TITLE: ANTIBACTERIAL ACTIVITY OF COFFEE SENNA (*Senna Occidentalis*) SEED

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Abstract

Chloroform, ethyl acetate, and aqueous extracts of *S. occidentalis* seed were evaluated for antibacterial potential by agar well diffusion and broth dilution techniques. Ethyl acetate extract exhibited the highest antibacterial activity by inhibiting the growth of *S. aureus; S. pyogenes; Enterococcus spp; L. monocytogenes; E. coli; K. pneumonia;* and *S. dysentria* with Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) ranging from 5 to 10 mg/mL and 10 to 20 mg/mL respectively. It was however inactive against *B. subtilis P.aeruginosa* and *S. typhi*. Components of the ethyl acetate extract were separated by column chromatography and subjected to antibacterial and phytochemical analysis. Fractions F7 and F11 had the highest antibacterial activities. Fractions F7 had a MIC range of 0. 62 to 1.25 mg/mL and an MBC range of 1.25 to 2.5 mg/mL while fractions F11 also had a MIC range of 0.62 to 1.25mg/mL and an MBC range of 2.5 to 5 mg/mL.

1.1 Introduction

Despite the tremendous progress in human medicine, infectious diseases remain one of the greatest health challenges of our time (WHO 2018). The emergence and rapid spread of multi- and pan-drug-resistant bacteria, combined with the drying up of the antibiotic pipeline in the pharmaceutical industry has significantly worsened the situation in recent years (WHO 2020). As a result, novel antibacterial agents with different mechanisms of action are urgently needed.

Like other living organisms, plants have their fair share of bacterial invasion and are capable of producing compounds that act as a defense against infectious bacteria (Franco et al., 2006;

Kovalskaya et al., 2011). Some of these compounds have been identified to belong to different classes of phytochemicals and has inspired a renewed interest in the development of new classes of antibiotic with a different mechanism of action from those in current use.

Senna occidentalis (L.) Link formally known as *Cassia occidentalis* (GBIF, 2011), commonly called Coffee Senna, Negro-Coffee, Stinking Weed (Joy et al., 2001) or Septic Weed is a small annual or biennial shrub that is a member of the *Senna* genus and belongs to the family Fabaceae (Leguminosae). It is a wild flowering plant found in many tropical countries. In Nigeria, it is very common along roadsides and in uncultivated lands.

Different parts of the *S. occidentalis* plant have been used in folk medicine as an excellent broad-spectrum internal and external antibacterial herb (Sadiq et al., 2012). The leaves are used to heal infected wounds (Taylor, 1996), while the seeds are dried, beaten up, and used as a coffee substitute. Hence, they are referred to as coffee Senna. Drink prepared from coffee Senna has a reputation for usefulness in the treatment of dermatitis infection, malaria, fevers, kidney, and bladder troubles, as well as general pains (Globinmed, 2014). Despite these uses, there is little research information on the antibacterial properties of *S. occidentalis* seed. hence this research was aimed at isolating and investigating the bioactive components of *S. occidentalis* seed as a potential source of novel antibacterial agent(s)

Fig 1 *Senna occidentalis* (L.) Link Plant with Flower and Matured Fruit

2.0 Materials and Methods

2.1 Bacterial isolates

Clinical bacterial isolates made up of five Gram-positive bacterial isolates; *Enterococcus spp, Bacillus subtilis, Listeria monocytogene, Staphylococcus aureus,* and *Streptococcus pyogenes;* and five Gram-negative bacterial isolates; *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi,* and *Shigella dysentria* were obtained from the Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital (ABUTH), Zaria and the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. All the bacteria were checked for purity and maintained at 4^0C in a nutrient agar slant.

2.2 Plant Collection and Identification

S. occidentalis plants with dried seeds were collected from uncultivated farmland opposite the Institute of Agricultural Research, $11^0$09'40.8" N $7^0$38'38.5" E, Ahmadu Bello University, Zaria. The plant was identified and authenticated with a voucher; 1611 at the Herbarium in the Department of Biological Science, Ahmadu Bello University, Zaria. The seeds were air-dried and stored in polythene bags until use.

2.3 Extraction of *S. occidentalis* Seed with Different Solvents

S. occidentalis seed extraction was carried out by cold maceration according to the method of Mahdi and Altikriti, (2010). The seeds were milled into fine powder. 200g of the pulverized seeds was defatted by adding two liters of N-Hexane and allowed to stand for two hours. The N-Hexane extract was decanted and the residue was exposed to air to enable the remaining N-Hexane to evaporate. 50g of the de-fatted seed powder was extracted separately with 500 mL of distilled water, ethyl acetate, and chloroform by separately soaking different 50g portions of the de-fatted seed powdered in each solvent for 24 hours. The solvents were decanted and filtered using Whatman filter paper No .1.

The extracts were evaporated to dryness in a water bath at 40^0C.

2.4 Antibacterial Activity of Crude Solvent Extracts of *S. occidentalis* Seed

2.4.1 Standardization of Bacteria

Clinical samples of bacterial isolates were subcultured on selective media according to the sample types and incubated for 24 hours. The Bacterial isolates were inoculated in 10mL sterile normal saline solution and incubated at 37^0C for six hours. After the incubation period, sterile normal saline solution was added to the growth medium to adjust its turbidity (by visual comparison) to match the turbidity of 0.5 McFarland turbidity standard (0.5 mL 1.75% (w/v) Barium Chloride dihydrate 99.5 mL1% (v/v) Sulphuric acid). This gave an approximate cell density of 1.5×10^8 colony-forming units per milliliter (CFU/mL) of growth medium.

2.4.2 Antibacterial Activity

Susceptibility of the bacterial isolates to the crude aqueous, ethyl acetate, and chloroform extracts of *S. occidentalis* seed was carried out on Muller Hilton Agar by agar well diffusion method as described by Hugo and Russel (1992). One gram of each extract was separately dissolved in 5 mL of 40% Dimethyl sulfoxide (DMSO) to obtain a stock concentration of 200mg/mL. Extract concentrations of 50mg/mL and 100mg/mL were prepared from the stock concentration. Muller Hilton Agar was prepared and sterilized according to the manufacturer's instructions. The media was allowed to cool to 45^0C. 20mL of the media was poured into sterile Petri dishes and allowed to cool and solidify. The sterile agar plates were seeded with 0.1mL of standardized bacteria. The inoculums were spread evenly over the surface of the agar with a sterile swab. A sterile standard cork borer of 4mm diameter was used to bore holes on the inoculated agar plates. 100µl of each extract concentration was added separately to the agar wells. Wells containing 40% DMSO instead of plant extract served as the negative control while Ciprofloxacin and Sparfloxacin standard antibacterial discs placed on the surface of the agar served

as the positive control. The inoculated plates were kept at room temperature for one hour to enable the extract to diffuse across the surface. The plates were then incubated at 37^0C for 24 hours after which they were observed for zones of inhibition of bacterial growth around the agar well. The zones were measured with a transparent ruler and the results obtained were recorded in millimeters.

2.5 Extraction of *S. occidentalis* Seed with Ethyl Acetate

About 600g of pulverized *S. occidentalis* seed was defatted with five liters of N-Hexane as previously described and extracted with six liters of ethyl acetate for 24 hours. The ethyl acetate extract was filtered and concentrated at 40^oC using a rotary evaporator. The recovered solvent was re-introduced into the seed residue and re-extracted for 24 hours. The extract was filtered and concentrated at 40^oC. The solvent recovered was again re-introduced into the residue for the third time and re-extracted for another 24 hours. The extract was filtered and again concentrated at 40^oC. The concentrated extracts from the three extraction steps were combined and dried to a constant weight in a water bath at 40^oC. Susceptibility of the bacterial isolates to 100 mg/mL of the crude ethyl acetate extract of *S. occidentalis* seed was carried out as earlier described.

2.6 Minimum Inhibitory Concentration of Ethyl Acetate Extract of *S. occidentalis* Seed

Minimum Inhibitory concentrations (MIC) of the ethyl acetate extract against susceptible bacteria isolates were determined using the broth dilution method as described by Egharevba *et al.,* (2010). 10mL of Muller Hilton broth (prepared according to manufacturer's instruction) were dispensed into separate test tubes, sterilized at 121^0C for 15 minutes, and allowed to cool. Bacterial isolates that were susceptible to the ethyl acetate extract were sub-cultured on selective media, incubated for 24 hours, inoculated in 10mLsterile normal saline solution, and incubated at 37^0C for six hours. After the incubation period, sterile normal saline solution was added to the growth medium to adjust its turbidity (by visual comparison) to match the turbidity of 0.5 McFarland turbidity standard. A stock

concentration of 80mg/mL of the ethyl acetate extract in sterile broth was prepared by dissolving 0.8g of the extract in a few drops of 40% DMSO and making up the mixture to10 mL with sterile broth. Twofold serial dilutions of the stock with sterile broth were prepared to obtain a working concentration of 40mg/mL, 20mg/mL, 10mg/mL, 5mg/mL, and 2.5mg/mL. 0.1mL of standardized inoculums were inoculated in the different concentrations of extract and incubated at 37^{o}C for 24 hours. A test tube containing the extract which was not inoculated with bacteria was incubated alongside to serve as a negative control. After the incubation period, the test tubes were observed for the presence or absence of turbidity as an indication of bacterial growth. The lowest concentration of the extract in the broth which showed no turbidity in the test tube was recorded as the MIC.

2.7 Minimum Bactericidal Concentration of Ethyl Acetate Extract of *S. occidentalis* Seed

Minimum Bactericidal Concentration (MBC) of the ethyl acetate extract of *S. occidentalis* seed was carried out as described by Egharevba et al., (2010), the contents of the MIC test tubes were sub-cultured on nutrient agar plates and incubated at 37^{0}C for 24 hours. After the incubation period, the plates were observed for the presence of bacterial colonies. Plates with the lowest concentration of extract and without bacterial colonies were recorded as the MBC.

2.8 Isolation of Antibacterial Agent from Ethyl Acetate Extract of *S. occidentalis* Seed by Column Chromatography

A solvent combination that best separates the various components of the extract was determined by Thin Layer Chromatography (TLC). A glass column (75cm by 3.5cm) was packed with 120g of silica gel (mesh size 60-120). Five grams of the ethyl acetate extract was dissolved in a minimum amount of ethyl acetate then mixed with a small amount of silica gel and allowed to dry. The dried mixture was gently loaded on the packed column; the column was eluted with absolute N-hexane as the mobile phase. The Polarity of the mobile phase was gradually increased by making it comprised of

5% absolute ethyl acetate and 95% absolute N-hexane. Upon getting to 100% ethyl acetate, 10% methanol was introduced up to 100% methanol. 100 fractions were collected in 50mLaliquots at a flow rate of 3mL/min. The contents of the fractions were monitored by TLC and fractions with similar TLC patterns were pulled together. 20mg/mL of each isolated fraction was screened for antibacterial activity. MIC and MBC were carried out.

2.9 Phytochemical Analysis of Crude and Fractions of Ethyl Acetate Extract of *S. occidentalis* Seed

The crude ethyl acetate extract of *S. occidentalis* seed as well as each fraction isolated from the crude extract were separately dissolved in ethyl acetate and spotted on several TLC plates. The spotted plates were developed in a solvent containing 7:3 N-hexane: ethyl acetate. The chromatograms were tested for the presence of phenols, tannins, alkaloids, flavonoids, anthraquinone, cardiac glycosides, sterol, and triterpenes as described by Jork et al., (1990)

3.0 Results

3.1 Antibacterial Activities of Extracts of *S. occidentalis* Seed against Gram-Positive Bacteria

Antibacterial activities of crude aqueous, ethyl acetate and chloroform extracts of *S.occidentalis* seed against Gram- positive bacteria are presented in Table 1. The antibacterial activities (which are represented by zones of inhibition of bacterial growth) indicate that the aqueous extract produced significantly higher ($P < 0.05$) zones of inhibition against *B. subtilis* than it did against *S. aureus, L. monocytogenes,* and *Enterococcus. Sp.* The aqueous extract, however, was inactive against *S. Pyogenes*. Ethyl acetate extract was significantly more effective ($p < 0.05$) against *S. aureus,* and *S. pyogenes,* than *L. monocytogenes,* and *Enterococcus spp* but was inactive against *B. Subtilis.* While the chloroform extract was significantly more effective ($p < 0.05$) against *S. Pyogenes* than *S. aureus* and *L. monocytogenes,* but was inactive against *B. Subtilis* and *Enterococcus spp*. All the five Gram-positive bacteria used were inhibited by Sparfloxacin, while Ciprofloxacin inhibited the growth of *S. aureus, S. Pyogenes,* and *B. Subtilis* but was inactive against *L. monocytogenes,* and *Enterococcus*

spp.

Table 1 Inhibition (mm) of Gram-positive Bacteria by Extracts of *S. occidentalis* Seed and Standard Antibacterial Drugs

		Test Organisms				
Extract	mg/mL	*Staphylococcus aureus*	*Bacillus subtilis*	*Streptococcus pyogenes*	*Listeria monocytogenes*	*Enterococcus spp*
Aqueous	50	21.00±0.00^{b}	22.67±0.58^{c}	-	20.00±0.00^{b}	18.00±1.0^{a}
	100	23.33±0.58^{a}	24.00±0.00^{b}	-	23.00±0.00^{a}	23.00±0.00^{a}
	200	23.67±0.58^{a}	30.67±0.58^{c}	-	23.67±0.58^{ab}	25.33±0.58^{ab}
Ethyl acetate	50	24.00±0.00^{d}	-	22.33±0.58^{c}	21.33±0.58^{b}	19.00±0.00^{a}
	100	25.00±0.00^{c}	-	26.00±0.00^{d}	23.33±0.58^{a}	24.00±0.00^{b}
	200	26.33±0.58^{b}	-	26.33±0.58^{b}	25.33±0.58^{a}	25.00±0.00^{a}
Chloroform	50	10.33±0.58^{a}	-	13.00±0.00^{c}	12.00±0.00^{b}	-
	100	14.00±0.00^{b}	-	15.33±0.58^{c}	12.00±0.00^{a}	-
	200	15.00±1.0^{a}	-	19.00±0.00^{b}	14.67±0.58^{a}	-
Ciprofloxacin	10(µg)	37.00	45.00	35.00	-	-
Sparfloxacin	10(µg)	35.00	40.00	32.00	35.00	37.00

- No inhibition. Values are expressed as means ± SD, (n = 3).

a,b,c,d,e Values with different superscripts in the same row are significantly different from each other at $P < 0.05$.

3.2 Antibacterial Activities of Extracts of *S. occidentalis* Seed against Gram-Negative Bacteria

Table 2 shows the antibacterial activities of crude aqueous, ethyl acetate, and chloroform extracts of *S. occidentalis* seed against Gram-negative bacteria. At 200mg/mL, the aqueous extract was most effective against *E. coli* but was not effective against *K. pneumonia* and *P. aeruginosa a*t 100mg/mL, the aqueous extract of the seed could only inhibit the growth of *E. coli* and *S. dysenteriae* but was inactive against *K. pneumonia, P. aeruginosa* and *S. dysenteriae.* While at 50mg/ mL, it only slightly inhibited the growth of *E. coli.*

Ethyl acetate extract of the seed was significantly ($p < 0.05$) most effective against *S. dysenteriae* while Chloroform extract was most effective against *E. coli*. All five Gram-negative bacteria were inhibited by Sparfloxacin while ciprofloxacin inhibited the growth of *E. coli, K. pneumonia, S. typhi,* and *S. dysenteriae* but was inactive towards *P. aeruginosa.*

Table 2 Inhibition (mm) of Gram-Negative Bacteria by Extracts of *S. occidentalis* Seed and Standard Antibacterial Drugs

Extracts	mg/mL	Test Organisms: *Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Salmonella typhi*	*Shigella dysenteriae*
Aqueous	50	09. 67±0.58	-	-	-	-
	100	16.00±0.00	-	-	12.67±0.58	-
	200	21.00±0.00^{c}	-	-	15.00±0.00^{b}	12.67±0.58^{a}
Ethyl acetate	50	20. 33±0.58^{a}	20.00±0.00^{a}	-	-	25.00±0.00^{b}
	100	23.33±0.58^{a}	27.00±1.0^{b}	-	-	28.00±0.00^{b}
	200	27.33±0.58^{a}	30.00±0.00^{b}	-	-	32.67±0.58^{c}
Chloroform	50	20.33±0.58^{c}	18.00±0.00^{b}	-	-	14.33±0.58^{a}
	100	21.00±0.00^{c}	20.00±0.00^{b}	-	-	15. 33±0.58^{a}
	200	23.67±0.58^{c}	22. 33±0.58^{b}	-	-	20.00±0.00^{a}
Ciprofloxacin	10(µg)	37.00	45.00	35.00	-	-
Sparfloxacin	10(µg)	35.00	40.00	32.00	35.00	37.00

- No inhibition. Values are expressed as means ± SD, (n = 3).
a,b,c,d,e Values with different superscripts in the same row are significantly different from each other at $p < 0.05$.

All the tested extracts were effective against both Gram-positive and Gram-negative bacteria. Ethyl acetate extract, however, was most effective in inhibiting bacterial growth and was therefore extracted in a larger quantity, subjected to MIC and MBC analysis and further purification.

3.3 Antibacterial Activity of Ethyl Acetate Extract of S. *occidentalis* Seed

Antibacterial analysis of the bulk ethyl acetate extract of *S. occidentalis* seed carried out to determine its potency gave results (Table 3) that were similar to the results previously obtained for the ethyl acetate extract of the seed.

Table 3 Inhibition (mm) of Bacteria by 100 mg/mL Ethyl Acetate Extract of *S. occidentalis* Seed

Test Organisms	Zones of Inhibition (mm)
Gram Positive	
Staphylococcus aureus	28.67 ± 0.58^{e}
Bacillus subtilis	-
Streptococcus pyogenes	26.00 ± 0.00^{bc}
Listeria monocytogenes	24.00 ± 0.00^{a}
Enterococcus spp	25.33 ± 0.58^{b}
Gram Negative	
Escherichia coli	26.33 ± 0.58^{cd}
Klebsiella pneumonia	27.00 ± 1.00^{d}
Pseudomonas aeruginosa	-
Salmonella typhi	-
Shigella dysenteriae	28.00 ± 0.00^{e}

- No inhibition. Values are expressed as means ± SD, (n = 3).
^{a,b,c,d,e} Values with different superscripts down the column are significantly different from each other at $p < 0.05$.

3.4 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Ethyl Acetate Extract of *S. occidentalis* Seed

Table 4 shows that a minimum concentration of 5mg/mL of ethyl acetate extract of the Seed is sufficient to inhibit the growth of *S. aureus, S. pyogenes, E. coli, K. pneumoni,* and *S.dysenteriae* while a minimum concentration of 10mg/mL was required to inhibit the growth of *L.monocytogenes* and *Enterococcus spp*. While Table 5 shows that the extract was bactericidal against *S. aureus, K. pneumonia,* and *S. dysenteriae* at a minimum concentration of 10mg/mL and bactericidal against *S. pyogenes, L. monocytogenes, Enterococcus spp.*, and *E. coli* at a minimum concentration of 20mg/mL

Table 4 Minimum Inhibitory Concentration of Ethyl Acetate Extract of *S. occidentalis* Seed

Test Organisms	MIC (mg/mL)
Staphylococcus aureus	5
Streptococcus pyogenes	5
Listeria monocytogenes	10
Enterococcus spp	10
Escherichia coli	5
Klebsiella pneumonia	5
Shigella dysenteriae	5

Table 5: Minimum Bactericidal Concentration of Ethyl Acetate Extract of *S. occidentalis* Seed

Test Organisms	MBC (mg/mL)
Staphylococcus aureus	10
Streptococcus pyogenes	20
Listeria monocytogenes	20
Enterococcus spp	20
Escherichia coli	20
Klebsiella pneumonia	10
Shigella dysenteriae	10

3.5 Antibacterial Activity of Fractions of Ethyl Acetate Extract of *S. occidentalis* Seed

The components of the ethyl acetate extract of *S. occidentalis* seed were isolated on a chromatographic column based on their polarity. The solvent mixture that best separated the components of the extracts on a TLC plate was N-Hexane/ethyl acetate in a ratio of 3:7. This ratio of the solvent mixture was used to monitor the fraction eluted from the column using TLC plates. One hundred (100) fractions in 50 mL aliquots were collected from the column and combined into 11 fractions based on the similarities of their Rf values. After evaporation of the solvents, fractions one to four were oily. They were therefore considered to be part of the de-fatting process and were not used for further analysis. Fractions 5 to 11 were solids of different physical appearances and were subjected to phytochemical screening and further antibacterial activity.

Antibacterial activities of 20mg/mL of fractions of ethyl acetate extract of *S. occidentalis* seed against Gram-positive and Gram-negative bacteria are presented in Table 6. The results show that fractions 6, 7, 8, 9, and 11 demonstrated excellent broad-spectrum anti-bacterial activity against *S. aureus, S. pyogenes, L. monocytogenes, Enterococcus spp, E. coli, K. pneumonia,* and *S. dysenteriae* but were inactive against *B. subtilis, P. aeruginosa,* and *S. typhi.* This pattern of antibacterial activity is similar to those exhibited by the crude extract (Table 1) while fractions seven and eleven exhibited significantly higher ($p < 0.05$) antibacterial activity compared to the others, fractions five and ten were inactive against all the bacteria isolates used.

Table 6 Zones Inhibition (mm) of Bacteria by Fractions of Ethyl Acetate Extract of *S. occidentalis* Seed

	Fractions (20mg/mL)						
Test Organisms	5	6	7	8	9	10	11
Gram Positive							
Staphylococcus aureus	-	22.00±0.00^{b}	25.67±0.58^{d}	24.00±0.00^{c}	20.67±0.58^{a}	-	24.00±0.00^{c}
Bacillus subtilis	-	-	-	-	-	-	-
Streptococcus pyogenes	-	21.00±0.00^{b}	23.33±0.58^{c}	20.00±0.00^{a}	22.67±0.58^{c}	-	23.33±0.58^{c}
Listeria monocytogenes	-	24.00±1.00^{d}	27.00±0.00^{e}	22.00±0.00^{b}	20.00±0.00^{a}	-	23.00±0.00^{c}
Enterococcus spp	-	28.00±0.00^{e}	28.00±0.00^{e}	20.00±0.00^{a}	21.00±0.00^{b}	-	23.67±0.58^{c}
Gram Negative							
Escherichia coli	-	22.00±0.00^{b}	26.00±0.00^{e}	23.33±0.58^{d}	22.67±0.58^{b}	-	20.00±0.00^{a}
Klebsiella pneumonia	-	25.67±0.58^{c}	30.00±0.00^{d}	24.00±0.00^{a}	24.67±0.58^{b}	-	25.00±0.00^{b}
Pseudomonas aeruginosa	-	-	-	-	-	-	-
Salmonella typhi	-	-	-	-	-	-	-
Shigella dysenteriae	-	24.00±0.00^{c}	28.00±0.00 ^{d}	22.33±0.58 ^{b}	21.00±0.00 ^{a}	-	21.00±0.00 ^{a}

- No inhibition. Values are expressed as means ± SD, (n = 3).
a,b,c,d,e Values with different superscripts across the row are significantly different from each other at $p < 0.05$.

3.6 Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Fractions of Ethyl Acetate Extract of *S. occidentalis* Seed

A minimum of 1.25mg/mL of fractions 6, 8, 9, and 11 were required to inhibit the growth of all susceptible bacterial isolates. A minimum of 0.62mg/mL of fraction seven was required to inhibit *L. monocytogenes, Enterococcus spp, K. pneumonia,* and *S.* dysenteriae while a minimum of 1. 25mg/mL of fraction F7 was required to inhibit S. aureus, S. pyogenes, and E. coli. (Table 7)

Table 7 Minimum Inhibitory Concentration (MIC) of Fractions of Ethyl Acetate Extracts of *S. occidentalis* Seed

	Fractions				
	6	7	8	9	11
Test Organisms	MIC (mg/ml)				
Staphylococcus aureus	1.25	1.25	1.25	1.25	1.25
Streptococcus pyogenes	1.25	1.25	1.25	1.25	1.25
Listeria monocytogenes	1.25	0.62	1.25	1.25	1.25
Enterococcus spp	1.25	0.62	1.25	1.25	1.25
Escherichia coli	1.25	1.25	1.25	1.25	1.25
Klebsiella pneumonia	1.25	0.62	1.25	1.25	1.25
Shigella dysenteriae	1.25	0.62	1.25	1.25	1.25

Table 8 Minimum Bactericidal Concentration (MBC) of Fractions of Ethyl Acetate Extracts of *S. occidentalis* Seed

Test Organisms	Fractions				
	6	7	8	9	11
	MBC (mg/ml)				
Staphylococcus aureus	5	2.5	2.5	5	2.5
Streptococcus pyogenes	5	2.5	5	5	2.5
Listeria monocytogenes	2.5	1.25	5	5	2.5
Enterococcus spp	2.5	1.25	2.5	5	5
Escherichia coli	5	2.5	5	5	2.5
Klebsiella pneumonia	2.5	1.25	2.5	5	2.5
Shigella dysenteriae	2.5	1.25	5	5	5

Comparing the MIC and MBC results (Tables 7 and 8) of the fraction and their zones of inhibition of bacteria growth (Table 6) indicate that fractions F7 and F11 exhibited the highest bactericidal activities.

3.7 Qualitative Phytochemical Analysis of Crude and Fractions of Ethyl Acetate Extract of *S. occidentalis* Seed

The presence of phenols, tannins, flavonoids, anthraquinone, cardiac glycosides, sterol, triterpenes, and Steroids were detected in the crude ethyl acetate extract of S. occidentalis seed (table 9)

able 9 Qualitative Phytochemical Analysis of Crude and Fractions of Ethyl Acetate Extract *S.occidentalis* Seed

Phytochemical	Crude	5	6	7	8	9	10	11
Phenol	+	-	+	+	+	-	-	+
Tannins	+	+	-	-	-	+	+	+
Alkaloids	-	-	-	-	-	-	+	+
Flavonoids	+	+	+	-	+	+	+	+
Anthraquinone	+	-	-	-	+	+	+	-
Cardiac glycosides	+	+	-	-	-	+	+	+
Sterols	+	+	+	-	-	-	-	-
Triterpenes	+	-	-	+	+	+	+	-
Steroids	+	+	+	-	-	-	+	+

+ = present**;** - = absent

4.0 Discussion

The three solvent extracts used inhibited more Gram-positive bacteria than Gram-negative bacteria, confirming the report by Lin et al., (1999) that plant extracts are usually more active against Gram-positive bacteria than Gram-negative bacteria.

Seven out of the 10 bacterial isolates which were classified as susceptible to the ethyl acetate extracts based on Performance Standards for Antimicrobial Susceptibility Testing of the Clinical and Laboratory Standards Institute (2017) are of serious public health concerns. *S. aureus, K. pneumonia, and Enterococcus spp.* are known to cause the majority of nosocomial infections and have been identified as particular threats. (Rice, 2008; Boucher et al., 2009). *L. Monocytogenes, S. aureus, S. dysenteriae,* and *E. coli* are known to cause food-related infections that not only cause diarrhea and discomfort but can be life-threatening. Most significantly, five out of the seven susceptible bacterial (*E. coli, K. pneumonia, S. dysenteriae, S. aureus, S. pyogenes,* and *Enterococcus Spp*) are included in the World Health Organizations list of antibiotic-resistant "priority pathogens" that pose the greatest threat to human health and for which new antibiotics are urgently needed (WHO 2017).

The presence of phenol in all five fractions with antibacterial activity and its absence in the two fractions without antibacterial activity is an indication that the major antibacterial component in the extract is most likely a phenolic compound. Phenols have multiple functions in plants; the most important of which is plant defense against pathogens and herbivore predators. Phenols are also well known for their antibacterial activities (Rauha et al., 2000) and have been applied in the control of human pathogenic infections (Doughari, 2012), The mechanisms of antibacterial action of the phenolic compound include enzyme inhibition possibly through reaction with sulfhydryl groups or through more nonspecific interactions with proteins (Cowan 1999). The site(s) and the number of hydroxyl groups on the phenol group are thought to be related to their toxicity to

bacteria due to evidence that increased hydroxylation results in increased toxicity and more highly oxidized phenols are more inhibitory towards bacteria (Scalbert 1991; Cowan 1999).

Alkaloids which rank among the most efficient and therapeutically significant plant substance (Okwu, 2005) were detected in fraction 10 and 11 but was not detectable in the crude extract by the method employed in the study. Alkaloids are known to intercalate with the cell wall and/or DNA (Cowan 1999) and also inhibit bacterial cell respiration and bacterial enzymes such as esterase, DNA, and RNA-polymerases (Kovacevic 2004).

Antibacterial properties of other compounds detected in the crude and the isolated fractions, like flavonoids and Tannins have been established by various researchers. Flavones glycosides with broad-spectrum antibacterial activity were isolated from the methanol extract of *S. occidentalis* seed by Yandava and Satnami (2011). Flavonoids are known to be synthesized by plants in response to microbial infection and are said to inhibit bacterial growth by complexing with extracellular soluble proteins and cell walls and also by binding to adhesins (Prashant et al., 2011). Tannins are synthesized and accumulate in plant tissue after a microbial attack (Bobbarala, 2012). Tannins are known to bind to adhesins, cause enzyme inhibition, substrate deprivation, and membrane disruption as well as form complexs with cell walls and metal ions (Cowan 1999). They are also known to cause bacterial colonies to disintegrate probably due to their interference with the bacterial cell wall thereby inhibiting bacterial growth (Cowan 1999).

5.0 Conflict of Interest

The authors declare no conflict of interest.

6.0 References

Bobbarala, V. 2012. Antimicrobial Agents. InTech, Croatia.

Boucher, H.W., Talbot, G. H., Bradley, J. S., Edwards, J. E., Gilbert, D., Rice, L. B. and Bartlett J. 2009. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical Infectious. Diseases. 48:1–12

Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbial Review 12: 564- 582

Doughari, J. H. 2012. Phytochemicals: Extraction Methods, Basic Structures and Mode of Action as Potential Chemotherapeutic Agents. In: Venketeshwer R (Ed.) Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, ISBN: 978-953-51-0296-0

Egharevba, H. O., Odigwe, A. C., Abdullahi, M. S., Okwute, S. K. and Okogun, J. I. 2010. Phytochemical Analysis and Broad Spectrum Antimicrobial Activity of Cassia Occidentalis L. (whole plant) New York Science Journal; 3(10):74-81. ISSN: 1554-0200.

Franco, O.L., Murad, A. M., Leite, J. R., Mendes, P. A., Prates, M. V. and Bloch, C. J. 2006. Identification of a cowpea gamma-thionin with bactericidal activity Federation of European Biochemical Societies Journal 273 pp 3489–3497 Global Biodiversity Information Facility. 2011. Backbone Taxonomy, http://www.gbif.org/species 15 October 2020

Globinmed. 2014. Global information hub on integrated Medicine cassia occidentalis http://www.globinmed.com 79122 21 October 2020

Hugo, W. B. and Rusell, A. D. 1992. Pharmaceutical Microbiology 5th ed. Blackwell Scientific Publication, Oxford London, pp. 258-297.

Jork, H., Funk, W., Fischer, W. and Wimmer, H. 1990. Thin-Layer Chromatography – Reagents and Detection Methods, Vols. 1a and 1b" VCH Weinheim, ISBN 3-527-27834-6 and ISBN 3-527-28205-X

Joy, P. P., Thomas, J., Mathew, S. and Skaria, B. P., 2001 Medicinal plants. Tropical Horticulture vol.2. Naya Prokash Calcutta India pp 449-632.

Kovacevic, N. 2004. Osnovi farmakognozije, Srpska školska knjiga, ISBN 86-83565-19

Kovalskaya, N., Zhao, Y., and Hammond, R. W. 2011 Antibacterial and Antifungal Activity of a Snakin-Defensin Hybrid Protein Expressed in Tobacco and Potato Plants. The Open Plant Science Journal 29-42 29 pp 1874-2947

Lin, j., Opoku, A. R., Geheeb-Keller, M., Hutchings, A. D., Terlanche, S. E., Jager, A. K. and Van, S. J. 1999 Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities journal of Ethnopharmacol 68 pp 267-74

Mahdi. S and Altikriti. Y. 2010 Extraction of natural products. http://www.fkog.uu.se/course/a/biolakt/biolakt-archive/BiolAkt%20201017 Sept 2018.

Okwu, D. E, 200 Phytochemicals, Vitamins and Mineral contents of two Nigeria Medicinal plants. International Journal of Molecular Medicine and Advance Science. 1(4) pp: 375-381.

Prashant, T., Bimlesh, K., Mandeep, K., Gurpreet, K. and Harleen, K. 2011 Phytochemical screening and Extraction: A Review Internationale Pharmaceutica sciencia http://www.ipharmsciencia.com 12 October 2020.

Performance Standards for Antimicrobial Susceptibility Testing 2017. 11th Ed 321 ISBN 1-56238

Rauha, J. P., Remesa, S., Heinonenb, M., Hopiab, A., Ka¨hko¨nenb, M., Kujalac, T., … and Vuorelaa, P. 2000 Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds International Journal of Food Microbiology 56 pp 3–12

Rice, L. B. 2008 Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. Journal of Infectious Diseases; 197 pp 1079–81

Sadiq, I. S., Shuaibu, M., Bello, A. B., Tureta, S. G., Isah, A., Izuagie, T., and Kamaru, M. B. 2012. Phytochemistry and Antimicrobial Activities of Cassia Occidentalis Used for Herbal Remedies Journal of Chemical Engineering Vol. 1, No. 1,

Scalbert, A. 1991. Antimicrobial properties of tannins. Phytochemistry 30: 3875–3883

Taylor, L., 1996 Tropical Plant Data base The Healing Power Of Rainforest Herbs: Fedegoso (cassia occidentalis) http://www.rain-tree.com 15 January 2020

WHO 2017 WHO publishes list of bacteria for which new antibiotics are urgently needed https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-newantibiotics-are-urgently-needed 10 December 2020.

WHO 2018 The Top 10 Causes of Death https://www.who.int/news-room/fact-sheets/detail/thetop-10-causes-of-death 30 November 2020

WHO 2020 antimicrobial resistance. https://www.who.int/news-room/factsheets/detail/antimicrobial-resistance 1 December 2020. Yandava, R. N. and Satnami, D. K. 2011 Chemical constituents from Cassia occidentalis Linn Indian Journal of Chemistry 50B pp 1112-1118