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Antioxidant and Antimicrobial Activity of *Psidium guajava* (Pomifera and Pyrifera) Aqueous Leaf Extract Varieties'

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: *Psidium guajava* (Pomifera (red) and Pyrifera (white)) is commonly used in traditional medicine to treat gastrointestinal disorders. In this study, the antioxidant and antimicrobial activity of methanol extracts of *P. guajava* (Pomifera (Red) and Pyrifera (White) leave varieties were investigated.
Methodology: The total phenolic (TPC) and flavonoid (TFC) contents and antioxidant capacities in terms of 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, were determined, and the correlations

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among the results were assessed. The antimicrobial activity was assessed through agar disc diffusion assays.

Results: Phytochemical screening showed that the extracts had alkaloids, glycosides, saponins, triterpenoids, phenols, and flavonoids in different abundances. The aerial part- (Pomifera (red) and Pyrifera (white)) extract contained 106.50 ±2.97 and 122.07 ±2.73 mg/g in QAE phenolic content, 2650.65 ±27.48 and 2486.82 ± 9.10 mg/g in RE of flavonoids, 17.86 ±0.19 and 10.16 ±0.07 mg/g in TAE of Tannins, 69.50 ± 0.14 and 10.50 ± 0.01 alkaloid content, and 7.25.50 ± 0.11 and 17.75 ± 0.09 saponin content and displayed significant antioxidant capacity in the DPPH (IC_{50} = 52.17 ± 7.30 *µ*g/mL) assay. By contrast, the *P. guajava* Pyrifera (white) extract contained high amounts of TPC (87.72 ± 7.75 mg GAE/g) and TFC (25.60 ± 1.57 mg QE/g). The leaf extract showed the highest inhibitory activity against highest activity was observed against *E. Coli*, *S.E,* and S. aureus (14, 15, and 16) respectively. *GC-MS revealed presence of* Sesquiterpenes, terpenes, and triterpenes. The leaves also contain vitamins of measurable amounts. Generally, the extracts evaluated showed potent antifungal activity against selected bacterial strains.

Conclusion: The results showed that *P. guajava* (Pomifera (red) and Pyrifera (white)) possesses different phytochemicals displaying significant antioxidant and antimicrobial activities, thus lending credence to its use in traditional medicine.

Keywords: GC-MS; Psidium guajava; total phenols; antioxidant activity; Staphylococcus aureus; antimicrobial activity.

1. INTRODUCTION

Medicinal plants are being studied more closely than ever before due to their ability to provide several advantages to society and humanity, particularly in the fields of medicine and pharmacology [1]. The plants' therapeutic properties are due to phytochemical elements that have specific pharmacological effects on the human body [2]. Phytochemicals are natural compounds found in plants such as medicinal plants, vegetables, and fruits that function in conjunction with nutrients and fibers to combat or prevent disease.

"Traditional systems of medicine have been using plants as sources of medicines. Some of the key bioactive compounds that are utilized include phytoestrogens, terpenoids, carotenoids, limonoids, phytosterols, glucosinolates, polyphenols, flavonoids, isoflavonoids, and anthocyanidins which are employed as nutraceuticals" [3]. "Modern science demonstrates that these phytochemical extracts have a specific pharmacological role in promoting human health. These include the anti-inflammatory, anti-allergic, antioxidant, antibacterial, antifungal, and antispasmodic role, including chemopreventive, hepatoprotective, hypolipidemic, neuroprotective, hypotensive, and antiaging properties" [4]. "Currently, medicinal plants are being investigated for their antioxidant properties, and the demand for natural antioxidants and food preservatives is increasing" [5].

"Guava refers to the name of the fruits of *Psidium guajava* (*P. guajava*) (Myrtaceae) a tree that is widely spread to various parts of the tropical and subtropical areas. The leaves of *P. guajava* are commonly used as active medicine for diarrhea which is also used for wounds, ulcers, and rheumatic pain, and are chewed to relieve toothache. These leaves have been reported to have antidiarrheal, antibacterial, anti-inflammatory, and anticancer activities" [6]. "It has been reported that galactose a type of lectin isolated from the ripe fruit of *P. guajava* binds with E. coli, preventing its adhesion to the intestinal wall and thereby preventing infection resulting in diarrhea. Another active compound is Quercetin which is a major flavonoid present in *P. guajava* leaves and has been reported to have antidiarrhoeal activity. Although the plant is widely used in traditional medicine, few studies have been conducted on the pharmacological activities of the plant. Phenolic and flavonoid compounds are widespread in the plant kingdom where they act as antioxidants and free radical scavengers" [7,8,9].

The objective of this study was to determine the presence of alkaloids, phenolic, flavones, flavonoids, glycosides, tannins, and saponins, and to quantitatively determine the total alkaloid, saponin, phenolic, tannin, and flavonoid contents, characterize the extract using Fourier-Transform and Gas Chromatography – Mass Spectrophotometer as well as determine the antioxidant and antimicrobial activities of the

aqueous extracts of *P. guajava* (Pomifera (red) and Pyrifera (white) Varieties.

2. MATERIALS AND METHODS

2.1 Requirements

Chemicals; 99.8% Methanol, 0.1 mM DPPH solution, 1N Folin-Ciocalteu reagent, 5% sodium carbonate, Gallic Acid, 5% sodium nitrite, 10% aluminium chloride, 4% sodium hydroxide, tannic acid, Mayer reagent, Wagner's reagent, Lead acetate, Alkaline reagent, Ferric chloride, Molisch's reagent, Alkaline reagent, Iodine solution, sodium hydroxide, all chemicals used to determine and quantify the presence of phytochemical constituents of *P. guajava* (Pomifera (red) and Pyrifera (white) extracts were of high purity.

Plant Material- Fresh Leaves of selected medicinal herb *Psidium guajava* (Pomifera (red) and Pyrifera (white)) varieties harvested from the Embu County, Kenya in the month of June, 2022. The collected leaves were thoroughly washed with running tap water to remove dusts and other unwanted materials accumulated on the leaves. The dust free leaves were shade, dried at room temperature for ten days. The dry leaves were then ground into the fine powder using a milling machine, weighed, packed and stored in clean dry plastic bags.

2.2 Phytochemical Screening

2.2.1 Detection of phenolic compounds

Detection of Phenolic Compounds was determined using the Lead acetate test. 50 mg of extract was dissolved in distilled water and 3 ml of 10% lead acetate solution added. The presence of phenolic compounds was indicated by a bulky white precipitate.

2.2.2 Detection of flavonoids

Detection of flavonoids was performed using the alkaline reagent test. An aqueous solution of the extract was treated with 10% ammonium hydroxide solution. Yellow fluorescence indicated the presence of flavonoids.

2.2.3 Detection of saponins

Detection of saponins was performed using the Frothing test. 50 mg of extract was diluted with distilled water to make up to 20 ml. The suspension was shaken for 15 min. A 2-cm layer of foam indicated the presence of saponins.

2.2.4 Detection of alkaloids

Alkaloids were detected were detected using the Hager's test. 50 mg of solvent-free extract was diluted with few ml of dilute hydrochloric acid and filtered. 2 ml Hager's reagent (saturated aqueous solution of picric acid) was added to a few ml of the filtrate. A prominent yellow precipitate indicated presence of alkaloids.

2.3 Quantitative Analysis

2.3.1 Total phenolic content

0.2, 0.4, 0.6, 0.8 and 1ml aliquots of working standard solution were pipetted out into the series of test tubes marked S_1, S_2, S_3, S_4 and S_5 respectively to make 2.5, 5.0, 7.5, 10.0, and 12.5 µg/ml concentrations. 50µl of phenolic extracts of samples were taken into series of test tubes and analysis performed in triplicates. The contents of all the test tubes were made up to 1ml with distilled water. Test tube marked 'B' with 1ml of distilled water served as the blank. 0.5ml of Folin-Ciocalteu (1N) was added to each test tube including the blank. The test tubes were vortex and allowed to stand for 5min at room temperature. 2.5ml of 5% sodium carbonate was added to all the test tubes including the blank. The test tubes were vortex and incubated in the dark at room temperature for 40min. The absorbance of the blue color developed against the reagent blank at 725nm was measured using spectrophotometer. The amount of total phenolics in the sample was calculated using equation1 below and expressed as mg/g of the Gallic acid Equivalent.

$$C = c_1 \, x \, \frac{V}{m} \quad \text{.....Equation 1}$$

Where C = total phenolic content in mg/g, in GAE (Gallic acid equivalent), c_1 = concentration of the Gallic acid established from the calibration curve in mg/ml, V = volume of extract in ml. and m = the weight of the plant extract in g.

2.3.2 Total tannins content

100 mg of Polyvinyl polypyrrolidone (PVPP) was weighed in 2-ml Eppendorf tubes. 500µL of plant sample and 500µL of distilled water was added. The tubes were incubated for 4 h at 4ºC. The Eppendorf tubes were centrifuged at 3000rpm for 10 min at 4ºC after incubation. The supernatant

contains only the non-tannin phenolic. 0.2, 0.4, 0.6, 0.8 and 1 ml aliquots of working standard solution was pipetted into the series of test tubes marked S_1, S_2, S_3, S_4 and S_5 respectively. 100µL of non-tannin phenolic extract of sample was added into series of test tubes. The analysis was performed in triplicates. The contents of all the test tubes was made up to 1 ml with distilled water. The test tube marked 'B' with 1 ml of distilled water served as the blank. 0.5 mL of Folin-Ciocalteu reagent (1N) was added to each test tube including the blank. Test tubes were vortex well and allowed to stand for 5 min at room temperature. After that, 2.5ml of 5% sodium carbonate was added to all the test tubes including the blank. Test tubes were vortex again and incubated in the dark at room temperature for 40min. The absorbance of the blue colour developed against the reagent blank at 725 nm was measured using spectrophotometer. The amount of tannins in the sample was calculated using equation1 below and expressed as mg/g of the Tannic Acid Equivalent.

$$C = c_1 \, x \, \frac{V}{m} \quad \text{.....Equation 2}$$

Where *C* = total tannin content in mg/g, in TAE (Tannic acid equivalent), c_1 = concentration of the Tannic acid established from the calibration curve in mg/ml, *V* = volume of extract in ml. and *m* = the weight of the plant extracting.

2.3.3 Flavonoid content

0.2, 0.4, 0.6, 0.8 and 1 ml aliquots of working standard solution was pipetted out into the series of test tubes marked S_1, S_2, S_3, S_4 and S_5, respectively to make 8, 16, 24, 32, and 40 µg/ml concentrations respectively. 50µL of extract of sample was taken into series of test tubes. The analysis was perfomed in triplicates. The contents of all test tubes was made up to 1 ml with distilled water. Test tube marked 'B' with 1 ml of distilled water served as the blank. 150µL of 5% sodium nitrite was then added to each test tube including the blank. The test tubes were vortex and incubated at room temperature for 5 min. Then 150µL of 10% aluminium chloride was added to all the test tubes including the blank. The test tubes were vortex again and incubated at room temperature for 6 min. 2 ml of 4% sodium hydroxide was added to all the test tubes. The contents of test tubes were made up to 5 ml using distilled water. The test tubes were vortex well and allowed to stand for 15 min at room temperature. The absorbance of the pink colour developed due to the presence of flavonoids against the reagent blank at 510nm was measured using the spectrophotometer. The amount of flavonoids in the sample was calculated using equation1 below and expressed as mg/g of the Rutin acid Equivalent.

$$C = c_1 \, x \, \frac{V}{m} \quad \text{.....Equation 3}$$

Where *C* = total flavonoid content in mg/g, in RAE (Rutin acid equivalent), c_1 is the concentration of the Rutin acid established from the calibration curve in mg/ml, *V* is the volume of extract in ml. and *m* is the weight of the plant extract in g.

2.4 Mineral Content

2.4.1 Calcium and magnesium content in the sample

5ml of sample solution was pipetted into a beaker. 5 ml of KCN solution and 10ml of ammonium chloride-ammonium hydroxide buffer was added into the sample solution followed by 2 drops of Solochrome Black 1 indicator and titrated against EDTA. The end point result that of wine red colour of the solution changed to pure blue colour. 5ml of blank solution was also titrated as above.

The amount of calcium and magnesium in the sample was calculated using the equation 4 and 5 below.

$$Ca\% = V \times N \times 100/wt \times 100/5 \times 20/1000 \text{.....} \quad \text{Equation 4}$$

$$Mg\% = V \times N \times 100/wt \times 100/5 \times 12/1000 \text{.....} \quad \text{Equation 5}$$

2.4.2 Quantitative analysis of vitamin B_{12} content

The concentration of cyanocobalamin was determined using UV/Vis by comparing the plant extracts with standards of known concentrations using the maximum absorbance at 361 nm according to Birgit Schelling, Mettler Toledo method [10].

2.4.3 Sample preparation

1g of sample was extracted in 80% methanol at 60℃ for 2 hours cooled and filtered into clean vials. The standard for analysis of vitamin B12

was prepared as follows; vitamin B12 solid standard; 0, 5,10, 20, 30 and 40mg/L standard curves were prepared by transferring 3ml of the standard to a quatz cuvette and the standards measured at the wavelength corresponding to the peak absorbance. The stock standards were prepared by dissolving 2.5mg in methanol and the working standards prepared by serial dilution. Methanol was used as a blank. All experiments were carried out in triplicates and the results expressed as mean ± standard deviation (SD) in mg/L/kg using the equation below.

$$Concentration\ per\ kg = \frac{Measured\ concentration\ \left(\frac{mg}{l}\right) x\ 1000\ g}{weight\ of\ sample} \quad \text{..... Equation 6}$$

2.5 Characterization

2.5.1 Fourier transform infrared

"The Shimadzu FTIR spectrophotometer (FTS-8000, Japan) was used to analyze functional groups present on the plant extract by the standard KBr method, with spectral resolution set at 4 cm^{-1} and the scanning range from 400 to 4000 cm^{-1}. The samples were ground with KBr in the ratio of 1mg to 10mg in a mortar and pestle, and 1mg of homogenous mixture placed in sample discs pressed using a hydraulic press and mounted into the FTIR machine for analysis" [11].

2.5.2 Gas chromatography mass spectrophotometer (GC–MS)

The samples were first cleaned up using sample cleanup procedure to remove interfering matrices, concentrate the analyte, and change the sample matrix to the GC grade before analysis. Briefly, solid phase extraction procedure involved use of C18 cartilage conditioned with 3ml of methanol then 3ml of sample was loaded to allow it flow slowly out of the cartilage giving it adequate time to interact with adsorbent. The sample was then left to dry in a stream of air for ten minutes. It was thereafter eluted with 3 mL methanol into a 4mL vial. "Thereafter concentrated using genetic concentrator, reconstituted with 1mL of methanol, filtered using nylon micro filters size 0.22uM into 1.5ml vials and taken to GC-MS for analysis. GC–MS analysis of crude *Psidium guajava* var. *pomifera and pyrifera* was evaluated using a shimadzu GC–MS. 5 g of the powdered plant samples were extracted with Acetonitrile, then solvent exchanged with 2, 2, 4-Trimethylpentane before GCMS analysis. GC–MS technique was used for identification of the chemical compounds present in the extracts and it was carried out on Agilent 5975 GC–MS operating in EI mode at 70 eV with a mass range of 40 – 400 m/z. A capillary column 30 m × 0.25 mm (id) and Helium gas was used as carrier gas with flow rate of 1.2 ml/min and oven temperature of 60°C"[12].

2.6 Antioxidant Activity

"Antioxidant activity was determined using the DPPH method with modification" [13]. Briefly, 0.1mM solution of DPPH was prepared in 80% ethanol by dissolving 0.038mg of DPPH. The solution in the 1L ethanol was covered with aluminum foil to protect from sunlight. 500mg/ml of extracts were prepared by dissolving 25mg of dry extract in 250 ml of ethanol. Concentrations of 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/ml of the extracts were prepared by serial dilution. The control standard ascorbic acid was also prepared the same way. The samples were then incubated a room temperature for 30 minutes. After that, the absorbance at 517 nm was measured, each sample of red and white *Psidium guajava* was measured three times, and the anti-radical activity was expressed as Ascorbic acid equivalent antioxidant capacity (TEAC) per g of *Psidium guajava*. The standard curve was generated using a concentration of 500, 250, 125, 62.5, 31.25, 15.625, and 7.8125 µg/ml of ascorbic acid. The percentage inhibition from the absorbance of the sample and negative control was calculated using the equation below.

$$\%\ Inhibition = \frac{(Absorbance\ of\ DPPH - Absorbance\ of\ Sample)}{Absorbance\ of\ DPPH}$$

The IC_{50} (Inhibition Concentration 50%) was calculated from the concentration and percentage inhibition graph.

2.7 Antimicrobial Activity

Antimicrobial activity was done using the Kirby-Bauer disk diffusion method using Mueller Hinton Agar (MHA)as the media.

2.8 Preparation of Mueller Hinton Agar

Generally, 19.0gm of Mueller Hinton Agar (MHA) powder was weighed using an electronic balance. The powder was dissolved in 500 ml of

distilled water. Mild heating was done to facilitate solubility of the Mueller Hinton powder. The media was covered using the aluminium foil and placed inside the autoclave for sterilization. The pressure and temperature was allowed to build to 15 pounds per square inch and 121ºC for 15 min. The media was cooled to 45ºC. The MHA was aseptically dispensed into the petri dishes and allowed to solidify. Then using a sterile cotton swab, four or five colonies were obtained and transferred into a 5 ml of 0.9% saline tube. Preparing a 0.5 McFarland standard solution as follows did the standardisation of a constant amount of bacteria.

2.9 Procedure for preparation of 0.5 McFarland Standard

0.048M $BaCl_2$ was prepared by weighing 1.17gm of H_2SO_4.H2O diluting to 100 ml with distilled water. Then 1% (v/v) of H_2SO_4 (0.25M) was prepared by adding 1.0 ml concentrated H_2SO_4 to 100 ml distilled water. 0.5 ml of the 0.048M $BaCl_2$ was then added to 99.5 ml of the 1% H_2SO_4 and mixed thoroughly. The McFarland Solution was then dispensed into the test tubes similar to those containing the bacteria to be standardised.

2.10 Procedure for Standardisation of Bacterial suspension

A thick black line was drawn horizontally across a white card using a black permanent marker. Then the bacterial suspension test tubes were agitated to suspend the bacterial cells. Finally, a test tube of the McFarland turbidity standard was held besides each bacterial suspension test tubes and the white card held behind the two test tubes to verify that the optimal density was approximately the same in both test tubes. To those suspensions that were denser than the turbidity standard, sterile physiological saline was added gradually till the optical densities were approximately similar. To those suspensions that were less dense than the standard, more organisms were added to the test tube from the culture plate.

A Whatman No.1 filter paper was punched out with the aid of a paper punch and placed on a petri dish. The discs were sterilized by autoclaving at 121ºC for 15 min and left to cool. The discs were impregnated with 0.1 ml of different concentrations of extract which were labelled as follows: 10%, 20%, 30% and the 4th disk contained a solution that served as a control.

A sterile cotton swab was dipped into the inoculum tube and rotated against the side of the tube (above the fluid level) using firm pressure to remove excess fluid. The dried surface of a Mueller-Hinton agar plate was inoculated by streaking the cotton swab three times over the entire agar surface. The lid was left slightly ajar to allow the plate to sit at room temperature for at least 3 to 5 minutes, for the surface of the agar plate to dry.

The impregnated disks and commercial antibiotic disks were dispensed evenly and pressed onto the surface of the inoculated agar plates using sterile forceps. Commercial antibiotic disks were used as positive control and the pure solvent (ethanol) impregnated disks were used as negative control. The MHA plates were sealed with parafilm and incubated at 37°C for 24hrs.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical analysis

Chart 1 shows the summarized phytochemical screening of chemical constituents of guava extracts under study on qualitative basis.

“The phytochemical screening of chemical constituents of guava extract revealed the presence of active compounds; tannins, flavonoids, phenols, saponins, and alkaloids which are known to exhibit medicinal and physiological activities. For instance, tannins are polyphenolic compounds that bind to proline rich protein that interferes with protein synthesis and has known to have antibacterial activity. Flavonoids are hydroxylated polyphenolic compounds known to be produced by plants in response to microbial infections to which this aspect has been extensively studied and found to have antimicrobial activity against an array of microorganisms *in vitro”* (Biswas, Rogers, McLaughlin, Daniels, & yadav, 2013). “Also they have a capacity to act as an antioxidant; it has been reported that the flavones and catechins seem to be the most powerful flavonoids for protecting the body against reactive oxygen species” (Panche, Diwan, & Chandra, 2016).

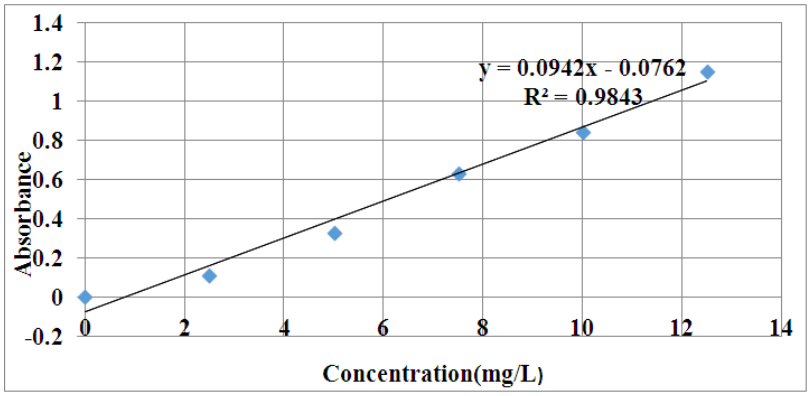
Chart 1. Summarized phytochemical screening of chemical constituents of guava extracts

Test	Observation
Tannins	+
Flavonoids	+
Phenols	+
Saponins	+
Alkaloids	+

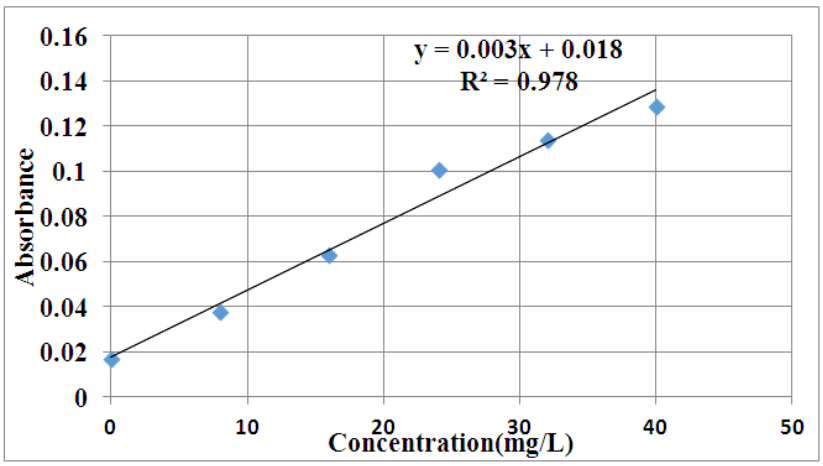
Key: + presence of constituents; - absence of constituents

3.1.2 Total flavonoid, phenolic, tannins, alkaloids and saponnin contents

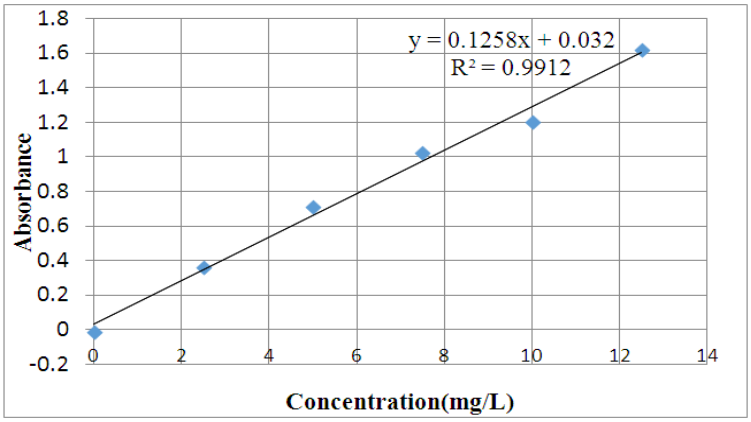
The total flavonoid, phenolic, tannins were obtained using the spectrophotometric method while alkaloids and saponnin contents were done using gravimetric method. Graphs 1, 2 and 3 below shows the standard calibration curves for phenolics, flavonoids and tannins.



Graph 1. Calibration Curve of Total Phenolic Content



Graph 2. Calibration Curve of Total Flavonoid



Graph 3. Calibration Curve of Total Tannin Content

Graphs 1, 2 and 3 above were drawn for the phenolic, flavonoids and tannins standards; gallic, rutin and tannic acids respectively. From the graphs, the R^2 values were 0.9843, 0.978 and 0.9912 respectively. The graphs were used to analyze the concentrations of phenolics, flavonoids and tannins in the dry plant samples and calculated using equations 1, 2 and 3 above and results are as shown in Table 1.

Table 1 shows the total phenolic, flavonoids, tannins, Alkaloids, and Saponin Content in two varieties of *Psidium guajava* leaf extracts. From the data collected, *psidium guajava* var. *pyrifera* had more phenolic and tannin contents compared to *Psidium guajava* var. *pomifera*. The flavonoid content in *P. guajava* var. *pomifera* was more compared to *psidium guajava* var. *pyrifera*. Besides, the alkaloid content was found to be relatively high in *P. guajava* (Pomifera) (red) than in *P. guajava* Pyrifera (white), while the saponin content was relatively high in *P. guajava* Pyrifera (white) than in *P. guajava* (Pomifera (red). These explains the medicinal properties of these leaves.

3.1.3 Fourier transform infrared analysis

The functional groups present on the plant extracts were determined using FTIR (FTS 8400) series located at the Jomo Kenya University of Agriculture and Technology.

From the spectra, the *Psidium guajava* var. *pomifera and pyrifera extracts* were found to have OH stretching at 3332 and 3325 cm^{-1}, CH_2 stretching 2934 cm^{-1}, C=O at 1598, and C-O-C at 1001 cm^{-1} respectively. All the extracts exhibited the presence of a broad peak for hydrogen - oxygen bonded –OH stretching in the functional group region. Presence of this functional groups can be attributed to the presence of tannins, flavonoids, alkaloids, and polyphenols-containing phytochemicals in the leave extracts of *P. guajava* var. *pomifera and pyrifera* [9]. Most studies show that a number of plant metabolites, including polyphenolic substances such as flavonoids and tannins and various herbal extracts, show antioxidant, anti-inflammatory, and antimicrobial activities.

Table 1. Total Phenolic, Flavonoids and Tannins, Alkaloids, and Saponin Content in Two Varieties of *Psidium guajava* Leaf Extracts

Secondary Metabiltes	Sample Name	
	***P. guajava* Pyrifera (white)**	***P. guajava* (Pomifera (red)**
Phenolics (mg/g in GAE)	122.07 ±2.73	106.50 ±2.97
Flavonoids (mg/g in RAE)	2486.82 ± 9.10	2650.65 ±27.48
Tannins (mg/g in TAE)	10.16 ±0.07	17.86 ±0.19
Alkaloids (mg/g of dry plant)	10.50±0.01	34.75±0.07
Saponins (mg/g of dry plant)	17.75±0.09	7.25.50±0.11

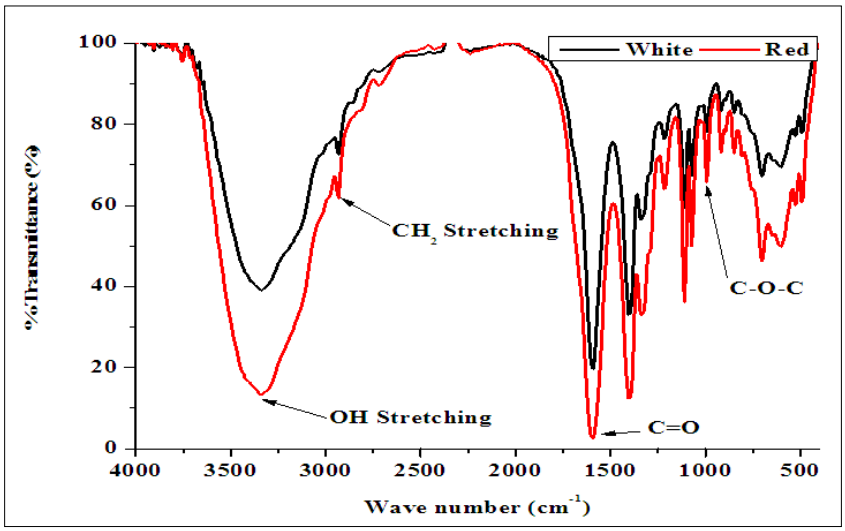


Fig. 1. FTIR of the *Psidium guajava var.* pomifera and pyrifera extracts

3.1.4 Gas chromatography – Mass spectrophotometer (GC-MS) analysis

Secondary metabolites present in *P. guajava* var. pomifera and pyrifera extracts were identified with the help of GC–MS and the results are depicted in Table 2.

"A number of volatile compounds belonging to alcohols, ketones, esters, aldehydes, terpenoids, and acids were identified in the Psidium guajava var. pomifera and pyrifera extracts based on the data obtained (Table 2). These volatile compounds are classified as alcohols, ketones, esters, amines, ethers or oxides, aldehydes, amides, phenols, heterocycles, and terpenes. Phytol is a diterpene alcohol derived from the degradation of chlorophyll, which is used in the synthesis of vitamins E and K, and it has promising antischistosomal properties in vitro and in a mouse model of schistosomiasis mansoni. Lupeol is an anti-cancer and anti-inflammatory dietary triterpene that aids in the stabilization of phospholipid bilayers in plant cell membranes. Isolated flavonoids and flavonoid glycosides have been shown to induce the formation of metal nanoparticles, such as Apiin (apigenin glycoside) extracted from Lawsonia inermis" [14].

3.2 Vitamin B12

The quantity of vitamin B_{12} was determined using UV-Visible method. The samples were extracted using methanol and the standards also prepared in the same way. The sample and the standards were run in a UV-Vis spectrophotometer at a wavelength of 361 nm and the standard curve below was obtained.

Table 2. Some secondary metabolites identified using GC–MS with the help of a NIST 17 spectral data base

Red and White compounds present				
RT	**RT Compound**	**MF**	**MW**	**Class**
5.903	terpineol	$C_{12}H_{17}F_3O_2$	250	Alcohol
7.781	Alfa-copaene	$C_{15}H_{24}$	204	Alcohol
8.738	2-propenoic acid 3-(2-hydroxyphenyl)- (E)	$C_9H_8O_3$	164	Hydroxycinnamic acid
9.5505	Lycopodan-8-one,5,12-dihydroxy-15-methyl-(5a,15S)-	$C_{16}H_{25}NO_3$		Ketone
11.088	Caryophyllenyl alcohol	$C_{15}H_{26}O$	222	Sesquiterpene Alcohol
12.6949	Globulol	$C_{15}H_{26}O$	222	Sesquiterpene alcohol
15.566	Cubenol	$C_{15}H_{26}O$	222	Sesquiterpene Alcohol
17.325	Rotundene	$C_{15}H_{24}$	204	Terpene
21.815	Phytol	$C_{20}H_{40}O$	296	Acyclic diterpene
40.4425	Lupeol	C30H50O	426	Triterpene

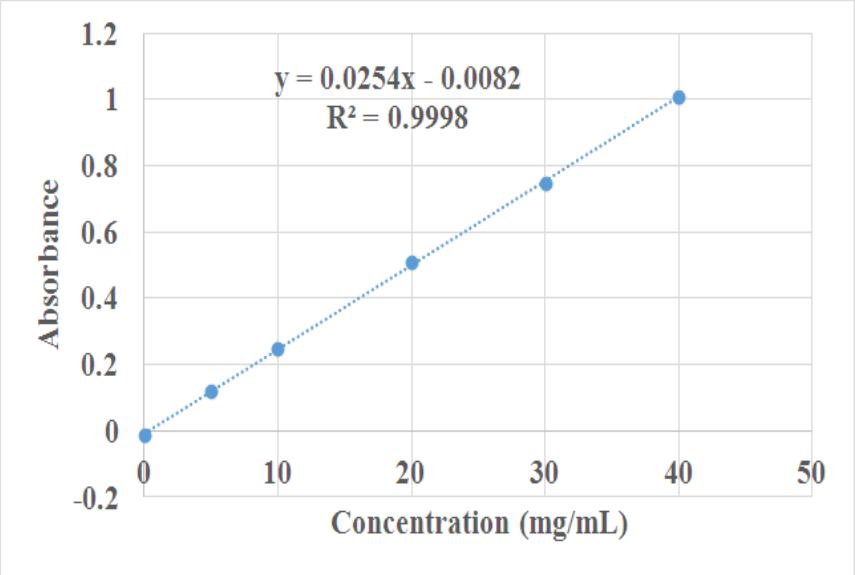


Fig. 2. Standard curve of vitamin B_{12}

Table 3. Quantity of Vitamin B_{12} in the plant extract of *Psidium guajava* (red and white) varieties

Sample Name	Concentration in mg/mL per kg
Psidium guajava (Pomifera (red)	315.8 ± 0.0
Psidium guajava Pyrifera (white)	236.8 ± 0.0

From the graph, the r^2 value was found to be 0.9998. the graph was used to run the sample extracts of red and white *P. guajava* Table 3.

From Table 2, the concentrations of vitamin B_{12} in the two plant samples were relatively high. However, the concentration vitamin B_{12} in *P. guajava (red)* variety was higher. This is depicted in the high amount of flavonoids, tannins as well as the phenols in the plants. The high content of these metabolites shows the *P. guajava* (red and white) aqueous extract have vitamin B_{12}.

3.3 Antioxidant Activity

From the graph (Fig. 2) the antioxidant activity is low compared to the standard. However, as the concentration of the extracts increases, the antioxidant activity also increases. Increase in the concentration means an increase in the radicals that are able to act on DPPH.

3.4 Antimicrobial Activity

Antimicrobial inhibition of the extract was evaluated using agar disc diffusion method and the results are depicted in the graphs in Figs. 4, 5, 6, and 7.

Figs. 4, 5, 6, 7 show the antimicrobial activity of extracts of *P. guajava* (Pomifera (red) and Pyrifera (white)). From the results obtained, the highest activity was observed against *E.C, S.T, S.A* and *B.C*, respectively [15]. "Bacterial destruction occurs due to the physical interaction between the cell wall and the extract and is more detrimental for gram negative bacteria due to absence of the thick peptidoglycan layer found in gram positive bacteria which acts as a protective layer. Due to this interaction, bacteria is denatured as interaction of metallic nanoparticles with protein thiol groups releases ions which lead to breakdown of bacterial cell surface leading to death" [5,16].

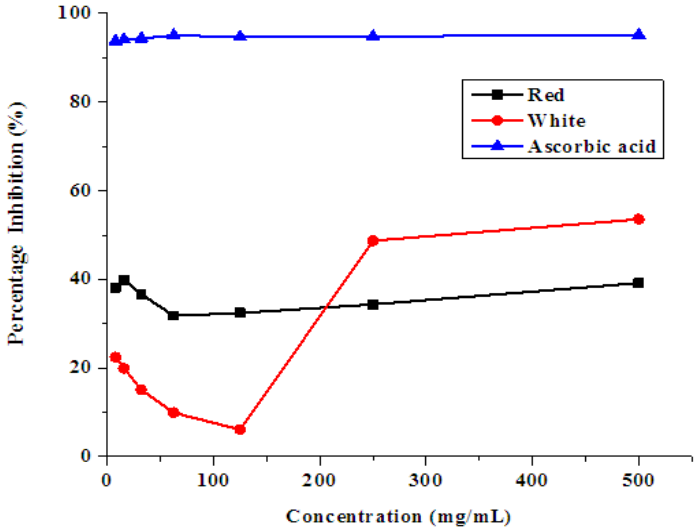


Fig. 3. Antioxidant Activity of the Red and white varieties

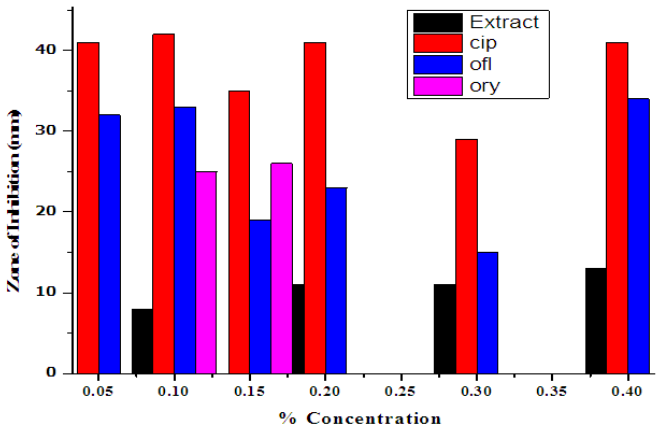


Fig. 4. Antimicrobial activity of extract, ciprofloxacin, ofloxin, and oryn against B.C

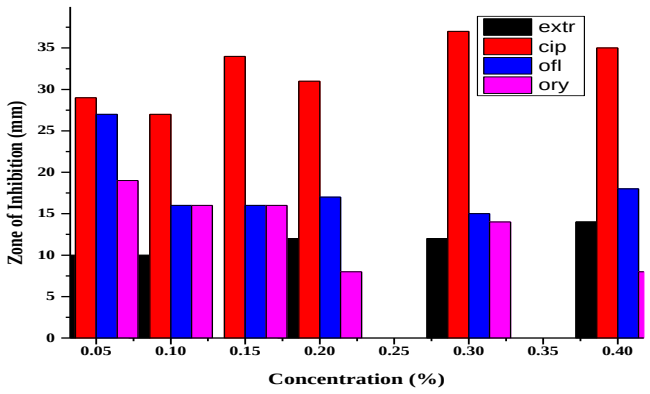


Fig. 5. Antimicrobial activity of extract, ciprofloxacin, ofloxin, and oryn against E C

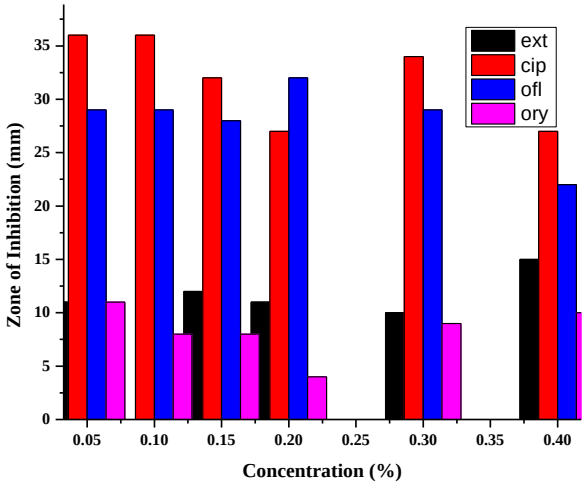


Fig. 6. Antimicrobial activity of extract, ciprofloxacin, ofloxin, and oryn against SA

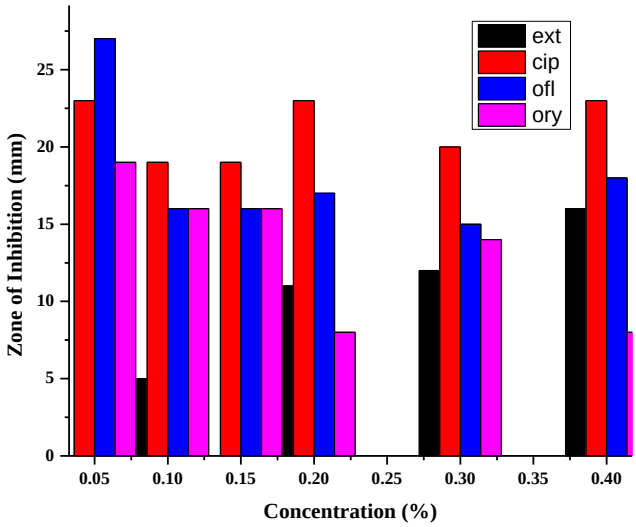


Fig. 7. Antimicrobial activity of extract, ciprofloxacin, ofloxin, and oryn against SE

4. CONCLUSION

These extracts contained 106.50 ± 2.97 and 122.07 ± 2.73 mg/g in GAE phenolic content, 2650.65 ± 27.48 and 2486.82 ± 9.10 mg/g in RAE of flavonoids, 17.86 ±0.19 and 10.16 ±0.07 mg/g in TAE of Tannins, 69.50 ± 0.14 and 10.50±0.01 alkaloid content, and 7.25.50±0.11 and 17.75±0.09 saponin content and displayed significant antioxidant capacity in the DPPH (IC_{50} = 52.17 ± 7.30 *µ*g/mL) assay. *GC-MS revealed presence of* Sesquiterpenes, terpenes, and triterpenes. The present study provides additional data for supporting the use of *Psidium guajava (*Pomifera (red) and Pyrifera (white)) extracts as natural antimicrobial and antioxidant agents. Future work will be performed to use these extracts in tea to prepare active drinks which can release antimicrobial and antioxidant agents to prevent stomach challenges such as ulcers and amoeba.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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