

RESEARCH PAPER

Identification and Quantification of Secondary Metabolites and The Antimicrobial Efficacy of Leaves Extracts of Some Medicinal Plants

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ABSTRACT:

Azadirachta indica, *Calotropis procera*, *Carica papaya*, and *Vernonia amygdalina* are among the most frequently used plants with proven ethnomedicinal applications in Ilorin, Nigeria. This study investigated the active constituents and antimicrobial efficacy of acetone and aqueous extracts of these plants against *Candida albicans*, *Escherichia coli* ATCC 259220, and *Staphylococcus aureus* ATCC 25923 by the disc diffusion technique. High-Performance Liquid Chromatography (HPLC) fingerprint of the constituents of acetone extracts of *C. papaya*, *A. indica*, and *V. amygdalina* was further investigated. Highest percentage yield (10.20 %) was obtained for acetone extract of *A. indica* while the lowest yield (10.20 %) was obtained for aqueous extract of *C. papaya*. Varying constituents viz. coumarins, glycosides, protein, saponin, anthraquinone, flavonoid, tannin and terpenoid were detected. Different levels of antimicrobial efficacy were exhibited by each of the plants while a synergistic effect was observed at 100 mg/mL concentration of acetone extract of the combination of all the plants. An array of compounds was separated at different peak heights corresponding to concentration of the compounds. Creptolepinone was detected in highest percentage (47.0 %) in acetone extracts of *C. papaya* while phorbol ester was detected in lowest percentage (1.2 %). Myricetin was detected in highest percentage (39.6 %) in acetone extracts of *A. indica* while alpha funebren was detected in lowest percentage (1.5 %). Vernodalin was detected in highest peak height (48.8 %) in *V. amygdalina* and andrographoside was detected in lowest peak height (0.6 %). This study depicts that the plants may be promising pharmaceutical candidates that can be used in the development of new therapeutic agents.

KEY WORDS: acetone; antimicrobials; azadirachtol; myricetin; vernodalin; *in vitro*.

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1.INTRODUCTION :

Many antimicrobial agents of natural or semi-synthetic sources are available to fight against infections caused by various pathogens (Rahman *et al.*, 2008). Medicinal plants are significant sources of natural antimicrobials in the development of novel drugs (Perry *et al.*, 1999). Herbal plants are abundant sources of bioactive molecules, which are categorized as aromatic substances and secondary metabolites including phenols or their oxygen-substituted derivatives

(Boligon *et al.*, 2012; Kumar *et al.*, 2010). The biomolecules can be extracted and administered in many forms, the most common of which is as a tisane or a tincture to treat several infections (El-Said and Al-Barak, 2011). Some of these bioactive molecules may also be screened and traded as raw materials for many herbal industries (Paul *et al.*, 2011).

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Many parts of medicinal plants are consumed as food or for medicinal purposes (Okoko and Ere, 2012; Romasi *et al.*, 2011). *C. papaya* Linn., a commonly cultivated perennial plant with economic importance all over tropical and subtropical countries (Pandey *et al.*, 2016; Reddy *et al.*, 2013), is commonly found in West Africa (Irvine, 1961). It is used traditionally in Nigeria, where it is commonly known as Gwanda, Okwuru-bekee and Ewe ibepe among the Hausa,

Igbo and Yoruba people, to treat various diseases such as cold, fevers, indigestion, diarrhea, eczema, and rheumatism. The leaf extracts, chopped leaves, and latex of *C. procera* have shown great promise as a nematicide in *in vitro* and *in vivo* studies (Khirstova and Tissot, 1995). Also, the roots and leaves of *V. amygdalina* are used therapeutically to treat kidney problems, stomach discomfort and other infections (Banso *et al.*, 1999; Tugume *et al.*, 2006).

Table 1. Medicinal plants tested in this study

Scientific name	Family	Common name	Local names			Traditional Uses
			Hausa	Igbo	Yoruba	
<i>V. amygdalina</i>	Asteraceae	Bitter leaf	Chusar-doki	Onugbu	<i>Ewuro</i>	Treatment of syphilis, gonorrhoea, antidiabetic
<i>C. procera</i>	Apocynaceae/ Asclepeceae	Giant milkweed	Tumfatiya		<i>Bomubomu</i>	peptic ulcer, treats stomach ache, cure skin diseases like measles, jaundice
<i>C. papaya</i>	Caricaceae	Pawpaw leaf	Gwanda	Okwuru-bekee	<i>Ewe ibepe</i>	Treatment of dysentery, diabetes, malaria, convulsion, gonorrhoea, syphilis
<i>A. indica</i>	Meliaceae	Neem	<i>Dogonyaro</i>		<i>Dogonyaro</i>	Treatment of malaria, jaundice, purgative

Despite the available information on these plants, an array of studies has reported variations in active metabolites of plants due to varying climatic condition of different locations of plants. This may consequently affect the qualities of plants and their medicinal potentials. As such, it is necessary to investigate the effect of solvents on the quantity and quality of secondary metabolites of some plants commonly used plants in the study region. Also, to investigate the effectiveness of these plants on common pathogens that are usually associated with infectious diseases.

2. MATERIALS AND METHODS

2.1. MATERIALS

2.1.1 Test Microorganisms and Standardization of Inoculum:

The clinical fungal isolate (*Candida albicans*) and reference bacterial strains (Gram-positive, *Staphylococcus aureus* ATCC 25923 and Gram-negative organism, *Escherichia coli* ATCC 25920) used in this study were obtained on Sabouraud Dextrose Agar slant and nutrient agar slant, respectively, from Microbiology Laboratory of University of Ilorin Teaching Hospital. They were kept at 4 °C and sub-cultured at 37°C for 24 hours on their respective agar before susceptibility testing. While the bacterial plates were incubated overnight at 37°C, the fungal plate was incubated for 48 h at 37°C. The inoculum was standardized by adjusting the McFarland density; the absorbance was adjusted at 580 nm and diluted to 0.5 McFarland turbidity equivalence using a densitometer to achieve the final concentration of 1.5×10^8 cfu/mL of the bacterial strains and 1.0×10^6 spores/mL of the fungal isolate at 530 nm (Ochei and Kolhatkar, 2008). These were used

within 20- 30 minutes of standardization (Wanger, 2007).

2.1.2 Collection of leaves of the medicinal plants

The medicinal plants used in this study were selected based on the frequency of their use and availability in Adeta area of Ilorin. Ilorin is located in the Southern Guinea Savannah of Nigeria with annual rainfall and precipitation of about 1300 mm. Other climatic data of the town is maximum temperature (38 °C), maximum relative humidity (77.50 %) and a 7.1-hour daily photoperiod of 1500 mm (Ejjeji and Adeniran, 2009; Olanrewaju, 2009). The study area, Adeta, was selected based on its topographical

2.2 Extraction of the crude extracts from leaves of the plants

After authentication, extraction was done following preparation of the leaves thus; the leaves were washed, under a running tap and air-dried for five days at room temperature as described by Akerele *et al.* (2008). The dried plant materials were milled separately to a fine powder with an electric miller (Master Chef Blender, Mode MC-BL 1980, China). The powdered material of *A. indica* was weighed (10g) into two separate beakers and was extracted separately by cold percolation method using 100 ml of acetone and aqueous (w/v). The same procedure was followed for *C. procera*, *C. papaya*, and *V. amygdalina* using acetone, and aqueous as the menstruum. The extractions were done for 48 hours with constant shaking at intervals. The homogenate was filtered through Whatman filter paper (Number 1) to yield the crude extract (Nenaah and Ahmed, 2011), which was subsequently evaporated to dryness using a rotary evaporator (Model RE Zhengzhou, Henan China). The crude extracts were weighed, stored in labeled sterile airtight containers and stored at 4 °C for further use. The yield (%) of the extract was determined using the formula:

$$\text{Yield (\%)} = (\text{Dry weight of extract} \div \text{Dry weight of plant material}) \times 100$$

2.3 Sterility testing of the crude extract

This was done using a modified method described by Lalitha (2008). Serial dilution of 1.0 g of each extract was made to reduce the concentration. A quantity (1 g) of the extract was inoculated into 10 mL Mueller Hinton (Hi-Media) broth. Clarity of the broth after incubation at 37 °C

parameters. According to Persson *et al.* (2005), the topographical parameters of a place can be used to delineate its crop management potentials. The area is located in Ilorin West, Nigeria (Latitude: 8.49 North; Longitude: 4.51 East; and Elevation: 339.00m/1112.20ft). Fresh healthy leaves of *A. indica*, *C. procera*, *C. papaya*, and *V. amygdalina* were collected from Adeta area, Ilorin, Nigeria. The taxonomy of each species was established and they were authenticated at Herbarium Unit of the Department of Plant Biology, University of Ilorin, Ilorin Nigeria where voucher specimens were deposited with reference numbers UILH/007/972 for *V. amygdalina*, UILH/001/1001 for *C. procera*, UILH/004/967 for *C. papaya* and UILH/003/860 for *A. indica*.

C for 24 hours indicated the absence of contaminants.

2.4 Preparation of stock solution of the crude extracts

Stock solution of the extract was obtained by dissolving 2.0 g of the extract into sterile test tube containing 20.0 mL of 5.0 % DMSO (95 mL of water added into 5 mL of DMSO). In another test tube a sterile tube containing 20.0 mL of 5.0 % DMSO was combined, 0.5g of each plant, to give 2.0 g of the combination of plants. Subsequently, different concentrations (100.0 mg/mL, 50.0 mg/mL and 25.0 mg/mL) were further prepared from the stock solution by doubling dilution method.

2.5 Preparation of antimicrobial discs containing the crude extracts

Paper discs (6.00 mm) were punched from Whatman filter paper (No. 1). The discs were sterilized in an autoclave for 15 minutes at 15 lbs pressure and allowed to cool. About five sterilized paper discs were aseptically arranged using sterile forceps in sterile Petri-dishes. Petri dishes containing discs were labeled 100 mg/mL, 50 mg/mL and 25 mg/mL, discs in each plate were aseptically impregnated with approximately 20 µl of their respective concentrations of each solvent extract using mechanical pipettes, and they were allowed to dry before storing in labeled airtight containers at 20 °C (Brooks *et al.*, 2004).

2.6 Preparation of antimicrobial discs containing the crude extracts

Paper discs (6.00mm) were punched from Whatman filter paper (No. 1). The discs were sterilized in an autoclave for 15 minutes at 15 lbs pressure and allowed to cool. About five sterilized paper discs were aseptically arranged using sterile forceps in sterile Petri-dishes. Petri dishes containing discs were labeled 100 mg/mL, 50 mg/mL and 25 mg/mL, discs in each plate were aseptically impregnated with approximately 20 μ l of their respective concentrations of each solvent extract using mechanical pipettes, and they were allowed to dry before storing in labeled airtight containers at 20 ° C (Brooks *et al.*, 2004).

2.7 Antibacterial and Antifungal susceptibility testing of the crude extracts

Susceptibility testing of the sterile crude extract was done for all the microbial isolates by the modified Kirby Bauer disc diffusion method (Lalitha, 2008; Parekh and Chanda, 2007). Following the Clinical and Laboratory Standards Institute (CLSI) guideline; 0.1 ml of the respective standardized inoculums of each test organism was uniformly spread onto sterile Mueller Hinton Agar (Hi-Media) plates for the bacterial strains and Sabouraud Dextrose Agar (SDA) for the fungal strains. The plates were allowed to dry, subsequently, discs impregnated with the crude extracts were aseptically placed onto the agar surface and gently pressed with the applicator to ensure contact of the discs with the medium. The crude extracts were allowed to pre-diffuse from the discs into the agar medium for 1 hour on the bench before incubation at 37 °C for 24 hours (for the bacterial strains) and for 48 hours at 30 °C (for the fungal strain). The diameter of clear zones around the discs were measured (mm) with the discs and recorded as the zones of inhibition.

2.8 Preliminary phytochemical

Qualitative phytochemical analyses of the crude extracts were done following the method described by Amadi *et al.* (2004).

2.8.1 Test for Saponins

The extract (1 mL) was diluted with 3 mL of distilled water, this was shaken for 15 min and the formation of a 1 cm layer of foam indicated the presence of saponin.

2.8.2 Test for Flavonoids

The extract (1 mL) was diluted with 1 mL of Sodium hydroxide (NaOH) and hydrochloric

acid (HCL). The development of yellow solution indicated the presence of flavonoids.

2.8.3 Test for Terpenoids

The extract (1 mL) was mixed with 2 mL of chloroform and 2 mL of concentrated sulfuric acid. The formation of a reddish-brown color at the interface indicated the presence of terpenoids.

2.8.4 Test for Coumarins

The extract (1 mL) was mixed with 1 mL of sodium hydroxide (10%). The yellow color indicated the presence of coumarins.

2.8.5 Test for Glycoside

To one mL of the extract was added 20 cm³ of water, heated for 5 minutes on a water bath and filtered through Gem filter paper (12.5 cm). Then 0.2 cm³ of Fehling's solutions A and B was mixed with 5 cm³ of the filtrate until it became alkaline (tested with litmus paper). A brick-red coloration on heating showed a positive

2.8.6 Test for Alkaloids

An equal volume of the extract was mixed with concentrated hydrochloric acid, and a few drops of Mayer's reagent were added. The presence of a green color or white precipitate indicated the presence of alkaloids.

2.8.7 Test for Tannins

The extract (1 mL) was mixed with 2 mL of distilled water followed by a few drops of ferric chloride (10%). A blue or green color indicated a positive result.

2.9 Sample preparation and HPLC analysis

The HPLC fingerprinting of each solvent extract was carried out following thus; powdered dried leaves (1.0 g) was macerated with acetonitrile/water (1:1; v/v, 10.0 mL), then centrifuged for 10 minutes at 3000 rpm and filtered. The crude extract from the filtrate was assayed directly by HPLC-UV; a modular Shimadzu (Nexeramx) LC-10 system comprised of an LC-10AD pump, a CTO-10A column oven, an SPD-10A UV-DAD detector, a CBM-10A interface, and an LC-10 Workstation was used. An LC-18 column (250 mm x 4.6 mm ID x 5 mm) from (Ubondapak, Bellefonte, USA) was employed at 30 °C. Separations were done in the isocratic mode, using acetonitrile: water (40:60; v/v) at a flow rate of 1.0 mL per minute with an injection volume ("loop") of 10 μ L, UV detection was at 254 nm.

2.10 Data Analysis

All the results were obtained in triplicates (n=3), the data were analyzed statistically and presented as mean \pm standard deviation.

3. RESULTS

3.1. Percentage yield after extraction

The percentage yield from 200 g of dry raw leaves of each plant after evaporation of the filtrate to dryness was calculated as powdered to

Table 2. Percentage yield of acetone and aqueous extracts of the plants used in this study

Plant Extract	<i>A. indica</i>		<i>C. procera</i>		<i>C. papaya</i>		<i>V. amygdalina</i>	
	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous
Yield from Raw Dry Powder (g)	29.5	23	29.2	21.6	20.9	20.4	28.6	21.6
Percentage Yield (%)	14.75	11.5	14.6	10.8	10.3	10.2	14.3	10.8

3.2. Qualitative phytochemical screening

The phytochemical screening results of aqueous and acetone extracts of the leaves of *A. indica*, *C. papaya*, *V. amygdalina*, and *C. procera* showed the presence of several phytochemical constituents (Table 3). Extracts of *A. indica* leaves showed the presence of varying phytochemical constituents; coumarins, alkaloid, glycosides, protein, saponin were present in the aqueous, and acetone extracts respectively while anthraquinone, flavonoid, and tannin were only present in acetone extract and not in aqueous extract. Terpenoid was present in aqueous extract but absent in acetone extract. The presence of these constituents varied in aqueous and acetone extracts of leaves of *C. papaya* as anthraquinone, alkaloid, flavonoid, glycosides, and saponin were present in aqueous and acetone extracts, respectively. Coumarins and tannin were only present in acetone extract but absent in aqueous extract while protein and terpenoid were only present in aqueous extract and absent in acetone extract. Aqueous and Acetone extracts of leaves of *V. amygdalina* revealed the presence of anthraquinone, alkaloid, coumarin, saponin, tannin, and terpenoid. Flavonoid was present in acetone extract only while glycosides as well as protein were present in aqueous extract only.

yield; 29.50 g (14.75 %) for acetone extract and 23.00 g (11.50 %) for aqueous extract of *A. indica*. *C. procera* was 29.20 g (14.60 %) of acetone extract and 21.60 g (10.80 %) of the aqueous extract. Dry powder of *C. papaya* was 20.90 g (10.30 %) of acetone extract and 20.40 g (10.20 %) for aqueous extract. *V. amygdalina* was 28.60 g (14.30 %) of acetone extract and 21.60 g (10.80 %) for aqueous extract.

Alkaloid, flavonoid, protein, saponin, and terpenoid were present in the aqueous and acetone extracts of *C. procera* leaves. Anthraquinone, coumarin, and glycoside were only present in the aqueous extracts and tannin was only present in the acetone extract.

3.3. In vitro antimicrobial screening

The susceptibility testing of the crude extracts is given in Table 5. Comparatively, among the tested extracts at 100 mg/mL, the highest zone of inhibition (16.06 mm) was given by acetone extract of the combination of the three plants (*C. procera*, *V. amygdalina* and *A. indica*) against *E. coli* while the lowest zone of inhibition (8.00 mm) was given by aqueous extract of *A. indica* against *S. aureus*. At the tested concentration of 50 mg/mL, acetone extracts of *A. indica* and *V. amygdalina* individually gave the highest zones of inhibition (17.00 mm) against *C. albicans* and *E. coli*, respectively. At 25 mg/mL concentration tested, the highest zone of inhibition (21.00 mm) was given by acetone extract of *V. amygdalina* against *E. coli* while aqueous extract of *C. procera* gave the least zone of inhibition (6.00 mm) against *S. aureus*.

Table 3. Phytochemicals in acetone and aqueous extracts of the plants

Constituents	<i>A. indica</i>		<i>C. papaya</i>		<i>V. amygdalina</i>		<i>C. procera</i>	
	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone	Aqueous	Acetone
Anthraquinones	–	+	+	+	+	+	+	–
Coumarins	+	+	–	+	+	+	+	–
Alkaloid	+	+	+	+	+	+	+	+
Flavonoids	–	+	+	+	–	+	+	+
Glycosides	+	+	+	+	+	–	+	–
Protein	+	+	+	–	+	–	+	+
Saponin	+	+	+	+	+	+	+	+
Tannin	–	+	–	+	+	+	–	+
Terpenoid	+	–	+	–	+	+	+	+

+ Presence

– Absence

Table 4. Diameter of zones of inhibition (mm) of the different plant extracts against the test microorganisms at different concentrations

Plants	Solvents	100 mg/mL			50 mg/mL			25 mg/mL		
		<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
<i>A. indica</i>	Acetone	15.00±0.81	16.00±1.18	15.00±0.63	11.00±2.03	12.7±0.63	17.67±0.26	20.00±1.13	11.5±0.53	16.00±2.16
	Aqueous	10.00±0.21	8.00±1.18	10.00±2.10	9.00±0.41	8.41±1.20	8.89±1.30	9.21±1.28	7.36±0.18	7.80±1.22
<i>C. procera</i>	Acetone	16.5±2.13	11.00±2.03	11.00±0.31	15.5±0.63	13.00±0.31	15.00±0.23	15.00±2.63	11.00±0.13	13.5±0.32
	Aqueous	10.20±1.84	8.12±0.84	10.00±1.29	8.23±1.21	8.10±1.34	9.38±0.13	7.42±0.28	6.00±1.02	7.82±0.22
<i>C. papaya</i>	Acetone	15.00±0.23	14.00±1.02	15.00±2.01	10.00±1.13	12.00±1.14	13.00±2.13	11.00±1.92	13.5±2.16	16.00±0.13
	Aqueous	9.10±2.01	10.10±0.18	9.42±1.82	9.00±1.41	8.84±1.20	9.91±0.84	8.01±0.28	8.28±1.14	8.82±0.88
<i>V. amygdalina</i>	Acetone	16.00±1.21	12.75±0.13	16.00±0.14	17.67±1.16	13.67±2.10	17.00±0.12	21.00±2.16	16.00±0.11	20.5±1.01
	Aqueous	12.84±1.26	10.52±1.68	10.02±1.86	10.94±1.48	9.32±0.26	9.92±1.82	9.42±1.87	8.96±1.24	8.38±0.83
Combination of the plants	Acetone	17.06±1.10	15.82±2.31	14.50±1.01	13.00±1.42	13.33±1.22	13.5±0.81	15.33±1.04	14.00±0.82	14.00±1.61
	Aqueous	12.91±1.82	12.13±1.14	11.02±1.86	12.14±0.18	10.12±1.28	10.19±0.14	9.22±1.13	10.10±1.15	9.42±1.36

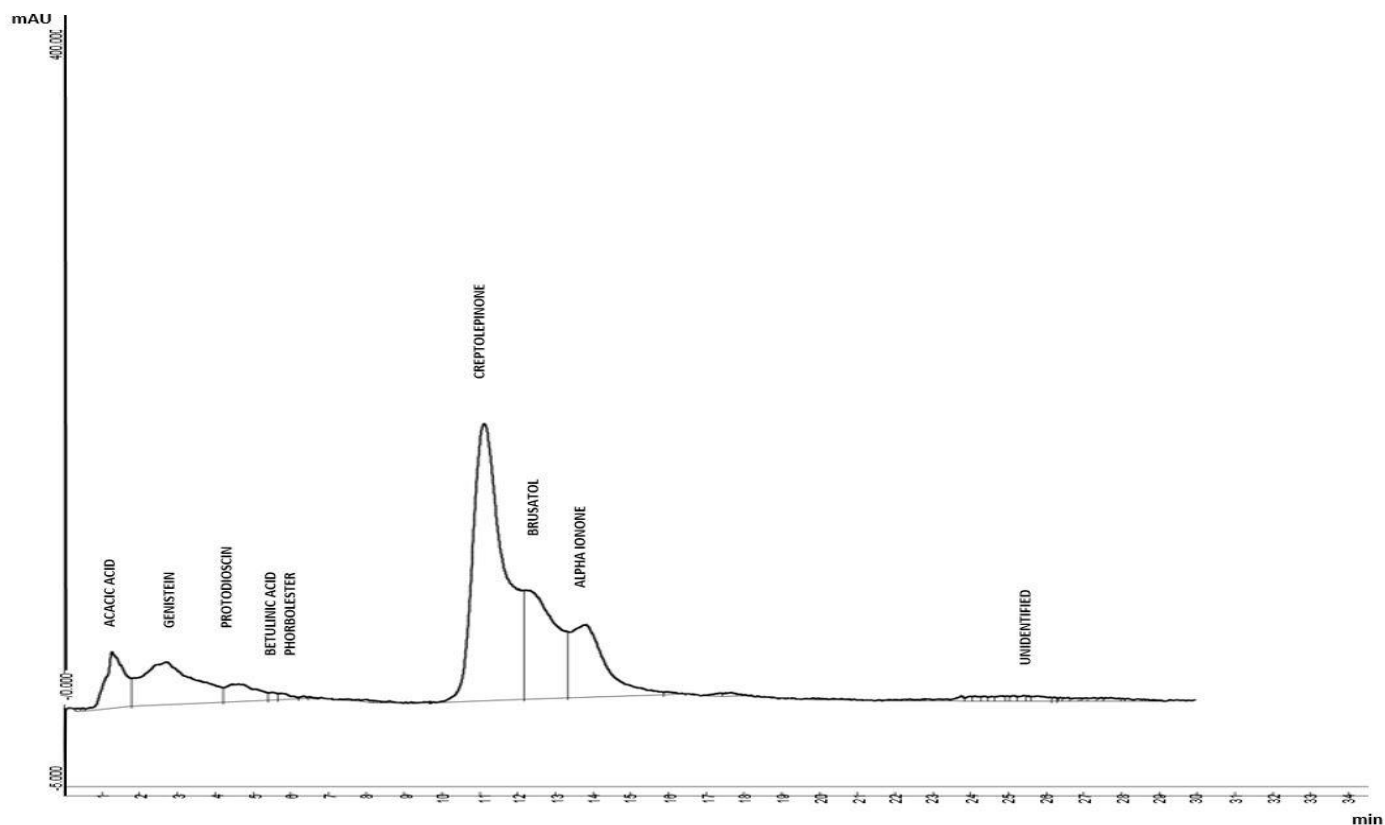
Table 5. Diameter of zones of inhibition (mm) of the control agents

Control	Zone of inhibition (mm)		
	<i>E. coli</i>	<i>S. aureus</i>	<i>C. albicans</i>
Aqueous	7.10±2.00	7.00±0.10	7.02±1.22
5% DMSO	10.10±1.21	11.10±0.22	9.14±0.01

3.4. HPLC analysis of acetone extracts of leaves of *V. amygdalina*, *A. indica*, and *C. papaya*

The concentration of components represented as peak heights, separated in leaves of *C. papaya*, *A. indica*, and *V. amygdalina* sample is shown in the chromatograms profile given in Figures 1, 2 and 3. The percentage of peak heights of compounds detected in *V. amygdalina* is presented in Figure 4; vernodalin (48.8 %) was detected in the highest amount while andrographoside (0.6 %) was the least compound

detected. Out of the seven fractions of compounds (Figure 5) separated in *A. indica*, myricetin (39.6 %), was detected in the highest quantity while alpha funebren (1.5 %) was detected in the least quantity. Out of the compounds detected in *C. papaya* (Figure 6) creptolepinone (47.0 %) was the most abundant while phorbol ester (1.2 %) was the least.

**Figure 1:** Chromatogram profile of Leaves of *C. papaya* by HPLC

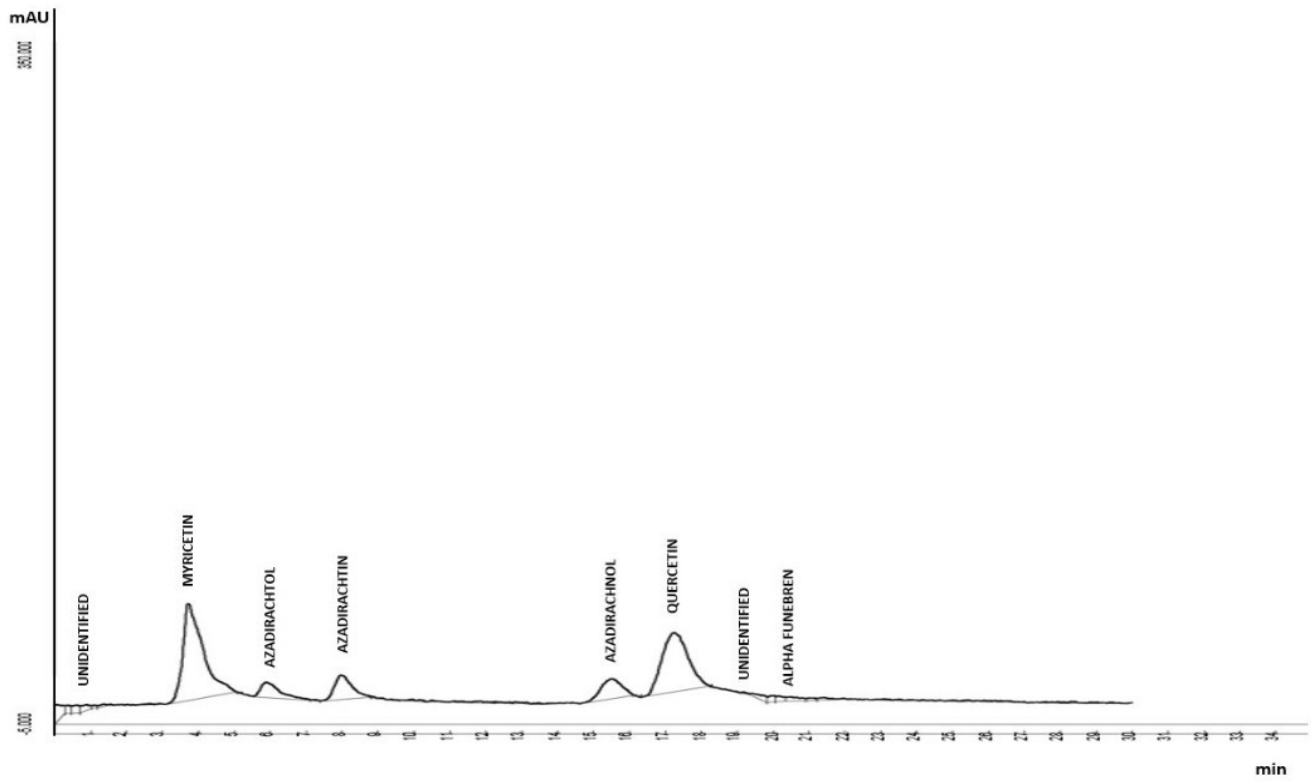


Figure 2: Chromatogram profile of Leaves of *A. indica* by HPLC

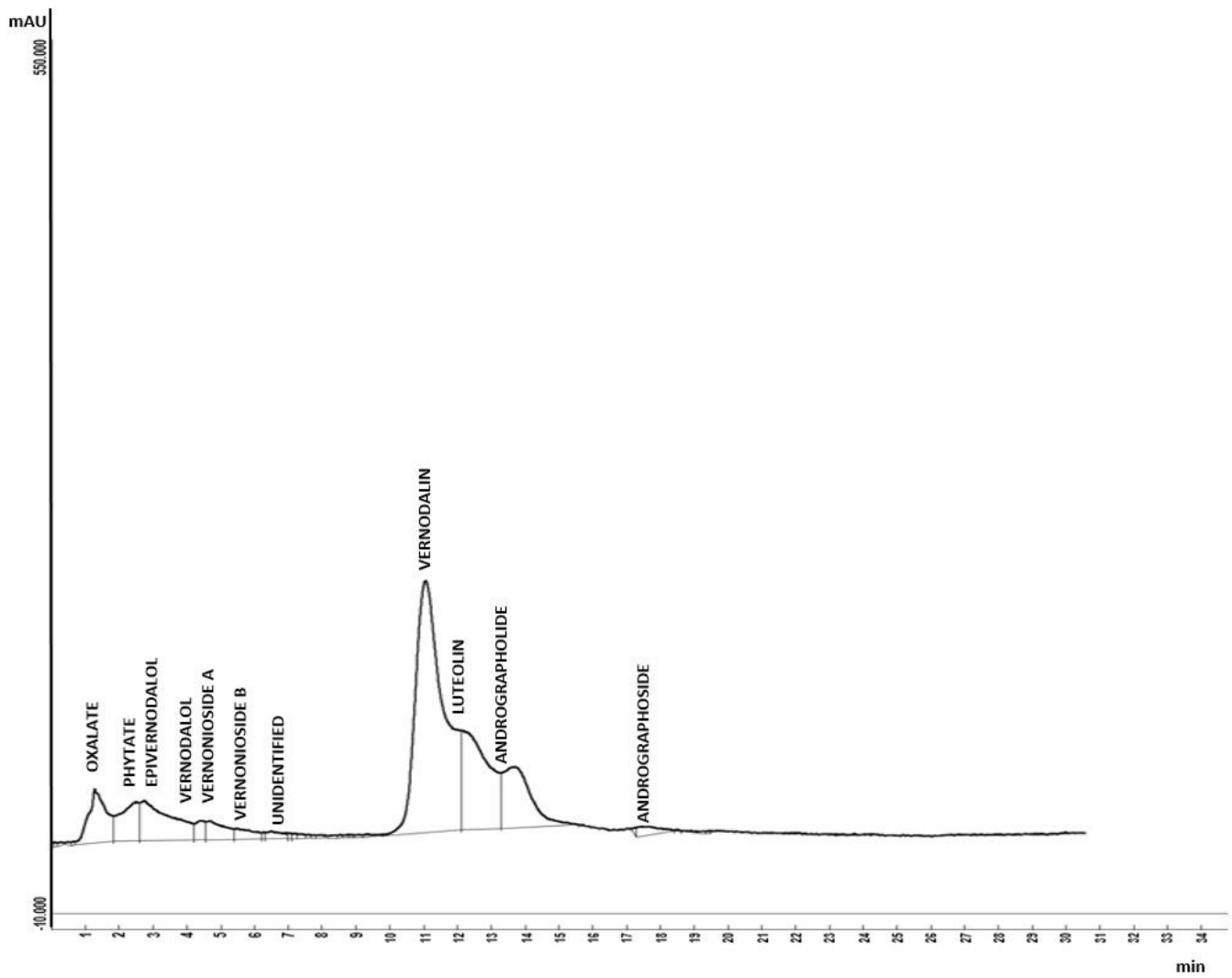


Figure 3: Chromatogram profile of Leaves of *V. amygdalina* by HPLC

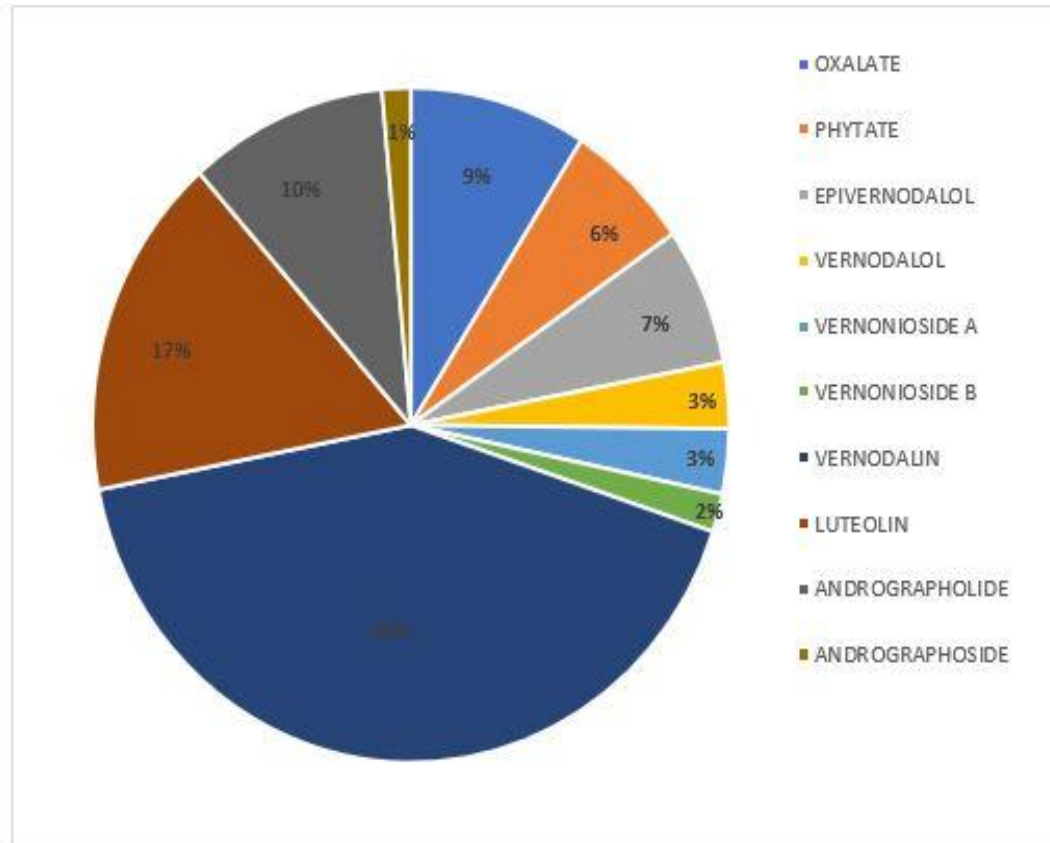


Figure 4: Percentage occurrence of compounds in detected in *V. amygdalina*

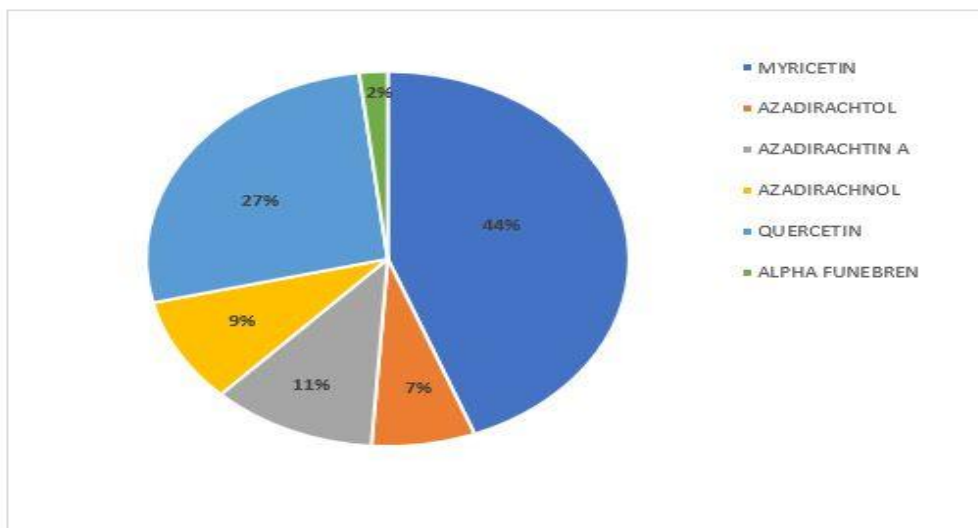


Figure 5: Percentage occurrence of compounds in detected in *A. indica*

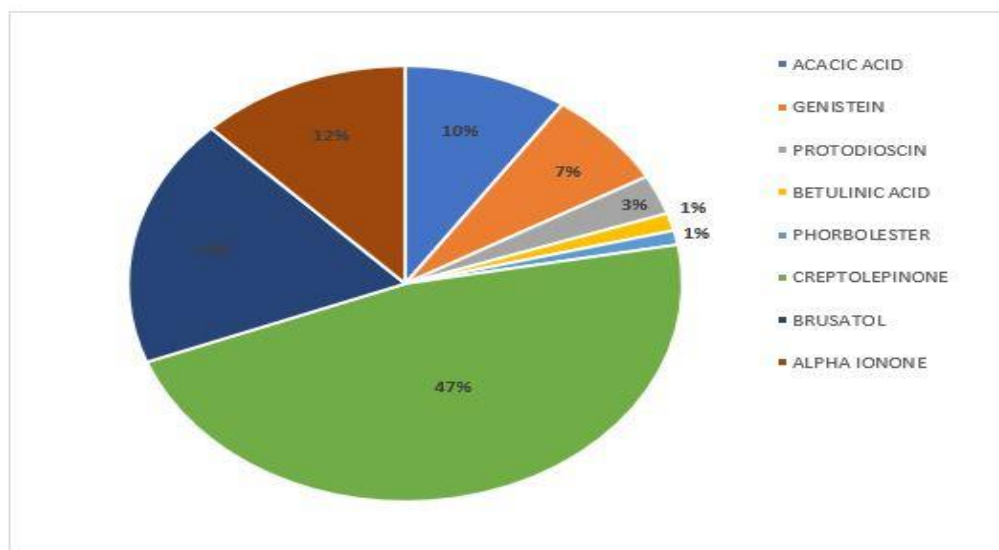


Figure 6: Percentage occurrence of compounds in detected in *C. papaya*

4. DISCUSSION

Medicinal plants are known to harbor inexhaustible compounds known as phytochemicals, which have been reported to be biologically active on the human body (Effraim *et al.*, 2007). Therefore, screening of plants for these phytochemicals has been of great interest to scientists, with the view to detect new drugs against several diseases. In the present study, acetone and aqueous were individually used as a solvent to extract components from *C. papaya*, *A. indica*, *V. amygdalina*, and *C. procera*. This was with the aim of obtaining a variable yield of compounds. As observed in this work, higher yield was obtained for acetone extract of all the plants than aqueous extract. This is in line with the statement that the choice of solvent used in the extraction of plants may affect the yield after extraction (Ncube *et al.*, 2008).

However, this study recorded the presence of secondary metabolites including alkaloid, coumarin, saponin, tannin, anthraquinone and terpenoid in all the plant extracts, but in no particular order. This may be because the solvents used for extraction are both polar solvents and are able to extract similar compounds from the plants. Ajaiyeoba (2002) reported that polar solvents (acetone and aqueous, inclusive) extract more active compounds than non-polar solvents. In another study, it is reported that the extraction of tannins and other phenolics was better in aqueous

acetone than in aqueous methanol (Okunade *et al.*, 2007). In addition, Enyi-Idoh *et al.* (2012) previously reported the presence of compounds such as alkaloid, saponin, tannin, and terpenoid in extracts of *V. amygdalina*. Imaga and Bamigbetan (2013) also reported the presence of flavonoid in extracts of *V. amygdalina*. Andarwulan *et al.* (2012), Sikanda *et al.* (2013) and Nguyen *et al.* (2015) variously reported the presence of flavonoids in *C. papaya* leave extracts, and that agrees with what was reported on *C. papaya* in this work.

The result of phytochemicals of *A. indica* in this study is in accord with former studies that reported the presence of tannins, saponins, flavonoids, alkaloids, glycosides in the ethanolic leaf extract of *A. indica* (Nguyen *et al.*, 2015). Presence of alkaloids, saponins and other phytochemicals in all the plant extracts may be indicative of the presence of broad-spectrum antimicrobial compounds which may be responsible for the antimicrobial properties exhibited by the plants. Previous studies equally showed that secondary metabolites of plants are significant sources of microbicides (Atangwho *et al.*, 2010). Edeoga *et al.* (2005), in his study, reported the physiological and antimicrobial effects of various phytochemicals. In their own study, Ireland and Dziejic (1986) reported that antimicrobial activities of plants may be attributed to the presence of compounds such as saponins. Another study reported that saponins has antimicrobial activity (Ncube *et al.*, 2008), while

Aliero and Aliero, (2008) and Amaral *et al.* (2009) variously reported the antibacterial activities of alkaloids and flavonoids. Flavonoids have also been confirmed to show antidiarrheal effects (Meite *et al.*, 2009), and this may account for the observed effectiveness of the studied plants against *E. coli*, a known causative agent of gastroenteritis and traveler's diarrhea. Furthermore, tannins have antibacterial properties as they are able to react with proteins to form stable water-soluble compounds that kill bacteria by cell membrane damage (Aliero and Aliero, 2008). Sodipo *et al.* (2000) reported that most phytochemicals are natural antibiotics, as they help the body in fighting infections caused by microorganisms.

In concurrence with the current study on the antimicrobial screening of plant extracts against *E. coli*, *S. aureus* and *C. albicans*, is the antibacterial evaluation of different plants against similar bacterial strains (Khan *et al.*, 2013). Hamadameen (2019) also reported the antibacterial susceptibility of *E. coli* ATCC 25922 while Saeed and Saadullah (2019) reported an antifungal susceptibility of *C. albicans* to some medicinal plants. Similar to our findings are the works of Eja *et al.* (2011), Ogunadare (2011) and Oshodi *et al.* (2004), who variously reported the antimicrobial potentials of *V. amygdalina* on *E. coli* and *S. aureus*. However, the observed variation in the levels of susceptibility of the test organisms to the plant extracts may be attributed to the differences in the cell wall structures of the test organisms. Prescott *et al.* (2001) reported that the potency of an antimicrobial agent varies with target species and Gram-negative outer membrane act as an extra barrier to many environmental substances including antimicrobial agents Tortora *et al.* (2004). In the report of Shittu *et al.* (2004) on *C. procera*, a significant reduction in the total viable microbial counts was observed.

Although all the plant extracts, irrespective of their type and concentration tested, demonstrated varying levels of antimicrobial activities by producing different zones of inhibition (even at the lowest concentration tested) around the test organisms. This may be ascribed to the varying quality of phytochemicals present in them. Iwalokun *et al.* (2004) reported that water could be an ideal solvent for extraction, this is notable in the appreciable levels of antimicrobial

activities exhibited by water extracts of the studied plants. However, the zone of inhibition produced by aqueous extracts was not as high as what was produced by acetone extracts of the plants. This may be justified by higher polarity of acetone than water, and consequently the solubility of more components in acetone. This is in line with the report of Uwah *et al.* (2013) that solubility of compounds occurs at different rates in solvents during extraction.

On an average, *V. amygdalina* displayed highest activity, followed by *A. indica*, *C. procera* and *C. papaya*. However, it was observed that the zones of inhibitions produced by the plant extracts was higher than what was produced when the control agents (aqueous and 5% DMSO) were used individually against the test organisms. This may be attributed to the mixed phytoconstituents in plant extracts compared to the single components of the control agents. Also, when the effects of combination of all the plants were tested at different concentrations against the test organisms, no remarkable effect was observed at 50 mg/mL and 25 mg/mL concentrations but a synergistic effect (an increase in the level of activities) was observed at 100 mg/mL concentration against *E. coli*. This finding concurs with several interesting outcomes that have been reported on the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of plant extracts reported by Gibbons (2003).

Quantitative analysis is a significant tool to detect the active components in plants (Fraise *et al.*, 2011). HPLC (High-Performance Liquid Chromatography) has emerged as the most popular, powerful and versatile form of chromatography used for the qualitative, semi-qualitative and quantitative phytochemical analysis of herbal drugs. Generally, the majority of these components are responsible for some particular pharmacological effects (Song *et al.*, 2007). The active components of *V. amygdalina* have long been shown to be mainly sesquiterpene lactones like vernodalin and vernoamygdalin and steroid glycosides like vernonioside B1 and vernoniol B. This agrees with our findings in which sesquiterpene lactone (vernodalinol) is among the compounds detected in our study. In various studies, sesquiterpene

lactones have been reported to possess a range of antimicrobial properties (Kupchan *et al.*, 1969; Luo *et al.*, 2011; Amodu *et al.*, 2013). Similar findings have also been reported on components detected in *V. amygdalina* as sesquiterpene lactones, vernolide, vernodalol (Erasto *et al.*, 2007). These support our finding, where Vernodalin was detected in the highest percentage of peak height and that may be attributed to the appreciable antimicrobial effect obtained by *V. amygdalina* extract against the test microorganisms.

Several studies have characterized bioactive compounds from *A. indica* as several types of sesquiterpene lactones (Farombi and Owoeye, 2011). This is in consistence with our study, as six bioactive compounds (Myricetin, quercetin, azadirachtol, azadirachtin A, azadirachnol, and alpha funebren) were detected in *A. indica*, with myricetin and quercetin detected in the highest amount. Sadeghian and Mortazaienezhad (2007) also reported the presence of Azadirachtin in aqueous leaves extracts of *A. indica*. In another study conducted by Soni *et al.* (2012), it was reported that 73.62 % concentration of Azadirachtin was detected in *A. indica*. Cseke *et al.* (1996) reported that Azadirachtin A, with the Empirical formula C₃₅H₄₄O₁₆ is the most abundant biologically active compound in *A. indica*. The antibacterial activities of *A. indica* leave observed in this work may therefore be attributed to the presence of these compounds.

The antimicrobial effect of *C. papaya* extract observed in this study coincides with the eight fractions of compounds (Cryptolepinone, brusatol, Acacic acid, genistein, protodioscin, betulinic acid, phorbolster) detected by HPLC assay. Genisten has been documented to be present in *C. papaya* (Tan *et al.*, 2012). Sawyer *et al.* (1995) reported that cryptolepine cause severe ultrastructural changes in bacteria and fungi. This finding was further validated by his findings in 2004, which demonstrated the occurrence of lysis in susceptible organisms following exposure to cryptolepine (Sawyer *et al.*, 2004). Acacic acid, a complex glycoside and brusatol (a quassinoid) detected in this work indicate further promising cytotoxic and antimicrobial potentials of *C. papaya*. Brusatol have been reported to have positive impacts on the treatment of various kinds of disease (Beutler, 2009).

5. CONCLUSION

This study has revealed that different solvent extracts of *A. indica*, *C. papaya*, *V. amygdalina*, and *C. procera* harbor varying metabolites and showed different levels of antimicrobial activities against the test pathogens. Therefore, this may serve as baseline information on the quality of plants sourced from Adeta area, Ilorin. It may also provide insightful lead towards the discovery of new bioactive natural products that may be used in the development of new therapeutic drugs against diseases caused by pathogens tested in this study. In line with the findings in this study, it is suggested that further research on the active constituents of plants' extracts should be further isolated, purified and tested individually and in combinations, to ascertain the type of effect (synergistic or antagonistic effects) produced by the constituents.

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Conflict of Interest (1)

Conflict of Interest Statement

We declare that we have no conflict of interest.

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