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Comparative Studies of Phytochemical and Antimicrobial Activity of *Carica papaya* L. Extracts against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*

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Abstract

Carica papaya extracts are known for their traditional medicinal uses. The ability of its parts to control the growth of common pathogens in the laboratory has been tested in different parts of the world using different varieties of *C. papaya*. This study was initiated to compare the phytochemical and antimicrobial activity of different plant parts extracts of *C. papaya* var. papaya GAV4 on *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans*. *C. papaya* plant parts were collected from a farm in Kiboswa (Kisumu): coordinates 0.0245°S and 34.7474°E, and then were transported to Maseno University Botany Laboratory. Seeds, green leaves and bark were washed thoroughly with tap water, rinsed in sterile water and dried after which they were ground using a grinder. From each of the three plant parts, three types of extracts were prepared using water, acetone and ethanol in the concentrations 25%, 50%, 75% and 100%. The antimicrobial activity of the extracts was tested on microbes growing on agar plates by inoculation with the different concentrations using diffusion method and replicated 3 times. Extracts were isolated using Soxhlet apparatus and MIC determined by serial dilution, zone of inhibition was measured in millimeters. Means from the measurements were separated and compared at significance level $P = 0.05$. Phytochemicals present included alkaloids, flavonoids, tannins, phenols, saponins, glycosides, anthocyanins and terpenoids while anthraquinones were absent. Ethanol bark extract on *C. albicans* showed higher inhibition and there were significant differences in inhibition among the plant parts and extracts used. In concentrations used, 25% was significantly different from 50%, 75% and 100%. The results obtained in this study confirm that *C. papaya* has antimicrobial activity on *E. coli*, *S. aureus* and *C. albicans*; and has also shown high potentials for use as a potential source of antibiotics to treat diseases caused by these microorganisms.

1. Introduction

In traditional system of medicine, plant preparations in the forms of decoctions, concoctions, macerations, or infusions are used to treat a wide range of diseases (Tsobou *et al.*, 2016). Current estimates indicate that about 80 million people worldwide still depend on plants for their health needs (Dwivedi *et al.*, 2020). Limited access to primary health care in developing countries has resulted into widespread use of herbal medicines due to the availability, accessibility, affordability and cultural acceptance across different ethnic backgrounds (Muhwana *et al.*, 2020). There is widespread use of broad-spectrum antibiotics which has led to the emergence of nosocomial infections caused by drug resistant microbes (Abubakar, 2009). Multi drug resistance and the presence of several virulence factors in the strains of many pathogens responsible for different diseases pose an increasing threat to disease treatment. There are several varieties of this plant spread throughout the world. Papaya also known as pawpaw

(*Carica papaya* Linn) is commonly known for its nutritional and medicinal values throughout the world (Alabi *et al.*, 2012). It is a giant herbaceous non woody plant resembling a tree from the family *Caricaceae* (Akujobi *et al.*, 2010).

Each part of papaya tree possesses economic value when it is grown on a commercial scale (Krishna *et al.*, 2008; Orchue and Momoh, 2013). Even though the active compounds are normally extracted from all parts of the plant, the concentration of these compounds varies from structure to structure (Aruljothi *et al.*, 2014). However, parts known to contain the highest concentration of the principles are preferred for therapeutic purposes and it can either be the leaves, stem, barks, roots, bulks, corms, rhizomes, woods, flowers, fruits, and the seeds (Kafaru, 1994; Emitaro *et al.*, 2020). Various parts of the papaya plant, which include the leaves, fruit, seed, latex, and root, are known to contain bioactive compounds that contribute to reported medicinal properties (Anibijuwon and Udeze, 2009; Aravind *et al.*, 2013).

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In Kenya there exists more than 65 varieties of *C. papaya* [Asundu *et al.*, 2010], yet most *C. papaya* farmers around Kiboswa (Kisumu) grow the *C. papaya* L. var. papayi GAV4 variety. It was therefore easy to obtain *C. papaya* L. var. papayi GAV4 plant materials for these studies. Extracts from different varieties of the same plant may respond differently to bioassay tests conducted on microorganisms due to varying physiological and chemical characteristics where they may occur (Nirosha and Mangalanayaki, 2013; Jyotsna *et al.*, 2014; Brij *et al.*, 2015). Therefore, evaluating *C. papaya* var. papayi GAV4 extracts for possible microbial control of *E. coli*, *S. aureus* and *C. albicans* was appropriate because research work carried out in other parts of the world was only conducted on other varieties of *C. papaya* (Nirosha and Mangalanayaki, 2013; Jyotsna *et al.*, 2014; Brij *et al.*, 2015). It is imperative therefore that before this study, little was known about the antimicrobial effect of bark, leaf and seed extracts of *C. papaya* var. papayi GAV4. The choice of *C. papaya* var. papayi GAV4 was guided by earlier findings that showed that it is the most abundant variety in Kiboswa (Kisumu) (Asundu *et al.*, 2010).

2. Material and methods

2.1 Study area

After identification of the plant (Figure 1) was conducted using a taxonomic key (Cowan, 1999), the materials used in this study were collected from a farm located at Kiboswa (Kisumu) within the geographical coordinates 0.0245°S and 34.7474°E. All plant parts including; fruits, leaves and bark were then transported to the botany laboratory at Maseno University (Maseno Kenya) located within the geographical coordinates 0.0°0S and 34.36°E where analysis was conducted.



Figure 1. *C. papaya* var. papayi GAV4 tree with ripe (yellow) and unripe (green) fruits.

2.2 Preparation of seed extract

Seeds were obtained when fruits earlier acquired from Kiboswa (Kisumu County), and taken to Maseno University botany laboratory were washed with clean tap water, rinsed in sterile distilled water and cut open using a kitchen knife, then left to dry

for 30 days at 25°C at the Botany Laboratory, Maseno University (Jyotsna *et al.*, 2014). The seeds were then powdered using a grinder to produce a fine powder (Figure 2).



Figure 2. *C. papaya* var. papayi GAV4 seed powder.

2.3 Preparation of leaf extract

Disease free green leaves of *C. papaya* var. papayi GAV4 earlier collected from Kiboswa were washed in tap water, rinsed in sterile distilled water, dried at 25°C for 20 days before being grounded into a green powder using a grinder to produce a fine powder (Jyotsna *et al.*, 2014) (Figure 3).



Figure 3. *C. papaya* var. papayi GAV4 leaves powder.

2.4 Preparation of bark extract

Diseases free papaya bark were cut from the tree using a sharp kitchen knife, washed in tap water, rinsed in sterile distilled water then dried at 25°C for 30 days before being ground to produce a brown powder using a grinder (Jyotsna *et al.*, 2014) (Figure 4).



Figure 4. *C. papaya* var. papayi GAV4 bark powder.

2.5 Ethanol extraction of seed, leaf and bark extracts

One hundred grams of powdered dried seeds, leaf and bark were weighed using a weighing machine and powder transferred into 500 ml glass conical flasks as earlier described by **Okunola et al. (2012)**. Ninety five percent of 500 ml ethanol was measured and poured onto the conical flask containing the seed, leaf and bark powder and stirred to produce mixtures that were allowed to stand for 24 hours before decantation and filtration through a Whatman filter paper No 1. (**Okunola et al., 2012**). The resulting filtrates were concentrated in a rotary evaporator at 79°C resulting in concentrates that were stored in the refrigerator at 4°C until required for use.

2.6 Water extraction of seed, leaf and bark extracts

One hundred grams of powdered dried seeds, leaf and bark extracts were transferred into 500 ml glass conical flask into which two hundred millimeters distilled water was poured then stirred to produce mixtures that were allowed to stand for 24 hours before decantation to produce filtrates using a Whatman filter paper No 1.

2.7 Acetone extraction of seed, leaf and bark extracts

One hundred grams of powdered dried leaves, seed and bark extracts were transferred into 500mls glass conical flask into which five millimeters of 95% acetone was poured then stirred to obtain mixtures that were allowed to stand for 24 hours before decantation and filtration through Whatman filter paper No 1. resulting in filtrates that were concentrated with a rotary evaporator at 45°C to produce concentrates that were later stored in the refrigerator at 4°C until required for use.

2.8 Test microorganisms

The test organisms that were used in this study were pure strains of human pathogenic organisms of clinical origin obtained from Centre for Disease Control (KEMRI/CDC) located in Kisian (Kisumu) and maintained on Mueller Hinton Agar (Oxoid, UK) medium as stock cultures in the laboratory refrigerator set at 4 °C. The isolates included pure strains of gram negative bacteria *E. coli* (ATCC 25922) gram positive bacteria *S. aureus* (ATCC 25923) and an imperfect yeast *C. albicans* (ATCC 1405). The *C. albicans* strain was grown in Nutrient agar (NA) media, *E. coli* grown in Nutrient agar (NA) media while the *S. aureus* was grown in Potato Dextrose agar (PDA).

2.9 Phytochemical compounds extraction and screening

Twenty-five grams of dried leaves, seed and bark powder were extracted in Soxhlet apparatus by using 25 ml of solvent having polarity of ethanol for 48hrs and then concentrated by evaporation. These prepared extracts were used for phytochemical screening for alkaloids, flavonoids, tannin, phenols, saponin, terpenoids, anthraquinones, Cardiac glycosides and anthocyanins as earlier described by **Mibei et al. (2012)**; **Musyimi et al. (2007)**; **Musyimi et al. (2017)**; **Opande et al. (2017)**; **Akinoyemi et al. (2005)** and **Opande et al. (2017)**.

2.9.1 Determination of alkaloids

Two grams of the extract were extracted by warming it for 2 minutes with 20ml of 1% H₂SO₄ acid in a 50ml conical flask on a water bath, with intermittent shaking. It was then centrifuged and the supernatant was pipetted off into a small conical flask. One drop of Meyer's reagent was added to 0.1ml supernatant in

a semi micro tube. A cream precipitate indicated the presence of alkaloids.

2.9.2 Determination of flavonoids

5ml of dilute aqueous ammonia solution were added to a portion of the plant extract followed by addition of concentrated sulphuric acid. Positive test was indicated by yellow colouration which disappeared on standing.

2.9.3 Determination of tannins

About 0.5 g of the dried powdered samples was boiled in 20ml of water in a test tube and then filtered through Whatman No. 42 filter paper. A few drops of 0.1% ferric chloride was added. A brownish green or a blue-black coloration indicated the presence of tannins.

2.9.4 Determination of phenols

Ferric chloride test was carried out where the extract was diluted to 5ml with distilled water. To this, a few drops of neutral 5% Ferric chloride solution was added. A dark green or a blue-black color indicated the presence of phenolic compounds.

2.9.5 Determination of saponins

About 2 g of the powdered sample was boiled in 20ml of distilled water in a water bath and filtered. Ten milliliters of the filtrate were mixed with 5ml of distilled water and shaken vigorously to form a stable persistent froth. The froth was mixed with 3 drops of olive oil and shaken vigorously, and then was observed for the formation of emulsion.

2.9.6 Test for anthraquinones

Powdered plant material was boiled with 10% HCl for a few minutes, then filtered and allowed to cool. This was then partitioned against equal volume of chloroform. Formation of rose-pink color upon addition of 10% aqueous ammonium solution, indicated the presence of anthraquinones.

2.9.7 Test for Cardiac glycosides

Five ml of extract was treated with 2ml of glacial acetic acid containing a drop of FeCl₃ solution. This was then underplayed with 1ml conc. H₂SO₄. A brown ring of the interface indicated a deoxy-sugar characteristic of cardenolides.

2.9.8 Test for Anthocyanins

2ml of 2M sodium hydroxide (NaOH) solution was added to few extracts in a test tube. The formation of blue-green colour compound confirmed the presence of anthocyanins.

2.9.9 Test for terpenoids

5ml of the extract was mixed with 2ml of chloroform followed by addition of 3ml of concentrated sulphuric acid to form a layer. Positive test was indicated by formation of a red colouration at the interface.

2.10 Antimicrobial susceptibility test for bacteria

The disc diffusion method on Mueller Hinton agar (**Yahaya et al., 2017**) was used to determine the antibacterial activity of the plant extracts. An overnight culture of the bacterium was diluted to 10⁵ cells/ml using a spectrophotometer at a wavelength of 625 nm. One milliliter of the bacterial suspension was introduced into sterile petri dishes and 20 ml of Mueller - Hinton agar at 40°C poured into the inoculated dishes before the plates were allowed to cool and solidify. A sterile filter circular discs, 8mm in diameter each were cut from Whatman No.1 filter paper

using a paper punch and each dipped in a known concentration of 25, 50, 75 and 100% of the extracts for about 2 minutes, then gently transferred to the centre of the inoculated agar media. Petri dishes inoculated with bacteria and fungi were kept for incubation for 24 hrs at 37°C and 25°C respectively. The diameter of inhibition zone was measured using 12.5 cm Vernier calipers. This was carried out in triplicates in a completely randomized design.

2.11 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts was determined by using Muller-Hinton broth dilution (Anibijuwon and Udeze, 2009) made and sterilized using an autoclave. Serial dilutions of the extract in liquid medium were prepared and 1.0 ml of the prepared broth dispensed into the test tubes labeled from 1 to 4 using sterile syringe and needle. A stock solution containing 100mg/ml of the extract was prepared. 1.0 ml of the solution was dispensed into the tube 1. Subsequently, from tube 1 solution was serially transferred until 4.0-1.0 ml of the solution was discarded from it. An overnight culture of each of the test isolates was prepared in sterile nutrient broth. 1 ml inoculum was transferred into each tube from tube 1 to tube 4. The final concentrations of the extract in each of the test tubes after dilution i.e. 100, 50, 25 and 12.5 mg/ml was incubated at 37°C for 24 hrs and examined for emergent growth.

To measure the MIC values, various concentrations of the stock, 25, 50, 75 and 100 mg/ml were assayed against the test bacteria where the minimum inhibitory concentration was defined as the lowest concentration able to completely inhibit any visible microorganism growth after overnight incubation with media (Prescott, 1999; Yahaya et al., 2017).

3. Results

3.1 Phytochemical screening

Information on the phytochemical constituents of plant materials are generally required for the discovery of novel drugs. The phytochemical screening of *C. papaya* plant materials carried out revealed the presence of alkaloids, flavonoids, tannins, terpenoids, anthraquinones, phenolic compounds and saponins in the extracts of leaf, seed and bark (Table 1), while anthraquinones were not detected in the seed and bark extracts.

Table 1. Screening for secondary metabolites in plant ethanol extracts of *C. papaya*

Phytochemical grouping	Leaves	Seeds	Bark
Alkaloids	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Phenols	+	+	+
Saponins	+	+	+
Anthraquinones	+	-	-
Cardiac glycosides	+	+	+
Anthocyanins	+	+	+
Terpenoids	+	+	+

Key: + Present; - Absent

3.2 Determination of the effect of *C. papaya* seed, leaf and bark extracts on growth of *C. albicans*, *E. coli* and *S. aureus*

The results obtained when the effect of *C. papaya* bark on growth of *E. coli*, *C. albicans* and *S. aureus* was conducted, indicated that the highest zone of inhibition was demonstrated against *S. aureus* was 9.82 mm by ethanol extracts of dried bark, while the lowest zone of inhibition was demonstrated against *C. albicans* measured 0.89mm as shown by water extracts of dried bark (Table 2). There was no visible inhibition exhibited by *C. albicans* on the acetone extracts, yet when comparison was made for the 3 extracts, there were significant differences among the extracts (Table 2).

Table 2. Diameter of zone of inhibition (mm) exhibited by *C. papaya* var. *papaya* GAV 4 bark extracts against *C. albicans*, *E. coli* and *S. aureus*

Test Microorganism	Ethanol extract	Water extract	Acetone extract
<i>E. coli</i> (ATCC 25922)	7.16a	5.96b	6.29b
<i>C. albicans</i> (ATCC 1405)	8.84 a	0.89b	0 c
<i>S. aureus</i> (ATCC 25923)	9.82 a	3.80 b	5.13 c
LSD at P=0.05	0.35		

Data presented are the means of three replicates. Means with the same letter down the same column are not significantly different at P=0.05.



Figure 5. Zone of inhibition for water extracts of bark on *E. coli*, and *C. albicans*

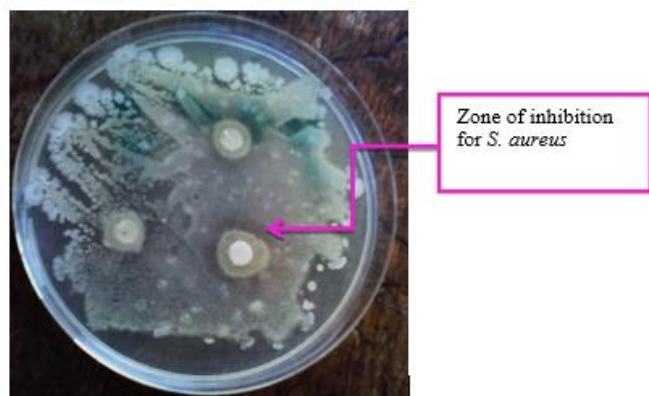


Figure 6. Zone of inhibition for water extracts of bark on *S. aureus*.

The effect of *C. papaya* water bark extract on growth of the 3 microorganisms (Figure 5 & 6) indicate that even the water extract has visible inhibitory effects against the growth of *E. coli*, *C. albicans* and *S. aureus* in the Petri dishes. Yet the effect of *C. papaya* seed extract on growth of *E. coli*, *C. albicans* and *S. aureus* when measured indicated that the highest zone of inhibition was demonstrated against *E. coli* at 8.13 mm by ethanol extract of dried seeds (Table 3). The lowest zone of inhibition was demonstrated against *E. coli* at a measurement of 5.09 mm by the acetone extracts of dried seed (Table 3). There was no inhibition at all for the acetone extract of *C. albicans* and *S. aureus*. There appeared to be no significant differences among the extracts.

Table 3. Diameter of zone of inhibition (mm) exhibited by *C. papaya* var. papayi GAV4 seed extracts against *E. coli*, *C. albicans* and *S. aureus*

Microorganism	Ethanol	Water	Acetone
<i>E. coli</i> (ATCC 25922)	5.13 a	4.51 b	2.97 c
<i>C. albicans</i> (ATCC 1405)	7.27 a	5.22 b	0 c
<i>S. aureus</i> (ATCC 25923)	8.87 a	5.78 b	5.93c
LSD at P=0.05	0.27		

Data presented are the means of three replicates. Means with the same letter down the same column are not significantly different at P=0.05.

Table 4. Diameter of zone of inhibition (mm) exhibited by *C. papaya* leaf extracts against *E. coli*, *C. albicans* and *S. aureus*

Microorganism	Ethanol	Water	Acetone
<i>E. coli</i> (ATCC 25922)	8.13 a	5.09 b	5.22 b
<i>C. albicans</i> (ATCC 1405)	7.8a	6.29b	0 c
<i>S. aureus</i> (ATCC 25923)	8.11 a	5.49b	0 c
LSD P=0.05	0.27		

Data presented are the means of three replicates. Means with the same letter down the same column are not significantly different at P=0.05.

When the effect of *C. papaya* leaves extract on growth of *E. coli*, *C. albicans* and *S. aureus*, was compared, the highest zone of inhibition was demonstrated against *S. aureus* with a measurement of 8.87 mm by ethanol extract of dried leaves

(Table 3). The lowest zone of inhibition was demonstrated against *E. coli* with a measurement of 2.97 mm by the water extract of dried seeds. There was no inhibition for the acetone extracts on *C. albicans* and *S. aureus* (Table 4). The results obtained indicated that there were significant differences among the extracts (Table 4).

The disc diffusion method on Mueller Hinton agar was used to determine the antimicrobial activity of *C. papaya* var. papayi GAV4 leaf, seed and bark extracts with different concentrations (Table 5). As shown in table 5, increase in the concentration has different effects on the microorganism, plant part and extract used. It is shown that ethanol, acetone and water leaf extracts did not inhibit the growth of *C. albicans* and acetone leaf extracts did not inhibit the growth of *S. aureus*. At 25% concentration, there was no inhibition of growth of *E. coli* by ethanol, water and acetone leaf extracts and water seed extracts.

3.3 Determination of Minimum Inhibitory Concentration (MIC)

Table 6 shows Minimum Inhibitory Concentration (MIC) of various extracts of leaf, seed and bark extracts of *C. papaya* var. papayi GAV4 on the microorganisms, the minimum inhibitory concentration was defined as the lowest concentration able to inhibit any visible bacterial or fungal growth, the MIC varies with the microorganism, plant part and extract used.

4. Discussions

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. They synthesize bioactive compounds which are of great potential in agriculture, antimicrobial and anti-insect activity (Emitaro et al., 2020). Phytochemical screening of the leaf, seed and bark extracts of the plant revealed the presence of flavonoids, alkaloids, saponins, tannins, phenolic compounds, glycosides and anthocyanins in agreement with other workers (Ekaiko et al., 2015a; Ekaiko et al., 2015b; Sikandar et al., 2013; Ayoola and Adeyeye, 2010), anthraquinones were only present in leaves but absent in seeds and bark. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Musyimi et al., 2007). For example, alkaloids isolated from plants have been found to have antimicrobial properties (Sikandar et al., 2013), and are one of the most efficient therapeutants that were isolated from the plant extracts during these studies. Flavonoids represent the common and widely distributed group of plant phenolics; their biological functions include protection against allergies, inflammations, platelets aggregation microbes, ulcer, viruses and tumors (Okwu and Okwu, 2004). The presence of tannins in the *C. papaya* can support its strong use for healing of wounds, ulcers, hemorrhoids, frost-bites and burns in herbal medicine (Igboko, 1983). Tannins have astringent properties which hasten the healing of wounds and inflamed mucous membrane (Igboko, 1983; Maduinyi, 1983).

The presence of phenolic compounds in the extracts of *C. papaya* shows that the extracts may have antimicrobial potential, because phenols and phenolic compounds have been extensively used in disinfections and remains the standard with which other bactericides are compared (Oakenful, 1981). The presence of saponins supports the fact that *C. papaya* extracts may have cytotoxic effects (Okwu and Okwu, 2004; Okigbo et al., 2009). Saponins exhibit broad range of pharmacological actions, such as ability to heal wounds and inflamed mucous membranes. Therefore, in view of the occurrence of phytochemicals in the extracts it is more appropriate to state that the antimicrobial activity of the *C. papaya* extracts may be attributed to the presence of the bioactive compounds.

Table 5. Effect of different concentrations of leaf, seed and bark extracts of water, ethanol and acetone on the growth (mm) of *E. coli*, *C. albicans* and *S. aureus*.

Plant part	Extract	Microorganism	Concentration mg/ml			
			25	50	75	100
Seed	Ethanol	<i>E. coli</i> (ATCC 25922)	11.33 a	10.56 b	5.78 c	8.11 d
		<i>C. albicans</i> (ATCC 1405)	10.78 a	10.67 a	10.56 a	12.22 b
		<i>S. aureus</i> (ATCC 25923)	14.22 a	12b	11.78 b	11.11c
	Water	<i>E. coli</i> (ATCC 25922)	0 a	8.11 b	8.89 c	8.67 d
		<i>C. albicans</i> (ATCC 1405)	8.67 a	9.44 a	9.67 a	8.56 b
		<i>S. aureus</i> (ATCC 25923)	10.78 a	12.44 b	11.89 b	9.22 c
	Acetone	<i>E. coli</i> (ATCC 25922)	9.60a	11.80 b	9.90 c	8.83 d
		<i>C. albicans</i> (ATCC 1405)	9 a	10.44 b	9.78c	9.78 c
		<i>S. aureus</i> (ATCC 25923)	9.78 a	10.00 a	10.89 b	9.89 c
Bark	Ethanol	<i>E. coli</i> (ATCC 25922)	8.78 a	6.44 b	7.56 c	7 d
		<i>C. albicans</i> (ATCC 1405)	0 a	0 a	4.44 b	0 b
		<i>S. aureus</i> (ATCC 25923)	0 a	0 a	9.33 b	9.67 c
	Water	<i>E. coli</i> (ATCC 25922)	5.56 a	5.8 a	5.33 b	5.22 b
		<i>C. albicans</i> (ATCC 1405)	9.44 a	0 b	7.78 c	8.70 d
		<i>S. aureus</i> (ATCC 25923)	0 a	10.11 b	10 b	8.78 c
	Acetone	<i>E. coli</i> (ATCC 25922)	6.78 a	6.60 a	5.75 b	6.22 c
		<i>C. albicans</i> (ATCC 1405)	8.44 a	7.78 b	7.44 c	7.88 d
		<i>S. aureus</i> (ATCC 25923)	2.78 a	9.78 b	10.89 c	4d
Leaf	Ethanol	<i>E. coli</i> (ATCC 25922)	10.33 a	9.89 b	5.78 c	5.44 c
		<i>C. albicans</i> (ATCC 1405)	0 a	0 a	0 a	0 a
		<i>S. aureus</i> (ATCC 25923)	6.67 a	3.56 b	2.71b	5.51c
	Water	<i>E. coli</i> (ATCC 25922)	0 a	6.44 b	0 c	8.44 d
		<i>C. albicans</i> (ATCC 1405)	0 a	0 a	0 a	0 a
		<i>S. aureus</i> (ATCC 25923)	10.56 a	10.67 a	0b	8.44 c
	Acetone	<i>E. coli</i> (ATCC 25922)	0 a	8.56 b	8.33 b	9.22 c
		<i>C. albicans</i> (ATCC 1405)	0 a	0 a	0 a	0 a
		<i>S. aureus</i> (ATCC 25923)	0 a	0 a	0 a	0 a

Data presented are the means of three replicates. Means with the same letter across the same column are not significantly different at $P=0.05$.

Table 6. Minimum Inhibitory Concentration (MIC) of various extracts of leaf, seed and bark extracts of *C. papaya* on *E. coli*, *C. albicans* and *S. aureus*

Microorganism	Plant part	Minimum Inhibitory Concentration MIC (mg/ml)		
		Water	Ethanol	Acetone
<i>E. coli</i> (ATCC 25922) P=0.05	Bark	0.1	0.05	0.1
	Seed	0.1	0.05	0.025
	Leaf	0.05	0.025	0.1
<i>C. albicans</i> (ATCC 1405) P=0.05	Bark	0.05	0.1	0.05
	Seed	0.05	0.1	0.05
	Leaf	-	-	-
<i>S. aureus</i> (ATCC 25923) P=0.05	Bark	0.025	0.025	0.025
	Seed	0.025	0.025	0.025
	Leaf	0.025	0.05	-

Key: - No inhibition

4.1 Inhibitory effects *C.papayavar.papayi* GAV4seed, leaf and bark extracts on *E.coli*, *C.albicans* and *S.aureus*

The confirmed presence of bioactive substances in these extracts is very important, such substances has been reported to confer resistance to plants against bacteria, fungi and other microorganisms, this therefore may explain the reasons for the demonstrated antibacterial activity by the plant extracts used in this study. Antimicrobial properties infer that any of these properties: i.e. anti-bacterial (antibiotics), anti-fungal (anti-mycotic), anti-cancerous (anti-oncogenic) or anti-viral is inherent (Baskaran et al., 2012).

The present study has clearly shown that the different parts of *C. papaya* possess antimicrobial potential against *S. aureus*, *E. coli* and *C. albicans*. In line with the present findings, several other studies have reported other varieties of *C. papaya* leaves (Kafaru, 1994; Rahman et al., 2011) have antimicrobial potentials. Additionally, the reports of other workers (Yahaya et al., 2017) have also shown that other varieties of *C. papaya* leaves and stem barks have significant antibacterial activity in extracts from different tree parts. Other workers concur with our findings that *C. papaya* had significant antibacterial activity (Nirosha and Mangalanayaki, 2013; Douhari et al., 2007). The gram-negative bacteria display some particularities that inhibit antibiotics penetration, as the lipopolysaccharide layer that determines the permeability and susceptibility to antibiotics.

In the antimicrobial test for bacteria, it was observed that the potency of the activity of *C. papaya* depends on the extraction solvent used; organic extracts such as ethanol were more effective than *C. papaya* in aqueous extracts may be as a result of the better solubility of the active components in organic solvents. The ethanol extracts clearly demonstrated a higher activity than the acetone and water extracts, the better efficacy of the ethanol extract against the acetone and extract may be because different solvents have different polarities, hence different degrees of solubility of the various phytoconstituents (Rahman et al., 2011). Based on the limited spectrum of activity of the other extracts compared with the ethanol extracts, it suggests that the active component is more soluble in ethanol than in the other solvents. This is in agreement with Aruljothi et al. (2014) and Ekaiko et al. (2015a) whose findings can be attested to other works by Okunola et al. (2012) that reported the effect of *C. papaya* on similar microorganisms including *Klebsiella pneumonia*, *Enterococcus* and *Proteus* spp. However, these results are in disparity with others (Sumathi and Gowthami, 2014) who reported that the zone of inhibition was observed only in leaf extracts.

4.2 Minimum inhibitory concentration of extracts on *E. coli*, *C. albicans* and *S. aureus*

The results obtained during this study have clearly shown that the MIC values varied from 0.025-0.1mg/ml for the three extracts. Lowest MIC value 0.025mg/ml was recorded against *E. coli* and *S. aureus* where against *C. albicans* the lowest MIC observed was 0.05mg/ml. these results indicates significant antimicrobial potential of extracts. High minimum inhibitory concentration observed for *Candida albicans*, high MIC may be an indication of low efficacy or that the organism has higher potential for developing resistance to the bioactive compounds in the plant, which is said to be related to a thick layer in their outer membrane which prevents the entry of inhibition substances (Chima et al., 2016). High MIC may also mean that a higher concentration of the extract is required to inhibit the organism's growth.

The low MIC value observed for *E. coli* and *S. aureus* is a good indication of high efficacy against these microorganisms. This

also means that lower concentration of the extract is required to inhibit the organism's growth. On the other hand, disparity in Minimum Inhibitory Concentration may be due to variable sensitivity to the chemical substances related to different resistant levels among strains.

5. Conclusions

Carica papaya var. *papayi* GAV4 leaves, seeds and bark contain alkaloids, saponins, tannins, glycosides, phenols, terpenoids, anthocyanins and flavonoids. Anthraquinones were absent in seeds and bark. This plant extracts showed antibacterial and antifungal activities against *S. aureus*, *E. coli* and *C. albicans*, thus an indication that the plant can be a potential source for production of drugs with a broad spectrum of activity. Additionally, the Minimum inhibitory concentration for *S. aureus* and *E. coli* was 0.025mg/ml while that of *C. albicans* was 0.05mg/ml.

Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial substances from plants are some of the future challenges that will be faced by workers studying new substances with antimicrobial properties. *C. papaya* var. *papaya* GAV4 may be recommended as a useful source to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost-effective. We suggest that in the search for new pharmaceuticals substances, screening of various natural organic compounds and the identification of active agents must be considered as a fruitful approach.

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Competing interests

The authors declare that they have no competing interest.

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