. thonningii* against *E. coli* were 1000 mg/ml for methanolic extracts and 50 mg/ml for aqueous extracts, while against *S. aureus*, the MBC values were 100 mg/ml for methanolic extracts and 50 mg/ml for aqueous extracts. For *A. occidentale*, the MBC values against *E. coli* were 200 mg/ml for both methanolic and aqueous extracts, and for *S. aureus*, the MBC values were 100 mg/ml for methanolic extracts and 50 mg/ml for aqueous extracts. In conclusion, both *P. thonningii* and *A. occidentale* demonstrated potential as sources of antibacterial agents against common food spoilage bacteria.

Keywords: *food safety, food preservatives, Piliostigma thonningii, Anacardium occidentalem*

Article History
Received: 07 Jun 2024
Accepted: 23 Jul 2024
Published: 12 Aug 2024

Scan QR code to view•

How to cite this paper: Maganda, J. B., Jafar, S., Jabaka, D. R., & Ukatu, V. E. (2024). Antibacterial Effects of Piliostigma thonningii and Anacardium occidentalem against some Food Spoilage Pathogens. *IPS Journal of Nutrition and Food Science*, *3*(3), 234–241. https://doi.org/10.54117/ijnfs.v3i3.63.

Introduction

Food spoilage is a metabolic process that causes foods to be undesirable or unacceptable for human consumption due to changes in sensory characteristics. Globally, food spoilage caused by microorganisms still widely affects all types of food and causes food waste and loss, even in developed countries. It has been estimated that the yearly losses of global food reach up to 40% due to various factors including spoilage by microorganisms (Gustavsson *et al.*, 2011). Bacteria, yeast, and molds are the common types of microorganisms responsible for the spoilage of a considerable number of food and food products (Lianou *et al.*, 2016). Once these microorganisms reach food products, they grow by utilizing the nutrients and produce metabolites that cause food spoilage (Parlapani *et al.*, 2017). Food borne disease is another pervasive food safety problem caused by consumption of contaminated food

products, which has been a significant safety concern to public health (Azziz-Baumgartner *et al*., 2005; Kirk *et al*., 2017). Most of food poisoning reports are associated with bacterial contamination especially members of Gram negative bacteria like *Salmonella typhi, Escherichia coli* and *Pseudomonas aeruginosa* (Solomakos *et al.*, 2008; Pandey and Singh, 2011). Other Gram positive bacteria including *Staphylococcus aureus* and *Bacillus cereus* have been also identified as the causal agents of food borne diseases or food spoilage (Braga *et al*., 2005).

Microorganisms are available naturally in the surrounding environment; therefore they can easily reach food during harvesting, slaughtering, processing, and packaging (Hatab *et al*., 2016). These microorganisms can survive under adverse conditions used in the food preservation such as low temperature, modified atmosphere packaging, vacuum packaging, as well as resist conventional pasteurization (Dimitrijevi´c *et al*., 2007; Provincial *et al.*, 2013; Saraiva *et al*., 2016; Säde *et al*., 2017). Thus, there is a considerable concern among consumers regarding the risk of using synthetic additives for human health, that led to decrease the use of these chemicals in food preservation (Gyawali and Ibrahim, 2014; Caleja *et al*., 2016). Therefore, new eco-friendly methodologies are required to reduce the growth of pathogenic bacteria and prolong the shelf-life of food products, without using chemical preservatives. Recently, many researchers investigated the possible utilization of some plant extracts as effective natural preservatives (Fernández- López *et al*., 2005; Suppakul *et al.*, 2016; Clarke *et al*., 2017). Traditionally, the crude extracts of different parts of medical plants, including root, stem, flower, fruit, and twigs, were widely used for treatments of some human diseases (Khan *et al*., 2013). Medicinal plants contain several phytochemicals such as flavonoids, alkaloids, tannins, and terpenoids, which possess antimicrobial and antioxidant properties (Talib and Mahasneh, 2010). The antimicrobial activities of some plant species have been widely researched. For example, the crude extracts of cinnamon, garlic, basil, curry, ginger, sage, mustard, and other herbs exhibit antimicrobial properties against a wide range of Gram-positive and Gram-negative bacteria (Alzoreky and Nakahara, 2003; Castro *et al*., 2008). In addition, it has been reported that the extracts from Chinese chives and cassia can effectively reduce the growth of *Escherichia coli* and other bacteria during storage of meat, juices, and milk (Mau *et al*., 2001). Also, antimicrobial activity of ethanolic *Punica granatum* extract and its fractions showed a highly antibacterial activity against Gram positive *(S. aureus* and *B. cereus*) and Gram negative bacteria (*E. coli* and *S. typhi*) causing food poisoning and these extracts can be used for prevention of food borne diseases or as preservative in food industry (Alzoreky, 2009; Mahboubi *et al*., 2015). Spices extracts used as food additives were potentially effective against some food poisoning bacteria and their antibacterial activity was investigated by several researchers (Ozcan and Erkmen, 2001; Nevas *et al*., 2004; Parekh and Sumitra, 2007; Abdulrahman *et al.*, 2010).

The growing interest in food bio-conservation has led to the use of new antimicrobial compounds of various origins, including those based on the use of microorganisms, their metabolites and natural extracts of plant origin (Ananou *et al.*, 2007).

There has been a constant increase in the search of alternative and efficient compounds for food preservation aimed at a partial or total replacement of antimicrobial chemical additives. Extract from many plants can be used as flavouring and seasoning agents in food and beverages (Ananou *et al.*, 2007).

Materials and Methods

Sample collection

The sample of fresh *Piliostigma thoninngii* and *Anacardium occidentale* leaves were collected from Federal University Birnin Kebbi permanent site. The leaves samples were washed with running tap water and dried in a shade for period of 3 days. The dried samples were grounded into fine powder using sterile mortal and pestle.

The plants were authenticated by Mr. Obadiah, Caleb Dikko, botanist from department of Biological Sciences Federal University Birnin Kebbi.

Media Preparation

The media used (Nutrient agar, Nutrient broth, Eosin methylene blue (EMB) agar and Mueller-Hinton agar) were weighed and prepared according to manufacturer's specification as follows:-

Nutrient agar

Nutrient agar powder (28g) was weighed and place into a conical flask containing 1000ml of distilled water to dissolve. The dissolved medium was heated to boiling using a hot plate. It was further autoclaved at a temperature of 121ºC for 15 minutes to obtain the complete sterility of the medium prepared. Sterility check was carried out by incubating the plates for 24 hours. Plates that show no growth (contaminants) were selected for use (Cheesbrough, 2006).

Nutrient broth

Nutrient broth powder (13g) was weighed and place into a conical flask containing 1000ml of distilled water to dissolve. The dissolved medium was heated to boiling using a hot plate. It was further autoclaved at a temperature of 121ºC for 15 minutes to obtain the complete sterility of the medium prepared. Sterility check was carried out by incubating the plates for 24 hours. Plates that show no growth (contaminants) were selected for use (Cheesbrough, 2006).

Eosin Methylene Blue (EMB)

Eosin Methylene Blue (EMB) agar powder (36g) was weighed and place into a conical flask containing 1000ml of distilled water to dissolve. The dissolved medium was heated to boiling using a hot plate. It was further autoclaved at a temperature of 121ºC for 15 minutes to obtain the complete sterility of the medium prepared. Sterility check was carried out by incubating the plates for 24 hours. Plates that show no growth (contaminants) were selected for use (Cheesbrough, 2006).

Mueller Hilton Agar

Mueller Hilton agar powder (38g) was weighed and place into a conical flask containing 1000ml of distilled water to

dissolve. The dissolved medium was heated to boiling using a hot plate. It was further autoclaved at a temperature of 121ºC for 15 minutes to obtain the complete sterility of the medium prepared. Sterility check was carried out by incubating the plates for 24 hours. Plates that show no growth (contaminants) were selected for use (Cheesbrough, 2006).

Extraction of Plant Sample

Here maceration method was used. Fifty gram (50g) of each powdered sample were weighed and soaked with 400ml of aqueous (distilled water) and 50g were also soaked with 400ml of methanol in a conical flask, and agitated occasionally to mix, and macerated for 72 hours at room temperature. Maceration intends to soften and break the plant's cell wall to release the soluble phytoconstituents (Handa *et al*. 2008). The crude extract of each sample were filtered and concentrated in an oven at 40ºC. The dried methanol and aqueous extracts were then packed in glass bottles with proper labeling and stored for future use.

Phytochemical Screening of the Extracts

The prepared extract of the plants samples were used to test various phytoconstituents present in them. Different chemical reagents are prepared and specific test, for specific phytochemicals were done. These various tests are qualitative and hence termed phytochemical screening. The tests is carried out by following standard procedures based on journal articles (Alamzed *et al*. 2013; Thusa and Mulmi, 2017; Talukdar and Chaudhary, 2010).

Test for tannin

To the diluted extract, 3-4 drops of 10% FeCl3 is added, blue color is seen for gallic tannins and the presence of catechol tannin turned the solution green (Talukdar and Chaudhary, 2010).

Test for reducing sugar

To 0.5 ml of plant extract, 1mL of water, and 5-8 drops of Fehling's solution was added and heated. The presence of reducing sugar is indicated by the appearance of brick red precipitation (Thusa and Mulmi, 2017).

Test for quinine

To the extract, freshly prepared FeSO4 solution (1mL) and ammonium thiocyanate was added then concentrated H2SO4 was added drop by drop. The deep red color indicate the presence of quinine (Thusa and Mulmi, 2017).

Test for glycosides

Molisch's Reagent Test: To the extract, 5 ml Molisch's reagent and concentrated H_2SO_4 was added. Violet color indicated glycosides (Alamzed *et al*., 2013).

Test for flavonoids

Shinoda test: 4 mL of extract solution, 1.5 mL of 50% methanol solution a small magnesium chunk was warmed. 5-6 drops of concentrated HCl was added, red color is observe for flavonoids (Talukdar and Chaudhary, 2010).

Test for terpenoids

In each sample, 0.2g was mixed with 2 mL chloroform, 3 mL conc. H_2SO_4. Reddish-brown coloration indicate the presence of terpenoids (Alamzed, *et al*. 2013).

Test for alkaloids (Meyer's test)

To 2 mL of extract, 1 mL of Meyer's reagent was added. The presence of pale yellow precipitate indicate the presence of alkaloids (Talukdar and Chaudhary, 2010).

Test for saponins

Two grams of powdered sample was boiled in 20 mL of distilled water. A 10 mL of filtrate, 5 mL of distilled water was quivered vigorously. The appearance of frothing indicate the presence of saponins (Alamzed, *et al*. 2013).

Test for volatile oils

Two mL extract was shaken with 0.1 mL of NaOH and a small quantity of dilute HCl. White precipitate indicate the presence of volatile oil (Talukdar and Chaudhary, 2010).

Test for cardiac glycosides

Five mL of the plant extract was treated with 2 mL of glacial acetic acid with one drop of FeCl3 solution. A violet ring may appear or a greenish ring may form just which showed the presence of cardiac glycosides (Talukdar and Chaudhary, 2010).

Test for steroids

One gram of the plant extract was dissolved in a few drops of acetic acid and a drop of conc. H2SO4 was added. The appearance of green color indicate the presence of steroids (Talukdar and Chaudhary, 2010).

Preparation of Different Concentration of Extracts

The different concentrations (400mg, 200mg, 100mg and 50mg) were prepare from the crude methanol and aqueous extracts of the plant samples by dissolving 4g, 2g, 1g and 0.5g into 10 ml of Dimethylsulphoxide (DMSO) each. These concentrations were used to test the antimicrobial activity of the crude extracts (Aska *et al.*, 2019).

Microorganisms Tested

Micro-organisms tested are *Staphylococcus aureus* and *Escherichia coli*. The clinical isolates are obtained from Microbiology Laboratory of Federal University Birnin Kebbi, Nigeria. The microorganisms were sub-cultured and re-identified using difference biochemical tests in microbiology Laboratory of Federal University Birnin Kebbi (FUBK) Nigeria.

Biochemical Test

Gram Staining

The gram staining reaction was done by placing a drop of distilled water on a greases-free clean slide. A colony was picked with the aid of a sterile wire loop from a cultured medium of about 18-24 hours and put onto the slide to form a smear. Afterwards, the slide was air-dried and then heat-fixed by passing through flame thrice. The smear was covered with a crystal violet stain for 60 seconds. The Stain which serves as the primary stain was washed with distilled water. After the

water has drained off, the slide was covered with iodine for about 60 sec. Again it was also rinsed off with distilled water, and the water was allowed to drain out. The 1odine serves as mordant. Then the slide was decolourized rapidly by the addition of 75% acetone alcohol and washed off with distilled water and allowed to drain out, then followed by the addition of safranin onto the slide for 45 sec which serves as a counter stain. The slide was rinsed off again with clean water, and the back was wiped out with clean cotton wool. It was left to air dry before viewing it under the microscope. A drop of immersion oil was added on the spot of the smear and it was viewed using xI00 objective lens (Cheesebrough, 2006).

Catalase test

The test was carried out by inoculating the isolates with sterile distilled water on a grease-free clean slide. Afew drops of hydrogen peroxide was added and Rapid Bubble formation indicate positive while no Bubble formation is negative (Cheesebrough, 2006).

Coagulase

A drop of distilled water was placed on a sterile slide. The colony of the test organism was emulsified on the drop, making a thick emulsion. A loop full of plasma was added to the suspension and mixed gently to observe a clumping reaction within 10 seconds (Cheesebrough, 2006).

Indole test

The test organism was cultured into peptone water, which contains tryptophan and incubated at 37ºC for 48hrs. One milliliter of Kovac`s reagent, which contains 4P-dimethylamine benzaldehyde was run down along the side of the test tube. The appearance of pink colour in the reagent layer within a minute indicated a positive reaction (Cheesbrough, 2006).

Urease test

The surface of a urea agar slant was streaked with a portion of a well-isolated colony. The cap loosened and incubated the tube at 35°-37℃ in ambient air for 48 hours. Development of an intense magenta to bright pink colour in 15 min to 24 h, indicates positive results, whereas no colour change indicates a negative result (Cheesbrough, 2006).

Inoculums Preparation

Inoculums were standardized to give a density of 10^6 colony-forming units (CFU)/ml. A loopful of the test organism was inoculated into 5.0 ml of nutrient broth and incubated at 37℃ for 24 h. 0.2ml from the 24-h culture of the organism was dispensed into 20 ml sterile nutrient broth and incubated for 3–5 h to standardize the culture to 10^6 CFU/ml (corresponding to 0.5 McFarland standards). Plates were inoculated within 15 min of standardizing the inoculums, to avoid changes in inoculum density (Abalaka *et al*., 2012).

Antimicrobial Sensitivity Test

The sensitivities of the isolated microbial species against the plants extracts were tested base on the agar well diffusion (Kirby–Bauer) technique (Bauer *et al*., 1966) as described by Saif *et al.* (2017).

Determination of Antimicrobial Activities of the Plants Extracts

The Agar well diffusion method was used to evaluate the antimicrobial activity of the plants extracts. Muller Hinton agar was prepared and allow to solidify in sterile Petri dishes before inoculating using spread plate method. After that, Agar wells were made with sterile cork borer and impregnated with the different concentrations (400, 200, 100 and 50 ml/g) of the plants extracts, and allowed for 30 minutes before incubating at 37ºC. All the Petri dishes were incubated at 37ºC for 16-18 hours, after incubation the wells were observed for growth inhibitions against the tested microorganisms by the tested extracts. The diameters of inhibition are measured and recorded in Millimeters (mm) (CLSI, 2016).

Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is the lowest concentration of the extracts which will inhibit growth as measured by observed turbidity in the test tube (CLSI, 2016). The minimum inhibitory concentration (MIC) was determined by Micro broth dilution method. Prepared nutrient broth was dispersed into test tubes and sterilized with autoclave at 121ºC for 15mins then the broth was allowed to cool. Two fold serial dilutions were used to obtain concentrations of 400mg, 200mg, 100mg, 50mg, 25mg, 12.5mg, 6.25mg and 3.12mg of the crude extracts. Four hundred milligram (400mg) was initially made by dissolving 4g of the crude extract in 10ml of DMSO to obtain 400mg from which subsequent concentrations was prepared using two fold dilutions. Methanol (70%) was be used as a negative control. The microorganisms sensitive to the tested extracts was inoculated and incubated for 24 hrs at 37℃ and the lowest concentration of the extracts that showed no visible growth was recorded as the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration

The lowest concentration that kills the organisms completely, where no bacterial growth is observed (MBC) CLSI (2016), MBC was determined by assaying the test tubes resulting from MIC determinations. A loopful of the content of each test tube were inoculated by streaking on a solidified nutrient agar plate and then incubated at 37 ℃ for 24 h and observed for bacterial growth (Usman *et al*., 2014).

Results

The results of biochemical tests carried out to re-identify the test organisms are hereby presented. *E.coli* and *S. aureus* were confirmed. *E.coli* were found to be rod shaped Gram negative and tested negative to citrate, indole and catalase tests but positive to coagulase and urease. *S. aureus* were found to be Gram positive cocci in shape and all the tests coagulase and catalase were found to be positive for *S. aureus* except on indole test that was found negative (Table 1).

The results of phytochemical analysis of the plants shows that phenols, alkaloids, flavonoids, glycosides, tannins, cardiac glycoside, and terpenoids were present in leaves extracts of *A. occidentale* and *P.thonningii* (Table 2). However, there was no quinine and steroids *P. thonningii* and only quinine is absent in *A. occidentale.*

The results of antibacterial activities of aqueous and methanolic leaves extract of *P. thonningii* on *S. aureus and E. coli* shows that the bacterial are sensitive to the extracts. The mean zone diameter of inhibition for *S. aureus* on the different extracts of *P. thonningii* was between 7mm to 22mm while that of *E. coli* was between 7mm to 26mm. (Table 3).

The results of antibacterial activities of aqueous and methanolic leaves extract of *A. occidentale* on *S. aureus and E. coli* also shows that the bacterial are sensitive to the extracts. The mean zone diameter of inhibition for *S. aureus* on the different extracts of *A. occidentale* was between 12mm to 26mm while that of *E. coli* was between 7mm to 25mm. (Table 4).

Table 1: Biochemical test of the re-identification of bacteria isolates.

Gram reaction	Shape	Citrate	Coagulase	Indole	Catalase	Urease	Organisms
G-ve	Rod	-	+	-	-	+	*E. coli*
G+ve	Cocci	+	+	-	+	+	*S. aureus*

Key: + =Positive. - =Negative G-ve =Gram negative G+ve =Gram positive

Table 2: Phytochemicals Screening of plants extracts

Phytochemical compounds	*P. thonningii*	*A. occidentale*
Tannin/ polyphenol	+	+
Quinine	–	–
Glycosides	+	+
Flavonoids	+	+
Terpenoids	+	+
Alkaloids	+	+
Saponins	+	+
Volatile oil	+	+
Steroids	–	+
Cardiac glycosides	+	+

Key: + = Present. - = Absent.

Table 3: Antibacterial activities of *P. thonningii* extracts on the test organisms

Test bacteria	Zone of inhibition in mm / Concentration of Extracts in mg/ml							
	Methanolic Extracts				Aqueous Extracts			
	400	200	100	50	400	200	100	50
S.aureus	16	13	8	7	22	18	14	11
E. coli	14	11	8	7	26	20	16	12

Table 4: Antibacterial activities of *A. occidentale* extracts on the test organisms

Test bacteria	Zone of inhibition in mm / Concentration of Extracts in mg/ml							
	Methanolic Extracts				Aqueous Extracts			
	400	200	100	50	400	200	100	50
S.aureus	20	18	12	10	26	20	18	15
E. coli	18	13	10	8	25	18	12	7

The MIC results of leaves extracts of *P. thonningii on S. aureus and E. coli* is presented on Table 5. The MIC values against *S. aureus* was 50mg/ml for methanolic extracts and 25mg/ml for aqueous extracts. Similarly the MIC values for *E. coli* was50mg/ml for methanolic extracts and 25mg/ml for aqueous extracts.

The results of MIC for *A. occidentale* extracts against *S. aureus and E. coli* were presented on the same Table (Table 5). The value for methanolic extracts on *S. aureus* was 50mg/ml and 25mg/ml for aqueous extracts. Similarly the MIC values for *E. coli* was 100mg/ml for both methanolic and aqueous extracts.

The MBC results of leaves extracts of *P. thonningii on S. aureus and E. coli* is presented on Table 6. The MBC values against *S. aureus* was 100mg/ml for methanolic extracts and 50mg/ml for aqueous extracts. Similarly the MBC values for *E. coli* was 100mg/ml for methanolic extracts and 50mg/ml for aqueous extracts.

The results of MBC for *A. occidentale* extracts against *S. aureus and E. coli* were presented on the same Table (Table 6). The value for methanolic extracts on *S. aureus* was 100mg/ml and 50mg/ml for aqueous extracts. Similarly the MBC values for *E. coli* was 200mg/ml for both methanolic and aqueous extracts.

Table 5: Minimum Inhibitory Concentrations (MIC) (mg/ml)

Test Bacteria	MIC values in mg/ml / Extracts			
	P. thonningii		*A. occidentale*	
	Methanolic	Aqueous	Methanolic	Aqueous
S. aureus	50	25	50	25
E. coli	50	25	100	100

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Table 6: Minimum Bactericidal Concentrations (MIC)

Test Bacteria	MIC values in mg/ml / Extracts			
	P. thonningii		*A. occidentale*	
	Methanolic	Aqueous	Methanolic	Aqueous
S. aureus	100	50	100	50
E. coli	100	50	200	200

Discussion

The bacterial obtained are been re-identified and confirmed to be *Staphylococcus aureous and Escherichia coli* through their shapes and several biochemical tests carried out as indicated. *E.coli* were found to be rod shaped Gram negative and tested negative to citrate, indole and catalase tests but positive to urease. *S.aureus* were found to be cocci in shape and all the tests catalase and coagulase were found to be positive for *S. aureus*.

The results of the various phytochemical screening tests obtained during the experiment indicated tannin, quinine, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, volatile oils, etc were the phytoconstituents found in plants. Several studies have reported rich variety of secondary metabolites in *A. occidentale* extracts (Rajesh *et al*., 2009). The pharmacological properties of medicinal plants have been attributed to their rich secondary metabolites. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Varaprasad*et al.*, 2009).

The antimicrobial activities of methanolic and aqueous extracts of *Piliostigma thonningii* and *Anacardium occidentale* at various concentrations against *Staphylococcus aureus and Escherichia coli* has been assessed in this study. The results revealed that the methanolic and water extract of the test plants are efficiently suppressing the growth of food pathogens and spoilage microorganisms with variable potency as shown. The aqueous extracts of *Piliostigma thonningii* at concentration of 400mg/ml has the highest zone of inhibition of 22mm against *Staphylococcus aureus* and 26mm against *Escherichia coli*, therefore, aqueous extracts are more effective than methanolic extracts which has 16 and 14mm zone of inhibition against *Staphylococcus aureus and Escherichia coli* respectively. And also, aqueous extracts of *Piliostigma thonningii* are more effective against *Escherichia coli* than on *Staphylococcus aureus.* Also, the aqueous extracts of *Anacardium occidentale* at concentration of 400mg/ml has the highest zone of inhibition of 26mm and 25mm against *Staphylococcus aureus and Escherichia coli* respectively. Therefore, methanolic extracts is more effective with 20 and 18mm zone of inhibition against *Staphylococcus aureous and Escherichia coli* respectively. Also, the aqueous extracts of *A. occidentale* were more effective against *S. aureus* than on *E. coli.*

The result of MIC in this study shows that for methanolic extracts of *Piliostigma thonningii*, MIC is 50mg/ml for both *Staphylococcus aureus* and *Escherichia coli*. While the MIC of aqueous extracts is 25mg/ml for both *Staphylococcus aureus* and *Escherichia coli*. For *Anacardium occidentale*, the MIC for methanolic extracts against *Staphylococcus aureus* and *Escherichia coli* is 50mg/ml and 100mg/ml respectively. While that of aqueous is 25mg/ml and 100mg/ml for *Staphylococcus aureus* and *Escherichia coli* respectively.

The antibacterial analysis in this study showed that there was no significant difference in the antibacterial effect of methanol and aqueous extract against the test bacteria. This result is in disagreement with report of (Arekemase *et al*., 2011) who reported that methanolic extract was more effective than aqueous extract. Also, there was no significant difference of effectiveness between the two plant samples.

The antibacterial effect of the methanol and aqueous extract against the test bacteria in this study could be attributed to the presence of the phytochemicals. Flavonoids have been reported to significantly affect the cell wall of the microorganisms which may invariably lead to the collapse of the cell wall and overall, affect the entire mechanism of the microbial cell (Nwinyi *et al.*, 2009). Alkaloids have also been reported to be involved in antimicrobial activities (Punitha*et al*., 2005).

The minimum inhibitory concentration (MIC) of extracts against test *S. aureus and E. coli* in this study are higher than MIC reported by (Arekemase *et al*., 2011).The authors reported MIC of 0.313 and 0.625 mg/ml for reference strain of *S. aureus* and *E. coli* and 1.25 mg/ml against *S. aureus* isolated from food as against the 25mg/ml recorded in this study. (Onuh *et al*., 2017) reported appreciable antimicrobial effect of the ethanol extract of *A. occidentale* against E. *coli*, *S. mutans*, *B. cereus*, *S. typhi*, and *C. albicans*. The authors also reported varying levels of phytochemicals in the leaves and stem bark of *A. occidentale*.

The result of the minimum bactericidal concentration (MBC) was similar to report of (Arekemase *et al*., 2011)who reported that the methanolic extract was found to be bactericidal to all the test bacteria, the aqueous extract was also found to be bactericidal to the test bacteria as shown in Table 6.

Conclusion

The results of the biochemical confirmation had confirmed *E. coli* and *S. aureus* as test bacteria. The phytochemical components determine were tannin, terpenoid, flavonoid, steroid, alkaloid, cardiac glycoside, glycoside, saponins and volatile oils. The results of antimicrobial activities of *P. thonningii* against *E. coli* indicated a zone of inhibition of 14mm for methanolic extracts and 26mm for aqueous extracts. Similarly the results of antibacterial activities of *P. thonningii* against *S. aureus* indicated zone of inhibition of 16mm for methanolic extracts and 22mm for aqueous extracts. Also, the results of antimicrobial activities of *A. occidentale* against *E. coli* indicated a zone of inhibition of 18mm for methanolic extracts and 25mm for aqueous extracts. Similarly the results of antibacterial activities of *A. occidentale* against *S. aureus* indicated zone of inhibition of 20mm for methanolic extracts and 26mm for aqueous extracts. The MIC values for *P. thonningii* against *E. coli* for methanolic and aqueous extracts are 50mg/ml and 25mg/ml respectively. Similarly the MIC values for *P. thonningii* against *S. aureus* for methanolic and aqueous extracts are 50mg/ml and 25mg/ml respectively. Also, the MIC values for *A. occidentale* against *E. coli* for both methanolic and aqueous extracts are 100mg/ml. Similarly the MIC values for *A. occidentale* against *S. aureus* for methanolic and aqueous extracts are 50mg/ml and 25mg/ml respectively. For the MBC, the value of *P. thonningii* against *E. coli* for methanolic and aqueous extracts are 1000mg/ml and 50mg/ml respectively. Similarly the MBC values for *P. thonningii* against *S. aureus* for methanolic and aqueous extracts are 100mg/ml and 50mg/ml respectively. Also, the MBC values values for *A. occidentale* against *E. coli* for both methanolic and aqueous extracts are 200mg/ml. Similarly the MBC values for *A. occidentale* against *S. aureus* for methanolic and aqueous extracts are 100mg/ml and 50mg/ml respectively.

Recommendation

Future research on this topic should be focus on how to improve and develop technologies on use of these plants either as preservatives or spices, in other to prolong the shelf life of foods and prevent the growth of spoilage and pathogenic microorganisms.

Acknowledgement

With much gratitude, I hereby acknowledge all who contributed positively to this research work, most especially Professor S.B Manga whose contributions through correction and advice made this research work a success.

Declarations

Competing Interest

The authors declare no competing interest.

Authors' Contributions

All listed authors contributed equally to the research process, literature writing, review and editing of the article.

Funding

There is no external funding for this article.

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