

Analysis of secondary metabolites and *in vitro* evaluation of extracts of *Carica papaya* and *Azadirachta indica* leaves on selected human pathogens

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Abstract

Extraction of the leaves of *Carica papaya* (family Caricaceae) and *Azadirachta indica* (family Meliaceae) were done using solvents with varying polarities (acetone, hexane and ethylacetate). The crude extracts were screened for phytoconstituents using the preliminary method and high-performance liquid chromatography (HPLC) for separation and quantification of the constituents. Susceptibility of three medically important microorganisms (*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Candida albicans*) to the solvent extracts was tested at 100 mg/mL, 50 mg/mL and 25 mg/mL concentrations the disc diffusion technique. Tannins, saponins, alkaloids, steroids, flavonoids and anthraquinone were present in all the solvent extracts of *A. indica* and *C. papaya*. Protein was present in all the solvent extracts of *A. indica* but absent in all the solvent extracts of *C. papaya*. Terpenoid was only present in hexane extract of *A. indica* but absent in other solvent extracts of *A. indica* and *C. papaya*. Flavonoid was present in all but only absent in ethylacetate extract of *A. indica*. Glycoside was present in all but absent in hexane extract of *A. indica*. Coumarin was only present in acetone extracts of both plants and absent in other solvent extracts of the two plants. Extracts of *Carica papaya* and *Azadirachta indica* displayed varying inhibitory activities (between 5.00-15.00 mm) against the organisms at all the tested concentrations. Acetone extract of *A. indica* produced zones of inhibition ranging from 5.00-14.00 mm while acetone extract of *C. papaya* produces a range of 4.00-10.00 mm. Also, hexane extract of *A. indica* produced inhibition range of 7.00-10.00 mm whereas hexane extract of *C. papaya* produces a range of 5.00-15.00 mm. However, ethylacetate extract of *A. indica* produced inhibition range of 5.00-13.67 mm, while ethylacetate extract of *C. papaya* produce a range of 5.00-15.00 mm. Different compounds quantified as different peaks by HPLC in the different solvent extracts of *Carica papaya* are acacic acid, genistein, protodioscin, betulinic acid, phorbol ester, creptolepinone, brusatol and alpha ionone while the fractions from the solvent extracts of *Azadirachta indica* are myricetin, azadirachtol, azadirachtin a, pentadecane, phytol, azadirachnol, quercetin, b caryophyllen, alpha ionone, ascaridole, trans-b.farnes. Results obtained in this work indicated that all the solvent extracts of *Carica papaya* and *Azadirachta indica* contained active phytoconstituents and the extracts displayed good potentials at preventing diseases associated with the microorganisms tested in this work.

Keywords: *Candida albicans*; *Escherichia coli*; Myricetin; Protodioscin; *Staphylococcus aureus*

Introduction

Indiscriminate use of antibiotics in human and veterinary healthcare system has led to the emergence of multi-drug resistant (MDR) strains of microorganism which pose greater threat to the global public health (Goyal *et al.*, 2008). The more realistic perception of the limitations of orthodox medicines in terms of cost, accessibility, effectiveness and safety (Shariff, 2001) has driven researchers to source for plants with medicinal properties for the development of new antimicrobial substances to which microorganisms have not yet developed resistance.

Most parts of the developing countries accept and rely on herbal medicine due to the belief of better compatibility with the human body and fewer side effects (Mohammad *et al.*, 2010). It has been estimated that about 35,000 to 70,000 plants are used as medicinally out of over 400,000 plants species reported worldwide (Bibi *et al.*, 2011). Plants have an almost limitless ability to synthesize aromatic compounds and secondary metabolites, most of which are phenols or their oxygen-substituted derivatives that can be used to treat chronic as well as infectious diseases (Boligon *et al.*, 2012).

Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Mahesh and Satish, 2008). *Azadirachta indica* commonly known as Neem, from the family Meliaceae is traditionally called *Dogonyaro* in most parts of Nigeria. Since the discovery of *Vernonia amygdalina* years back, there has been enormous information on the medicinal value of this plant. However less than 10% of the world's flora has been studied chemically in detail to determine their active constituents (Ekam *et al.*, 2010).

Carica papaya Linnaeus is an herbaceous succulent perennial plant with self-supporting stems. It is from the family Caricaceae, a common plant used medicinally in Nigeria and it is commonly called *Ibepe* in Yoruba, *Gwanda* in Hausa and *Okwulu Oyibo* in Igbo. In traditional medicines, papaya leaves have been used as a treatment for malaria (Titanji *et al.*, 2008). Also, scientific reports have revealed that *C. papaya* possesses immunomodulatory, antimicrobial, anti-inflammatory, antihelminthic, anticancer, wound healing, anti-fertility, abortifacient, diuretic, anti-hypertensive and antimalarial properties (Gurunga and Škalco-Basnet, 2009; Amazu *et al.*, 2010).

Even though hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Mahesh and Satish, 2008). Also, many researchers have reported the influence of different extraction solvents and techniques on the content of natural antioxidants in extracts (Michiels *et al.*, 2012). Hence, this study involves testing for the effects of different solvents on the active constituents and the antimicrobial potentials of *Azadirachta indica* and *Carica papaya* which are some of the well tolerated, readily available and affordable plants locally in Nigeria.

Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *Carica papaya* and *Azadirachta indica* were collected from Apalara Area, Ilorin, Kwara state, and authenticated at Herbarium Unit of Department of Plant Biology, University of Ilorin where were voucher specimens were deposited (*Azadirachta indica* - U/LH/003/860 and *Carica papaya*-U/LH/004/967).

These plant materials were washed with tap water in order to remove the dust, dried under the shade at room temperature for 2-3 days and powdered with electric blender (Master Chef Blender, Model MC-BL 1980, China). About 10 g powdered material of each plant species was variously soaked in 100 mL solvent of acetone, hexane and ethylacetate extract for 48 hours with regular shaking and then filtered (Alain *et al.*, 2010). The filtrates were then evaporated under reduced pressure ($45 \pm 10^\circ\text{C}$) using the rotary evaporator (Model RE

Zhengzhou, Henan, China). All extracts were stored in different sterile and labeled glass bottle at room temperature until screened. Percentage yield of the extract was calculated and recorded. They were kept in the refrigerator at 4 °C until needed for analysis. Sterility of the extract was tested by serially diluting 1 g of each extract up to 10^{-1} . A quantity (2 ml) of the extract solution was inoculated into 10 mL of Muller Hinton broth, incubated at 37 °C for 24 hours and checked for clarity as an indication of sterility. Stock solutions of corresponding crude extracts were prepared in dimethyl sulfoxide (DMSO) (Perez *et al.*, 1990), from which 2-fold serial dilutions was used to prepare three different concentrations (100 mg/mL, 50 mg/mL and 25 mg/mL) of each solvent extract of the plant extract.

Pathogenic microorganisms

The pathogenic microorganisms *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 259220 and *Candida albicans* were obtained from Microbiology Laboratory of University of Ilorin Teaching Hospital, Ilorin, Nigeria. The bacteria were collected on nutrient agar slant while fungus was collected on Sabouraud dextrose agar. Each was re-cultured on fresh nutrient agar and Sabouraud Dextrose Agar plate for purity. Their morphology and Gram staining were carried out to confirm their identity (Ochei and Kolhatkar, 2008).

Preparation and loading of discs

Paper discs were prepared from Whatman No. 1 filter paper. The discs were labeled and autoclaved for 15 minutes at 15 lbs pressure. They were allowed to cool, transferred into air tight Bijou bottles and kept in the refrigerator. Approximately 20 µl of each of 100 mg/mL concentration of each solvent extract was loaded on the discs using a mechanical pipette. More discs were loaded with same amount from 50 mg/mL and 25 mg/mL of each plant extract. All the impregnated discs were allowed to fully dry for 24 hours under a laminar flow cabinet (Basri and Fan, 2005), they were stored in airtight Petri dishes at 20 °C till they were ready for use (Jawetz and Adelberg, 2004).

Antibacterial and antifungal activities of acetone, ethyl acetate and hexane extracts of leaves of A. indica and V. amygdalina

Standardization of inoculums was achieved by aseptically using sterile wire loop to transfer colonies from the agar slant into 5 mL Mueller-Hinton Broth (MHB) and Sabouraud Dextrose Broth (SDB) for bacteria and fungus respectively. These were further incubated at 37 °C for 18 hours and 30 °C for 48 hours respectively. The inoculums were standardized by adjusting to 0.5 McFarland turbidity (mixture of 99.5 ml of 1% sulphuric acid and 0.5 ml barium chloride) equivalents to 1.5×10^8 CFU/ mL for bacteria and 2×10^5 spores / mL for the fungus (Baris *et al.*, 2006).

Then, susceptibility of the pathogenic bacteria (*Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922) and fungi (*Candida albicans*) to the different solvent extracts of *A. indica* and *C. papaya* was tested by a modified disc diffusion assay described by Ncube *et al.* (2008). About 1 ml each of standardized bacterial and fungal inoculums were separately introduced aseptically into separate sterilized Petri dishes then overlaid with 20 ml Mueller-Hinton agar (MHA; Becton-Dickson, USA) for bacteria and Sabouraud dextrose agar (SDA) for the fungus respectively. The inoculated plates were swirled for even distribution of the inoculums within the agar medium then allowed to gel. The impregnated discs with the plant extracts were distributed on the inoculated agar surface (22 mm from each other and 14mm from the edge of the plate) using sterile forceps. The discs were gently pressed for uniform contact with the surface of the medium; the plates were allowed to stand on the bench for 30 minutes for proper diffusion of agent from the disc into the inoculated agar and incubated at 37 °C for 24 hours for bacteria and at 30 °C for 24 hours for *fungus*. Commercially available antibiotics, Gentamycin at 30 µg (Becton- Dickson, USA) and an antifungal drug (griseofulvin) were taken as a positive control and dissolved in distilled water (30 µg / 6 µL) while DMSO was incorporated as negative control. Clear zones around the discs were measured using with the aid of Vernier

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caliper (mm) and recorded. This was done in triplicates and the average of the three replicates was calculated and recorded.

Phytochemical screening

Phytochemical screening for the presence of tannins, steroids, saponins, glycoside, terpenoid, protein, flavonoids, anthraquinone, alkaloid and coumarin was carried out according to the method described by Trease and Evans (2007).

HPLC method

HPLC analysis was carried out using the modified method of Springfied *et al.* (2005). A modular chromatographic system Shimadzu (Nexeramx) LC-10 comprising a LC-10AD pump, a CTO-10A column oven, a SPD-10A UV-DAD detector, a CBM-10A interface and a LC-10 Workstation was used. A 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 mobile phase acetonitrile: water (40:60 v/v) at a flow rate of 1 mL /min; with an injection volume ("loop") of 10 µL, UV detection was at 254 nm.

Results

Percentage yield of acetone, hexane and ethyl acetate extracts of Carica papaya and Azadirachta indica after evaporation

Acetone (12.40%), hexane (14.6%) and ethylacetate (8.04%) extracts of *A. indica* gave higher percentage yield, while low percentage yield was obtained for acetone (10.2%), hexane (10.0%) and ethylacetate (8.0%) extracts of *C. papaya* (Figure 1).

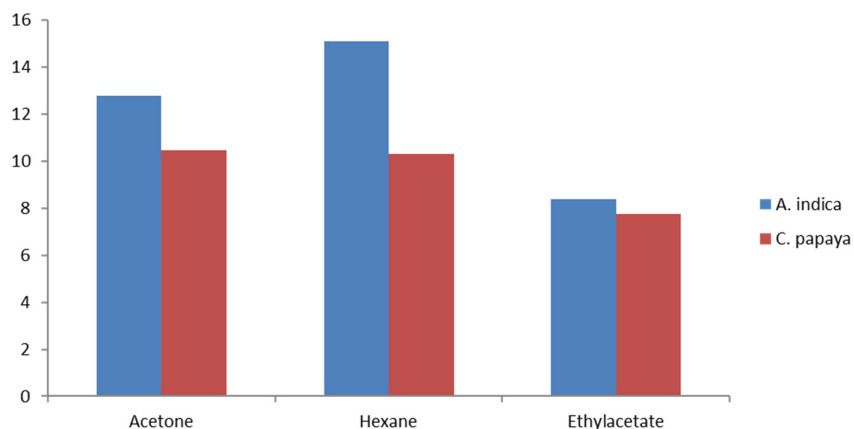


Figure 1. Percentage yield of acetone, hexane and ethyl acetate extracts of *Carica papaya* and *Azadirachta indica* leaves

Table 1 shows the result of phytochemical screening of all the solvent extracts of *A. indica* and *C. papaya*. Classes of compounds: tannin, steroid, saponin, anthraquinone and alkaloid were present in all the solvent extracts of *A. indica* and *C. papaya*. However, protein was present in all the solvent extracts of *A. indica* but absent in all the solvent extracts of *C. papaya*. Terpenoid was only present in hexane extract of *A. indica* but absent in all other solvent extracts of the plants. Flavonoid was present in all but absent in ethylacetate of *A. indica*. Glycoside was also present in all but absent in hexane extract of *A. indica*. Lastly, coumarin was only

present in acetone extracts of both plants and absent in other solvent extracts of the two plants (Tables 2 and 3).

Table 1. Preliminary phytochemical constituents of acetone, hexane and ethylacetate extracts of *Carica papaya* and *Azadirachta indica* leaves

Composition	Acetone		Hexane		Ethylacetate	
	<i>A. indica</i>	<i>C. papaya</i>	<i>A. indica</i>	<i>C. papaya</i>	<i>A. indica</i>	<i>C. papaya</i>
Tannin	+	+	+	+	+	+
Steroid	+	+	+	+	+	+
Saponin	+	+	+	+	+	+
Glycoside	+	+	–	+	+	+
Terpenoid	–	–	+	–	–	–
Protein	+	–	+	–	+	–
Flavonoid	+	+	+	+	–	+
Anthraquinone	+	+	+	+	+	+
Alkaloid	+	+	+	+	+	+
Coumarin	+	+	–	–	–	–

Table 2. Constituents of different solvent extracts of *Azadirachta indica* separated by HPLC

Component	Acetone		Ethylacetate		Hexane	
	Retention (Mins)	Height	Retention (Mins)	Height	Retention (Mins)	Height
Myricetin	3.700	49.672	3.700	61.812	3.700	51.309
Azadirachtol	5.883	7.817	5.883	11.408	5.883	10.159
Azadirachtin A	7.996	12.452	7.966	14.708	7.966	12.939
Pentadecane	*	*	9.116	2.910		
Phytol	*	*	*	*	*	*
Azadirachnol	15.500	10.242	15.500	12.686	15.500	12.609
Quercetin	17.233	30.220	17.233	35.938	17.233	35.708
B. Caryophyllen	*	*	19.166	5.261	19.166	4.861
Alpha Ionone	*	*	*	*	*	*
Alpha Funebren	20.500	2.131	20.500	2.493	20.500	1.974
ASCARIDOLE			21.416	1.959		
Trams.B.Farnes	*	*	*	*	*	*

Table 3. Constituents of different solvent extracts of *Carica papaya* separated by HPLC

Component	Acetone		Ethylacetate		Hexane	
	Retention (mins)	Height	Retention (mins)	Height	Retention (mins)	Height
Acacic acid	1.266	29.889	1.266	28.971	1.266	28.070
Genistein	2.700	22.482	2.700	19.927	2.700	17.419
Protodioscin	4.600	9.166	4.600	4.589	4.600	2.427
Betulinic acid	5.500	4.067	*	*	*	*
Phorbolester	5.733	3.297	5.733	7.123	*	*
Creptolepinone	11.116	146.389	11.116	139.485	11.116	137.631
Brusatol	12.216	57.652	12.216	44.250	12.216	40.454
Alpha ionone	13.816	38.442	13.816	15.417	13.816	8.795

Figures 2 and 3 showed the results of susceptibility test of the extracts on the test pathogenic microorganisms. All the solvent extracts of *A. indica* and *C. papaya* were generally observed to have irregular pattern of activities at all the tested concentrations. Although in Figure 2, the activity of *A. indica* was highest for acetone extracts at 25 mg/mL concentration against *E. coli* and lowest for acetone and ethyl acetate at 50 mg/mL against *E. coli* respectively.

In Figure 3, highest activity was obtained for ethylacetate extract (at 50 mg/mL) and hexane extract (at 25 mg/mL) of *C. papaya* against *E. coli* and *Candida* respectively while lowest activity was obtained for 50 mg/mL of hexane extract (against all the test organisms), ethyl acetate (against *E. coli*) and at 25 mg/mL of acetone extract against *E. coli*.

Figure 4 shows the comparative results obtained for the control agents (gentamicin and griseofulvin) and the highest zones of inhibition obtained for the solvent extracts of *A. indica* and *C. papaya* against the test organisms. Highest zone of inhibition (28 mm) was obtained by gentamicin, followed by griseofulvin and *C. papaya* respectively (18 mm) while *A. indica* gave the least zone of inhibition (15 mm).

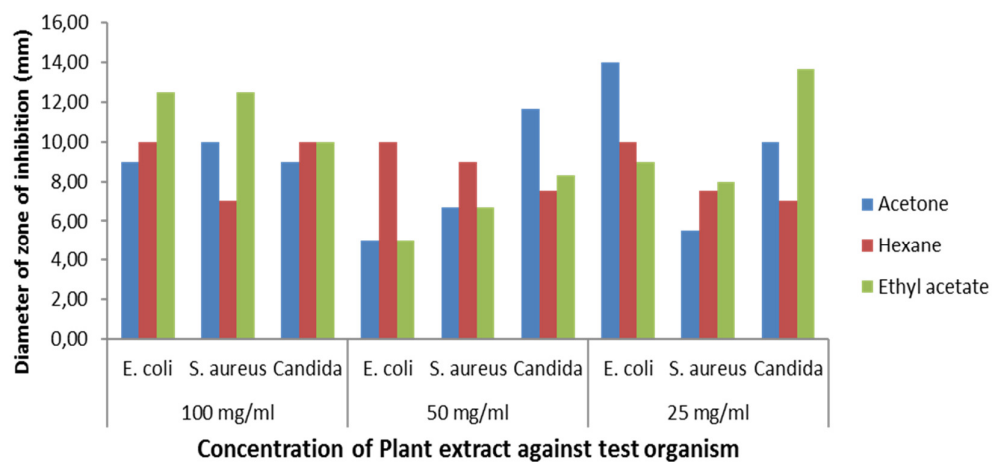


Figure 2. Average zones of inhibition of different concentrations of *A. indica* leaves against the tested microorganisms

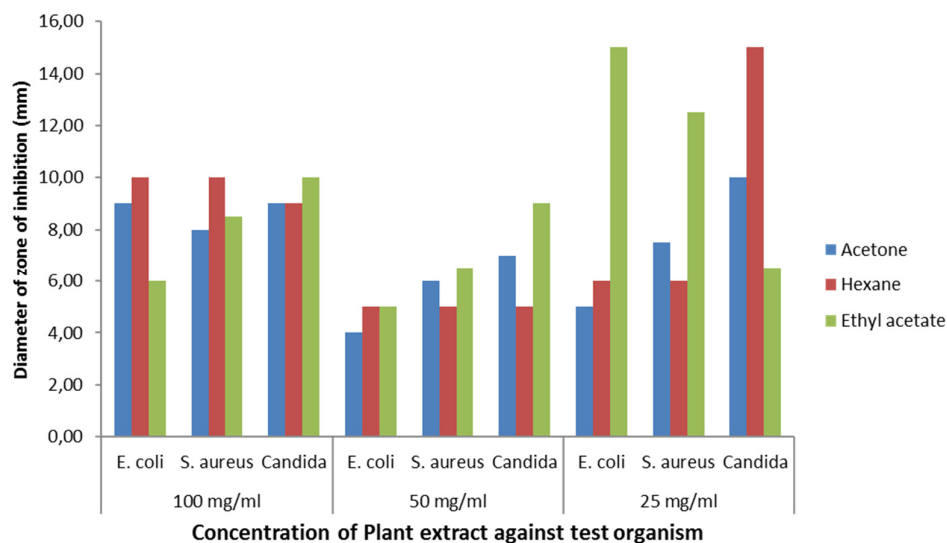


Figure 3. Average zones of inhibition of different concentrations of *C. papaya* leaves against the tested microorganisms

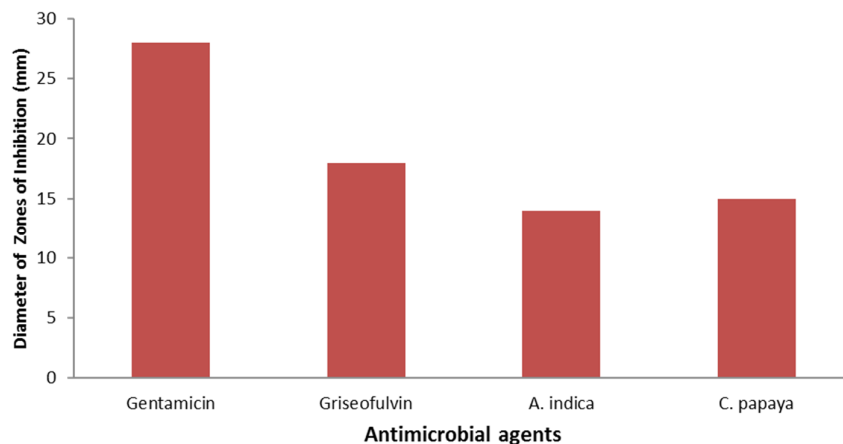


Figure 4. Comparison between highest effects produced by plant extracts and the commercial drugs

Chromatogram of hexane extracts of *C. papaya* leaves as visualized in the form of peak showed six fractions of compounds namely: acacic acid, genistein, protodioscin, creptolephone, brusatol and alpha ionone. These compounds separated based on their unique chemistry; the peaks had the heights of 28.070, 17.410, 2.427, 137.631, 40.454, 8.705%. Creptolephone had highest sharp peak 137.631 at 11.116 min. retention time (Rt). Brustanol had the next sharp peak 40.14 at retention time (Rt) 12.216 min. The height peak of acacic acid was 28.070 at 1.266 Rt. The next chromatogram peak of 17.410 denoted genistein at Rt 2.700, the height of alpha ionone was 8.705 at Rt 12.210 and 2.427 height for protodioscin at Rt 4.600 (Figure 5).

Chromatogram of hexane extracts of *Azadirachta indica* leaves as visualized in the form of peak showed seven fractions of compounds namely: myricetin, azadirachtol, azadirachtin a, azadiractnol, quercetin, b-caryophyllen, alpha funebren. These compounds separated based on their unique chemistry; the peaks had the heights of 51.309, 10.159, 12.939, 12.609, 35.708, 4.861, 1.974%. Myricetin had highest sharp peak 51.309 at 3.700 min. retention time (Rt). Quercetin had the next sharp peak 35.708 at retention time (Rt) 17.233 min. The height peak of azadirachtin a was 12.939 at 7.966 Rt. The next chromatogram peak of 12.609 was azadiractnol at 15.500 Rt, azadirachtol had height peak of 10.159 at Rt 5.883. B-caryophyllen height peak as 4.861 at 19.166 Rt and Alpha funebren had height peak of 1.974 at 20.500 Rt (Figures 6, 7, 8, 9, 10).

Discussion

Leaves of *A. indica* and *C. papaya* used in this study were air dried to get rid of moisture which may affect the solubility of subsequent separation by extraction and the secondary metabolites in plants may become unstable as a result of moisture, especially if the plant is to be used as an antimicrobial agent. It has been reported that plants are usually air dried to a constant weight to get rid of moisture (Dilika *et al.*, 1996; Baris *et al.*, 2006). Higher percentage yield was obtained for all the solvent extracts of *A. indica* than extracts of *C. papaya*. In order to extract different phenolic compounds from plants with a high degree of accuracy, various solvents of differing polarities must be used (Wong and Kitts, 2006). Takazawa *et al.* (1982) also stated that there is a need to employ broad range of extractive solvents in the extractions of possible phytochemicals from medicinal plants. Hence, this study involved the use of acetone, hexane and ethyl acetate. Parekh *et al.* (2006) reported that traditional healers use primarily water for extraction but plant extracts from organic solvents have been

found to give more consistent antimicrobial activity compared to water extracts. All the solvents used for extraction of biomolecules from plants are chosen based on the polarity of the solute of interest. Although, the solvents used in this study are not very commonly used, acetone for example has been used by a number of authors (Basri and Fan, 2005; Mathkega *et al.*, 2006). The most commonly used solvents for investigations of antimicrobial activity in plants are methanol, ethanol, and water (Parekh *et al.*, 2006; Bisignino *et al.*, 1999; Salie *et al.*, 1996; Rojas *et al.*, 2006). In a study conducted by Masoko and Eloff (2006) on antifungal activities of different solvent (hexane, dichloromethane, acetone and methanol) extracts of *Combretum* species, it was discovered that acetone and methanol extracted more chemical compounds from the leaves than other solvents.

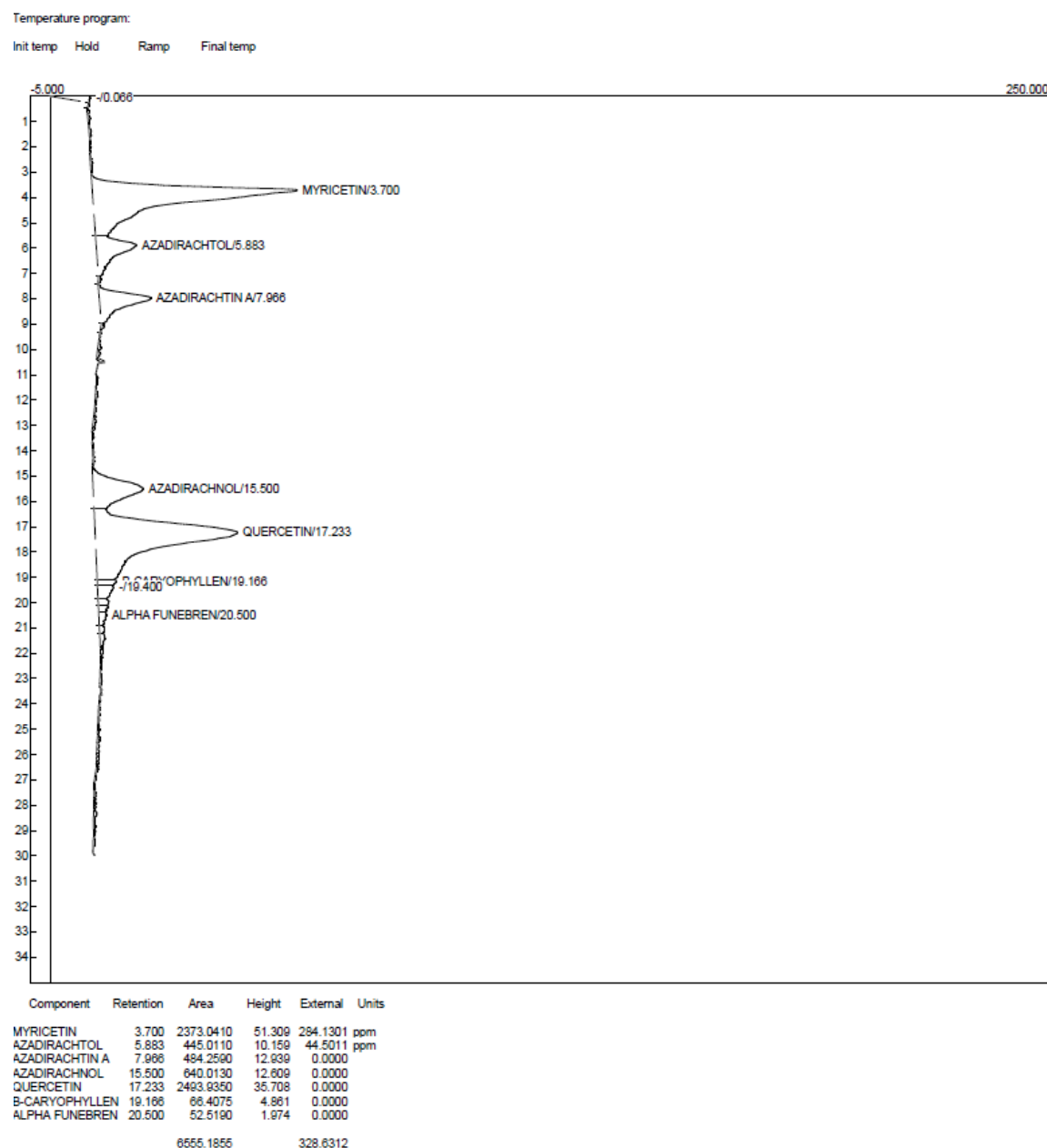


Figure 5. Chromatogram of acetone extract of *A. indica* leaves

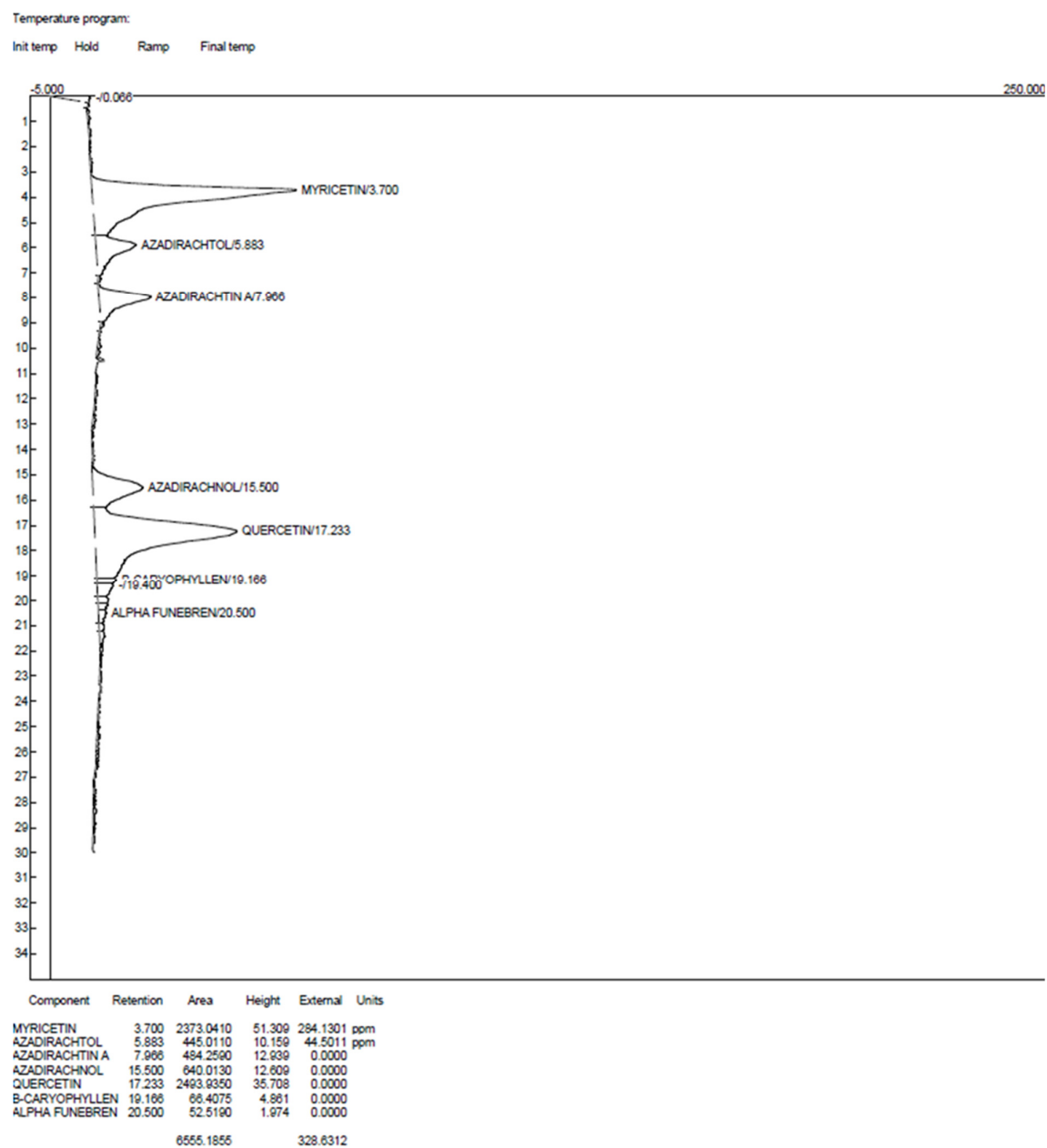


Figure 6. Chromatogram of hexane extract of *A. indica* leaves

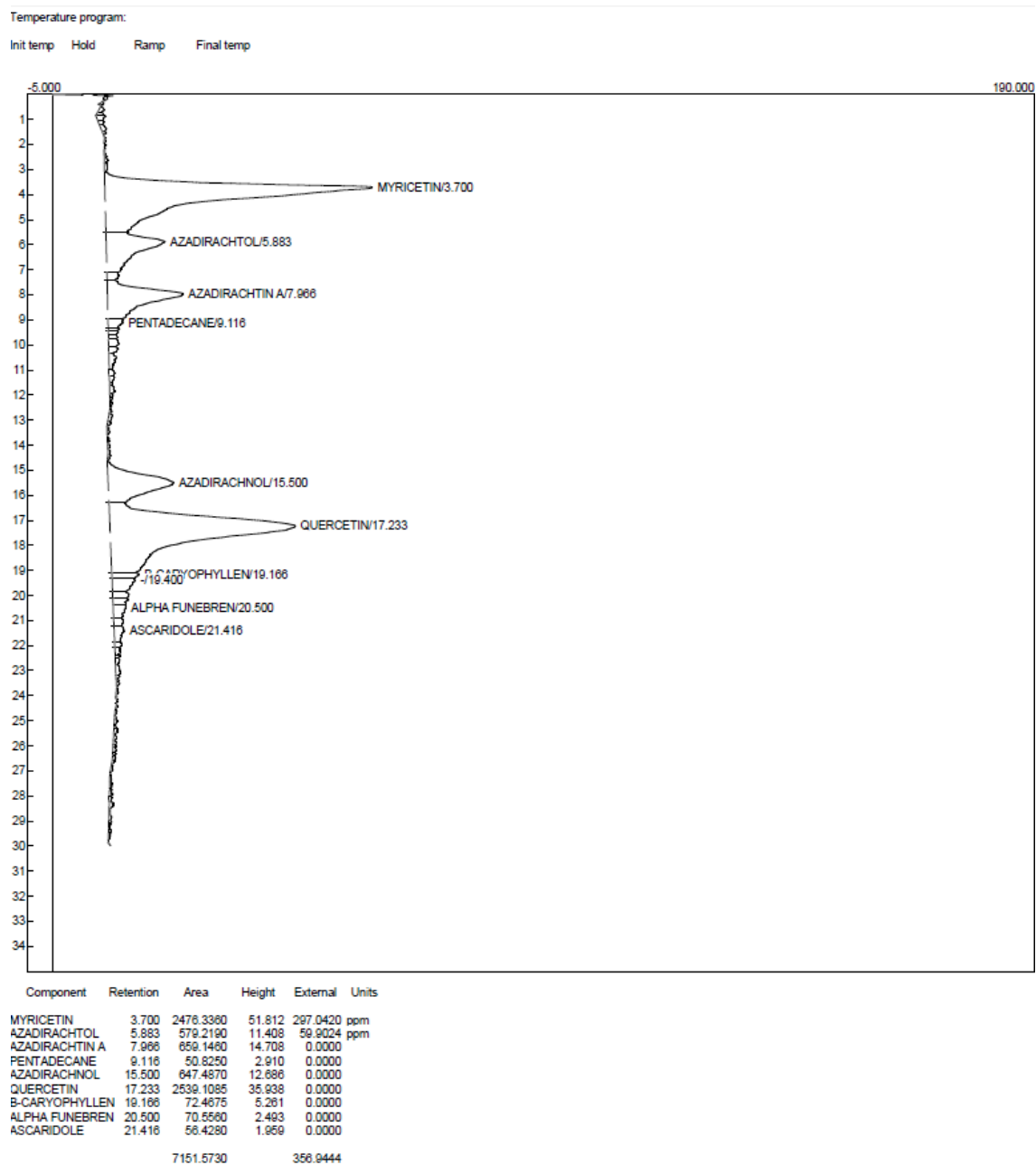


Figure 7. Chromatogram of ethylacetate extract of *A. indica* leaves

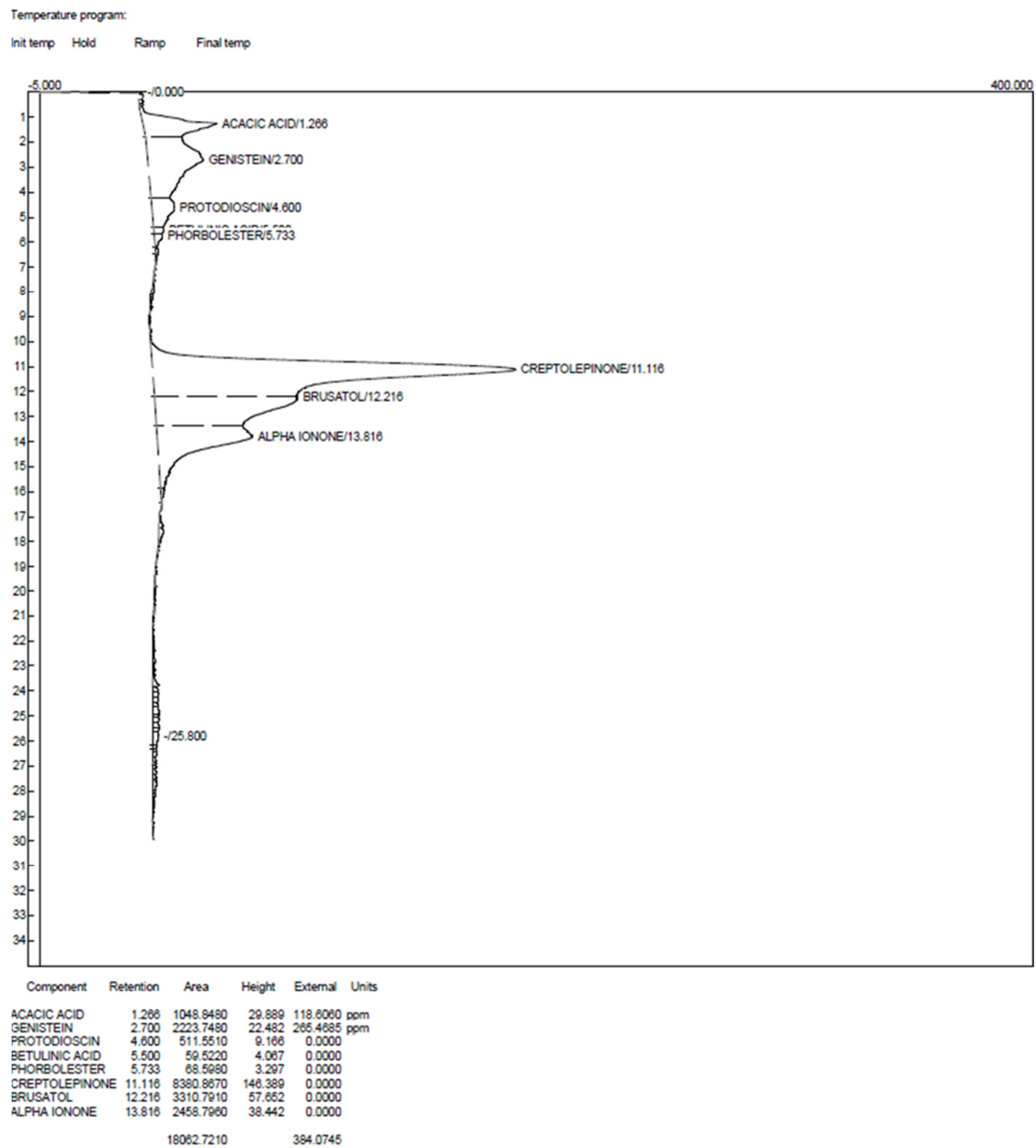


Figure 8. Chromatogram of acetone extract of *C. papaya* leaves

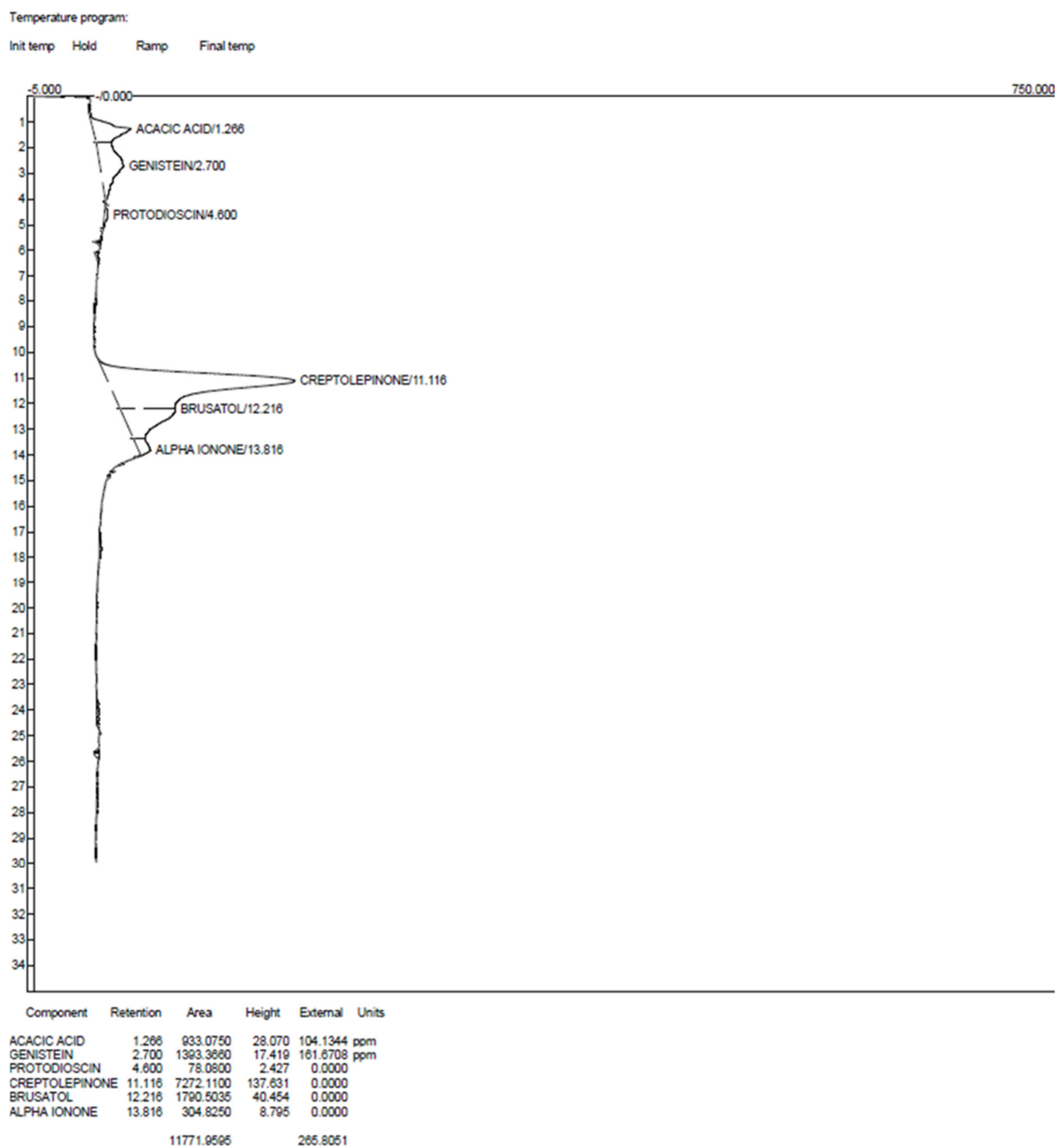


Figure 9. Chromatogram of hexane extract of *C. papaya* leaves

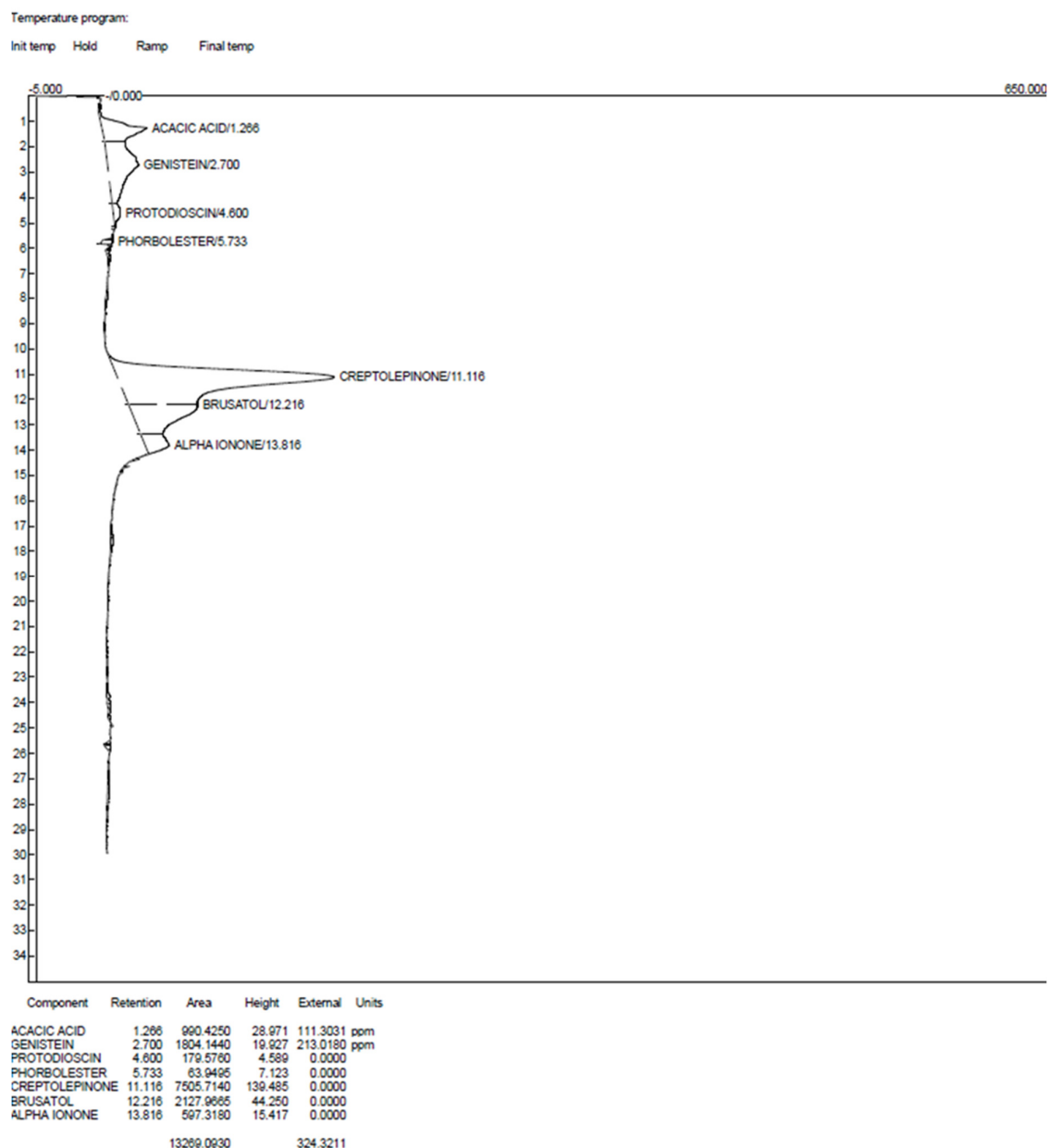


Figure 10. Chromatogram of ethyl acetate extract of *C. papaya* leaves

Eloff (1998) examined a variety of extractants for their ability to solubilize antimicrobials from plants, their rate of extraction, their ease of removal, their toxicity in bioassay, among other things, and acetone received the highest overall rating. Anokwuru *et al.* (2011) reported that acetone and N, N dimethylformamide (DMF) are highly effective at extracting antioxidants, while Koffi *et al.* (2010) reported that methanol was more effective at extracting large amount of phenolic compounds from walnut fruits when compared to ethanol.

Findings in this work recorded strong occurrence of antimicrobial compounds (saponins, steroid, tannin, anthraquinone, glycoside, protein, flavonoids). Tannin, alkaloid, steroid, saponin and anthraquinone were present in all the solvent extracts of *A. indica* and *C. papaya*. Enyi-Idoh *et al.* (2012) also reported that *Vernonia amygdalina* have ample quantities of saponins, flavonoids, alkaloids and tannins. It has been reported that phytochemical substances such as alkaloids, saponins, tannins, flavonoids, alkaloids, flavonoids, and terpenoids are bioactive bases responsible for antibacterial property by exerting bacteriostatic and bactericidal effects on some bacteria (Obadoni and Ochuko, 2001; Okigbo and Ogbonnanya, 2006; Funmilayo *et al.*, 2010). According to Ogundare *et al.* (2006), saponins, flavonoids, tannins and anthraquinones found in bitter leave were reported to have very potent antibacterial effect.

Variable antimicrobial activities were recorded for different solvent extracts of *A. indica* and *C. papaya* against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 259220 and *Candida albicans*. Although, solvent extracts of the plants showed no regular pattern of activities against the test pathogens and this concurs with the report that different organisms respond differently to different and same concentrations of a specific medicinal plant (Arekemase *et al.*, 2013). Antimicrobial activities observed in this work maybe imputable to the antimicrobial compounds detected in this work. All extracts tested exhibited dose dependent antimicrobial activities against the test organisms (*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *C. albicans*). Arekemase *et al.* (2013) has also demonstrated antimicrobial activity of *Vitellaria paradoxa* extract against bacteria. The lowest concentration of the extracts tested was displayed inhibitory effects against the test organisms; the effects produced by commercial antibiotics was still higher than what was obtained for the plants under study. This maybe because plants comprise array of compounds which are in mixtures in contrast to the pure compound contained in the commercial agents (Gatsing *et al.*, 2010). Findings in this work are in line with the work of Eja *et al.* (2011), who reported significant antimicrobial activities of *Vernonia amygdalina* on *E. coli* and *Staphylococcus aureus*.

Antimicrobial activities demonstrated by hexane and ethylacetate extracts of *C. papaya* may be attributed to the presence of flavonoid which was not present in ethylacetate extract of *A. indica* and glycosides which were also not present in hexane extract of *A. indica*. Although, terpenoid was only present in hexane extract of *A. indica* but absent in all other solvent extracts.

Several fractions of flavonoids and phenolic compounds were comparatively separated in the different solvent extracts of *C. papaya* and *A. indica* as different peaks and quantified by HPLC. Variation in the retention times within different extracts indicated the presence of phytochemicals in *C. papaya* extracts as acacic acid, genistein, protodioscin, betulinic acid, phorbol ester, creptolepinone, brusatol and alpha ionone while the fractions from the solvent extracts of *Azadirachta indica* are myricetin, azadirachtol, azadirachtin a, pentadecane, phytol, azadirachnol, quercetin, b. caryophyllen, alpha ionone, ascaridole, trams.b.farnes. However, this variation has also been reported in scientific literature (Asen, 1984). It has been reported that ethanolic extracts of Ivorian plants extracted higher concentrations/amount of phenolics compared to acetone, water, and methanol (Koffi *et al.*, 2010).

Conclusions

Results obtained in this work indicated that all the solvent extracts of *Carica papaya* and *Azadirachta indica* contained numerous active phytoconstituents and the extracts displayed good potentials at preventing diseases associated with the microorganisms tested in this work. Hence, it can be concluded that the plants are useful medicinally as broad-spectrum antimicrobial agents. The antimicrobial properties of these plants can therefore be credited to the presence of active constituents suggesting that the plants can serve as potential

sources of alternative antimicrobial agents. This work suggests further research on the dosage and safety of these medicinal plants on vital organs of the consumer.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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