CHAPTER FIVE

IN VITRO ANTIMICROBIAL ASSAY OF SOME MEDICINAL PLANTS FROM ETHIOPIA AGAINST PLANT AND FOOD-BORNE PATHOGENS

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Abstract

Thirty-seven extracts of 23 plant species collected from three citrus growing regions of Ethiopia were screened for their activity against seven plant pathogens and five food-borne pathogens. In total, 21 extracts from 13 plant species showed some degree of antimicrobial activity to at least one pathogen. Of these, seven species, i.e. *Achyranthus aspera*., *Tribulus terrestris*, *Withania somnifera*, *Acacia seyal*, *Dolichos oliver*, *Cissus quadrangularis* and *Mirabilis jalapa* are species with no known previous reports of antimicrobial activity against the tested pathogens. The minimum inhibitory concentration value of eight selected plant extracts with antimicrobial activity against both fungal and bacterial pathogens ranged between 1:2 and 1:5 (v:v), indicating significant differences in their composition of active compounds. Thin layer chromatography was used for separation of the chemical compounds. None of the extracts inhibited *Escherichia coli* or *Erwinia carotovora*. On the other hand, three plant extracts inhibited a bacterial strain with complete resistance to all antibiotics tested. *Acacia seyal*, which demonstrated broad-spectrum antimicrobial activity, contained substantial concentrations of soluble phenolic compounds. Further determination of the active chemical ingredients is crucial for health improvement studies and postharvest disease control.

Key words: Plant extracts; Antibacterial; Antifungal; Phenolic compounds

5.1 INTRODUCTION

Plants are indispensable sources of medicinal importance used in both Western type pharmaceutical products and local medicinal preparations. The traditional use of plant material for treatment of human ailments dates back to prehistoric times (Cowan, 1999). According to the World Health Organisation, 80% of the world's population relies on traditional medicines to meet their daily health requirements (Maffi, 1999). However, from the estimated 250 000 species of higher plants described to date, only 5-15% have been studied for their potential therapeutic value (Rojas et al., 2003; Steep, 2004).

Ethiopia is a tropical country with a high floral diversity and endemism (Brenan, 1978). According to Tewoldebirhan (1991), there are about 7 000 species of higher plants in Ethiopia, of which 12% are endemic. More than 80% of the Ethiopian population depends on traditional remedies (Dawit and Ahadu, 1993), derived mainly (95%) from plant material (Dawit, 1986). The nationwide use of plants as a sole source of traditional medicine provides promising opportunities for the search of ethnobotanical specimens based on traditional knowledge.

Several researchers have studied the ethnobotanical (Dawit and Ahadu, 1993; Giday, 2001; Desissa and Binggeli, 2002), phytochemical (Abegaz and Woldu, 1991; Dagne and Abate, 1995) and antimicrobial activities (Habtemariam et al., 1993; Mammed, 2002) of a variety of medicinal plants. However, despite the broad spectrum of plants studied to date, no publications dealing with the potential of Ethiopian medicinal plants used for their antimicrobial activity could be found. The present study was aimed at screening potentially useful medicinal plants from Ethiopia for their antimicrobial potential to control major plant pathogens. In addition with the growing importance of food safety, it was decided to also evaluate the potential inhibitory activity of these extracts against major food borne pathogens.

In this chapter, we report on 37 extracts of 23 medicinal plants from three citrus growing regions of Ethiopia, which are also agriculturally important areas in terms of soil type and weather. Information about agro-ecology and cultural uses of medicinal plants are also included.

5.2 MATERIALS AND METHODS

5.2.1 Plant material

Twenty-three cultivated and wild medicinal plant spp. were collected from three citrus growing areas in Ethiopia, i.e. Somali, Oromia and Amhara Regional States between September 2002 and January 2003 (Table 5.1). Information about regionally important plants used in medicine was collected by consulting and interviewing local traditional healers. Plant samples including leaf-, stem-, root- and seed parts were collected, washed with tap water, air dried, packed into brown paper bags and transported to the herbarium of Alemaya University for identification. Identities of plant species were confirmed by Dr. Lisanework Nigatu from the Department of Plant Science (Alemaya University) and voucher specimens were stored and labelled in the collection. Dry samples were then brought to Plant Pathology Laboratories, University of Pretoria, South Africa with permit number (P0017192) for phytochemical analyses and biocontrol studies. Strict quarantine handling, processing and plant destruction protocols were followed during and after processing of samples. Plant rights and traditional knowledge have been protected within the University's ethical criteria requirements guidelines.

5.2.2 Plant material extraction

Dried, undamaged plant parts (leaves, stems, roots and seeds/fruits) were selected and reduced to powder in a Satin coffee grinder (Russell Hobbs, Germany). The powdered samples were stored at ambient temperature in glass bottles until further use. Two solvents, i.e. methanol/ acetone/ water (7:7:1 v:v) (Regnier and Macheix, 1996) with some modification and distilled water alone (Bautista-Banos et al., 2003) were used for extraction purposes. One part of the dried plant powder was suspended in 20 parts of solvent mixture followed by three successive extractions. The first and second extraction suspensions were mixed using a VM-300 vortex mixer (Labotec, Johannesburg) and placed on a rotary shaker (Stuart Scientific, United Kingdom) for 1 h at 170 rpm. Samples were centrifuged in a micro-centrifuge (Sigma, Germany) at 3913 x g for 10 min. The third extraction was placed overnight on the rotary shaker (Stuart Scientific) and centrifuged (Sigma). For each plant sample, the supernatants from three extractions were combined, concentrated under vacuum at room temperature (23 ºC) and freeze-dried. Distilled water was added to the concentrate to make up 10 ml of stock solution. The suspensions were filter sterilised

through a syringe filter (0. 22 µm pore size) into sterilised containers. Suspensions were either used immediately or kept at -4 ºC for later use.

5.2.3 Test pathogens

Three fungal pathogens [(*Penicillium digitatum* Sacc. (UPPed-1), *Geotrichum candidum* Lk ex Pers. (UPGec-1) and *Phytophthora nicotianae* Breda de Hann (UPPhn-1)], six bacterial plant pathogens [two strains each of *Erwinia carotovora* (UPErc-1 and UPErc-2) and *Xanthomonas campestris* pv. *mangiferaeindicae* (UPXac-1 and UPXac-2), and one strain each of *Pseudomonas syringae* pv. *syringae* (UPPss-1), *Ralstonia solanacearum* (UPRas-1)] and five food-borne pathogens [*Escherichia coli* (UPEsc-1), *Salmonella typhimurium* (UPSat-1), *Shigella sonnei* (UPShs-1), *Staphylococcus epidermidis* (UPSte-1) and *Streptococcus faecalis* (UPStf-1)] were obtained from the culture collection of Plant Pathology Laboratories University of Pretoria, South Africa. The pathogens were subcultured and maintained on Potato Dextrose Agar (PDA) (Biolab, Johannesburg) for fungi and Standard-1 nutrient agar (STD-1 NA) (Biolab) for bacteria. Fungal cultures were incubated for 7-14 days at 25 ºC under UV light until sporulation. Spores were harvested from the plates using a sterile swab and 20 ml of ¼ strength Ringer's solution (Merck, Johannesburg). A fungal spore concentration of 10^5 spores ml^{-1} was prepared using a haemacytometer. Agar blocks (3 x 3 mm size) from these cultures were used in all further trials. For bacteria, densities of cultures grown in Nutrient Broth (NB) (Biolab) on a rotary shaker (Stuart Scientific) for 24 h at 25 ºC were determined using a Petroff-Hauser counting chamber. A standardised concentration of 10^8 cells ml^{-1} was used in all subsequent tests.

5.2.4 *In vitro* antimicrobial assay

Two assay techniques, i.e. the agar plate (Thornberry, 1950) with slight modification and agar well diffusion assay (Rojas et al., 2003), were used to evaluate the antimicrobial activity of plant extracts against fungal and bacterial pathogens.

5.2.4.1 Agar plate technique

This method was selected to screen plant extracts for their efficacy against the fungal pathogens *P. digitatum*, *G. candidum* and *P. nicotianae*. This technique avoids volatilisation of active plant extract compounds. Aliquots of 9 ml PDA were made up in test tubes, autoclaved and cooled down to 50 ºC, after which 1 ml of plant extract was added aseptically, poured into a Petri dish (90 mm diameter) and swirled to cover the base. Fungal agar blocks (3 x 3 mm) from the cultures prepared

as described in section 2.3 were transferred to the centre of the plates. Plates were incubated at 25 ºC for 7-14 days and evaluated every two days for growth inhibition. The experiment was performed in triplicate and percentage inhibition of pathogen growth was determined according to Skidmore (1976), using the following formula: Percentage inhibition = (C - r) x 100/C, where r = fungal radial growth measured on the treated plate and C = radial growth measured on the control plate.

5.2.4.2 Agar well diffusion

This technique was used to determine the toxicity of extracts against bacterial pathogens, which multiply sufficiently to detect growth or inhibition within 24-48 h of incubation. Bacterial broth cultures were prepared to a density of 10^8 cells ml^{-1} as described in Section 2.3. Aliquots of 100 µl were spread evenly onto individual STD-1 NA agar plates. On each plate, four equidistant wells were made in the agar with a 0.5 mm diameter sterilised cork borer, 2 mm from the edge of the plate. Fifty µl of each plant extract was transferred to a respective agar well and plates were incubated at 25 ºC for 24-48 h. The same volumes of antibiotics [Streptomycin (Sigma) (0.2 mg/ml), Tetracycline (Sigma) (2%), Novobiocin (Sigma) (2%) and Rifampicin (Rolab, Johannesburg) (2%)] were used as positive controls. Extraction solvents [methanol, acetone and sterilised distilled water] were included as negative controls. Experiments were performed in triplicate. The formation of clear inhibition zones around the wells were regarded as positive results and measured in mm.

5.2.5 Determination of minimum inhibitory concentration of selected plant extracts

The minimum inhibitory concentration (MIC) of each plant extract was determined using the method described by Barbour et al. (2004). Eight plant extracts that showed a wide range of antimicrobial activity in 2.4 were used for further tests. One ml of each plant extract, prepared as described in 2.2, was serially diluted in sterile NB. The plant extract volume to broth medium ratio (v:v) was prepared at 1:2; 1:2.5; 1:3; 1:3.5; 1:4 and 1:5. Each plant extract dilution was inoculated with 20 µl of the standard concentration of pathogen inoculum prepared as described in section 2.3. Culture tubes were incubated at 25 ºC for 24 h (bacterial isolates) and 72 h (fungal pathogens) and were evaluated visually for presence or absence of growth. The lowest plant extract concentration retaining its inhibitory effect (absence of turbidity) was regarded as the MIC value of the extract. Control flasks with uninoculated medium were incubated in parallel. The extraction solvents

methanol, acetone and sterilised distilled water were regarded as negative controls, whereas antibiotics were incorporated as positive controls. Experiments were performed in triplicate.

5.2.6 Phytochemical analysis:

5.2.6.1 Determination of total soluble phenolics, free acids, bound ester and glycoside

Crude Extract

Crude extracts (CE), prepared as described in 5.2.2, were used to quantify the amount of total soluble phenolics. (De Ascensao and Dubery, 2003)

Extraction of free acids

To extract the free acids (FA), 1.25 ml of the CE was acidified with 25µl trifluoroacetic acid. An equal volume of diethyl ether was added and the mixture shaken and allowed to stand briefly to allow separation of fractions. The organic phase was removed and placed in a new Eppendorf tube. This procedure was repeated four times. The separated upper phase layers combined and diethyl ether evaporated under vacuum. Two hundred and fifty micro liters of methanol was added and the extracts were stored at 4 ºC until further use. (De Ascensao and Dubery, 2003)

Extraction of bound esters

Sodium hydroxide (2N) was added to plant extracts prepared as described previously (5.2.2) at the rate of 2% w/v (i.e 0.2 g/ 1.25 ml). The mix was vortexed and kept in the dark for 4 h. After 3 h, samples were kept in an icebox and an equal volume of HCl (1M) was added in order to acidify the mixture. Three-fold volume of diethyl ether was added; the mixtures shaken and the samples allowed to settle to enhance the separation of hydrolysed ester-bond phenolic compounds. The procedure was repeated three times. The organic phases were combined and diethyl ether evaporated under vacuum. Two hundred and fifty micro liters of methanol was added and the extracts were stored at 4 ºC until further use. (De Ascensao and Dubery, 2003)

Extraction of glycosides

The process involved hydrolysis of glucose-conjugated compounds. Sixty milliliter of concentrated HCl (10N) was added to an Eppendorf tube containing 1ml of the crude extract. Samples were kept in a water bath for 1 h at 96 ºC. Five hundred micro liters of diethyl ether was added while the sample was kept in the icebox. The extraction with an equal volume of diethyl ether was repeated

three times and the separated upper phase layers were combined together. The organic phases were combined and dried under vacuum. Two hundred and fifty micro liters of methanol was added and the extracts were stored at 4 ºC until further use.

The total contents of soluble phenolics in medicinal plants were determined using a modification of the Folin-Ciocalteu's Phenol reagent (Bray and Thorpe, 1954). The extracts of 37 plants were evaluated using the 96 wells ELISA-plates (Merck, Germany). In each well, 25 µl of the Folin-Ciocalteu reagent (Sigma) was added to 175:5 µl (v:v) of distilled water and the test plant extract respectively. After three min., 50 µl of 20% sodium carbonate was added into each well. Four wells were used for each sample, randomly placed on the ELISA plate, and the experiment was done in duplicate. Plates were incubated at 40 ºC for 30 min. Phenolic measurements were taken with an ELISA reader version 1.3.1 (Multiscan Ascent VI. 24 354-0973, Finland). The absorbance of a blank consisting of distilled water was subtracted from all sample readings. Data were calculated as gallic acid equivalent in µg g^{-1} using the standard curve (y= 1.3527 x + 0.0109, R^2=0.9989). (De Ascensao and Dubery, 2003)

5.2.6.2 Thin layer chromatography

The same eight plant extracts used in 2.5 were used for further evaluation. The following solvent combinations were tested to obtain the best separation of phenolic compounds: toluene/ ethyl acetate (1:1), chloroform/ methanol/ ethyl acetate/ acetone/ water (50:20:20:5:3.5), ethyl acetate/ formic acid/ water (3:1:3), butanol/ ethanol/ water (5:1:2), toluene/ acetic acid (4:1), chloroform/ ethyl acetate/ formic acid (5:4:1), butanol/ acetic acid/ water (6:1:2), acetic acid (10%), methanol/ butanol/ ethyl acetate/ dichloromethane (1:1:1:1), ethyl acetate/ acetic acid/ water (3:1:3), ethyl acetate/ acetic acid/ formic acid/ water (50:5.5:5.5:13) and chloroform/ acetone/ formic acid (9:2:1), of which, toluene/ ethyl acetate (1:1), butanol/ ethanol/ water (5:1:2), and ethyl acetate/ acetic acid/ formic acid/ water (50:5.5:5.5:13) gave the best result. The TLC plate (pre-coated aluminium, SIL G-100) was loaded with 10 µl of each sample. Spots were visualized with a CAMAT 50HZ UV lamp at 254 and 366 nm. Three separation solvent systems from the preparation described [were selected. Of these again, toluene/ ethyl acetate (1:1) was further used for separation of soluble FA, GLY and EB of *A. seyal* and *W. somnifera* plant extracts selected for their antifungal potential without antibiosis. The visibility of compounds was amplified by spraying ammonia vapour onto the plates and the R_f values of the separated spots were determined.

5.2.6.3 High performance liquid chromatography analyses

Fresh preparation and six months old extracts of *A. seyal* and *W. somnifera* prepared as described in 5.2.2 were used. Identification and quantification of individual phenolic compounds of CE, FA, GLY, EB, of the two plants extract: *A. seyal* and *W. somnifera* were done by high performance liquid chromatography (HPLC).

The samples were analysed on a Hewlett Packard HPLC equipment (Agilent 1100 series) equipped with a 20 µl loop injection valve (Agilent) and connected with a UV detector at 280, 325 and 340 nm. A Luna 3u C18 reverse phase column (250 x 4.60 mm) was used. Acetonitrile and water (pH 2.6 acidified with phosphoric acid, H_3PO_4) were used as eluents with a gradient program from 7% acetonitrile/ water at 0 minutes to 20% at 20 minutes increasing to 23% at 28 minutes, 27% at 40 minutes, 29% at 45 minutes, 33% at 47 minutes and 80% at 50 minutes. Twenty microliters of each sample [(CE, 20x; FA, 10x; Gly, 10x and EB, 10x diluted) were injected and chromatogramed at a flow rate of 1 ml min^{-1}. Data were analyzed using the Hewlett Packard software. The phenolic compounds in the extracts were identified by comparison with the reference compounds such as, gallic acid, caffeic acid, ferulic acid, syringic acid, quercetin, umbelliferone, naringin, hydroxy benzoic acid, 3,4, dihydroxy benzoic acid, sinapic acid, vanillic acid, *ρ*-coumaric acid, salicylic acid, scopoletin, catechin, kaempferol, chlorogenic acid, luteolin and fisetin.

HPLC data was analysed qualitatively by comparing the presence and absence of peaks in chromatograms obtained with the different treatments. For unidentified compounds, the area of the peak (mAU*s) was used to evaluate quantitative differences among treatments while for the known compounds; the amount (µg/ml) was used for comparison between treatments. Data from the areas of unidentified compounds was subjected to normality and homogeneity of variances tests then log (x+1) transformed prior to analysis.

5.2.7. Statistical analysis

One-way analysis of variance (ANOVA) was performed using the SAS computer program (version 8.2, 2001). Treatment means were compared with Tukey's HSD multiple range test at a 5% level of significance.

5.3 RESULTS

5.3.1 Medicinal plant species

The 37 plant extracts prepared from 23 plant species collected from Ethiopian citrus growing regions are shown in Table 5.1. From preliminary trials, methanol/ acetone/ water was identified as the best solvent system compared to aqueous extraction. The most effective plant species regarding antimicrobial activity were found in Hursso, Somali National Regional State. Plant leaves were found to be more inhibitory (44.2%), followed by stem (27.9%), root (14%) and seeds (10.8%) extracts.

Table 5.1 Plant species collected in Ethiopia, their location, plant parts used in the study and known usage as described by local healers

Plant species	Family	Plant type	Location	GPS coordinates	Altitude (m asl)	Plant part used				Local use(s) in Ethiopia*** as described by traditional healers interviewed
						Leaf	Stem	Root	Seed	
Acacia seyal Del. var. Seyal	Mimosaceae	Tree	Hursso[d]	N: 9.614 E: 41.643	1062.5	✓	✓	✓	-	Intestinal disorder, bleeding and conjunctivitis
Achyranthus aspera L.	Amaranthaceae	Herb	Errer[c]	N: 9.573 E: 41.38	996	✓	-	✓	-	Intestinal disorder (dysentery)
Agave sisalana L.	Agavaceae	Herb	Tisabalima[f]	N: 11.459 E: 39.628	1492	✓*	-	-	-	Insecticide
Artemisia afra Jacq. Ex. Willd	Compositae	Herb	Merssa[g]	N: 11.668 E: 39:.663	1602	✓	-	-	-	Hemorrhage (topical and decoction drink)
Azaridachta indica A. Juss	Meliaceae	Tree	Errer[c]	N: 9.575 E: 41.384	996	✓	-	-	-	Stomach ache (bloating) and insect repellant
Calotropis procera Ait. Dry	Asclepiadaceae	Herb	Errer[c]	N: 9.575 E: 41.384	996	✓	✓	-	-	Chronic skin infection and hemorrhage
Cissus quadrangularis L.	Vitaceae	Herb	Tisabalima[f]	N: 11.459 E: 39.628	1492	-	✓**	-	-	Insectcide and fungicidal
Convolvulus sp.	Convolvulaceae	Herb	Errer[c]	N: 9.550 E: 41.389	1084	-	✓	✓	-	Snake bite, decoction drink
Cucumis meeusei A. Rich	Cucurbitaceae	Herb	Alemaya University[h]	N: 9.00 E: 37.968	1890	-	-	✓	-	Skin burn and discharge of after birth
Dolichos oliveri Schweinf.	Fabaceae	Herb	Hursso[d]	N: 9.614 E: 41.643	1062.5	✓	-	✓	-	Epilepsy and sinus
Euphorbia abyssinica JF Geml.	Euphorbiaceae	Tree	Abomissa[e]	N: 8.491 E: 39.835	1600	-	✓	-	-	Wound healing (topical) and worm expel
Lablab purpureus L.	Fabaceae	Herb	Tisabalima[f]	N: 11.459 E: 39.628	1492	✓	-	-	-	Weed control and nitrogen fixation
Millettia ferruginea (Hochst) Baker	Papilionoideae	Tree	Tisabalima[f]	N: 11.459 E: 39.628	1492	✓	-	-	-	Insecticide
Mirabilis jalapa L.[a]	Nyctaoginaceae	Herb	Hursso[d]	N: 9.614 E: 41.643	1062.5	-	-	✓	-	TB[b], Cancer
Nicotiana tabacum L.	Solanaceae	Herb	Tisabalima[f]	N: 11.459 E: 39.628	1492	✓	✓	-	✓	Insecticide
Table … continued	Portulacaceae	Herb	Errer[c]	N: 9.573 E: 41.38	996	✓	-	-	-	Breast and knee tumours (surface application)
Ruta chalepensis L.	Rutaceae	Herb	Merssa[g]	N: 11.668	1602	✓	✓	-	-	Stomach ache and intestinal disorder

				E: 39.663						
Solanum incanum L.	Solanaceae	Herb	Alemaya University[h]	N: 9.00 E: 37.968	1890	✓	-	-	✓	TB[b]
Solanum nigrum L.	Solanaceae	Tree	Hursso[d]	N: 9.614 E: 41.643	1062.5	✓	-	-	✓	Gastritis, cancer and haemorrhage
Tribulus terrestris L.	Zygophyllaceae	Herb	Errer[c]	N: 9.573 E: 41.38	996	✓	✓	-	-	Induce uterus contraction and discharge of urine
Tagetes minuta L.	Asteraceae	Herb	Tisabalima[f]	N: 11.459 E: 39.628	1492	✓	-	-	-	Insecticide
Tamarindus indica L.	Caesalpiniaceae	Tree	Ghibe Valley[i]	N: 8.248 E: 37.540	995	✓	-	-	✓	Stomach ache and intestinal disorder
Withania somnifera L. Dunal	Solanaceae	Herb	Hursso[d]	N: 9.614 E: 41.643	1062.5	✓	✓	-	-	Epilepsy cure

Legend: [a] = Cultivated plant

[b] = Tuberculosis

[c] = East of Addis Ababa, the capital, 400 km (train) or 560 km (road)

[d] = East of Addis Ababa, 420 km (train) or 540 km (road)

[e] = South east of Addis Ababa, 160 km (road)

[f] = North east of Addis Ababa, 450 km (road)

[g] = North east of Addis Ababa, 490 km (road)

[h] = East of Addis Ababa, 500 km (road)

[i] = South west of Addis Ababa, 185 km (road)

*= Modified leaf

** = Modified stem

*** = According to traditional healers around the area

5.3.2 *In vitro* antimicrobial assay

Some degree of antimicrobial activity, at least to one pathogen, was shown by 21 extracts from 13 species. Of these extracts, 11 showed selective toxicity to fungal pathogens, while two of them inhibited bacterial growth. Eight of the extracts showed broad-spectrum activity against both fungal and bacterial pathogens (Table 5.2 and 5.3). In the *in vitro* semi-qualitative experiment, leaf and root extracts of *A. seyal*, root extracts of *M. jalapa*, leaf extracts of *T. minuta* L., leaf extracts of *W. somnifera* and seed extracts of *Solanum incanum* L. showed broad spectrum antimicrobial activity to the microbial pathogens challenged. The bacterial inhibition zones were in the range of 4-30 mm. Maximum inhibition was detected with *M. jalapa* against *S. epidermidis*. The latter pathogen was found most susceptible to over 80% of plant extracts evaluated (Table 5.2). Two species of bacterial pathogens (*E. carotovora* $_1$ and *E. coli*) were not affected by any of the plant extracts. On the other hand, some bacterial pathogens showed resistance to the antibiotics used in the control experiment. *Xanthomonas campestris*$_2$ was resistant to all antibiotics tested, while strain UPXac-1 was not inhibited by streptomycin. Similarly, *R. solanacearum* showed resistance to streptomycin, whereas *E. carotovora*$_2$, *P. syringae* and *S. sonnei* were resistant to novobiocin (Table 5.2). Sterilized distilled water, methanol and acetone did not have any inhibitory effect against the pathogens.

5.3.3 Determination of minimum inhibitory concentration of selected plant extracts

The MIC values of the eight plant extracts which showed inhibitory activity against some pathogens tested are shown in Table 5.4. The MIC values of extracts ranged between 1:1 and 1:5 (v:v) dilution ratio. The MIC of *A. seyal* ranged between 1:2 for *S. sonnei* and 1:4 for *S. epidermidis* and *X. campestris*, whereas the MIC of *W. somnifera* ranged between 1:3 for *S. epidermidis* and 1:3.5 for *S. faecalis* and *X. campestris*. Similarly, the MIC of *M. jalapa* root extract ranged between 1:2 (*S. sonnei*) and 1:5 (*S. epidermidis*).

5.3.4 Thin layer chromatography and R_f values of selected plant extracts

Of the twelve separation solvent systems evaluated, three were selected as most effective. The R_f value of these plant extracts are given in Table 5.5. Butanol/ ethanol/ water (5:1:2) resulted in a high band separation with almost all extracts except for *A. seyal*. Extracts such as *T. indica*, and *M. jalapa* showed no band separation activity to other solvent systems used. Unlike the other extracts evaluated, *T. minuta* and *S. incanum* exhibited fractional separation in the three solvent systems selected.

Table 5.2 Plant extract toxicity assay against plant and food borne bacterial pathogens tested

Plant species	Plant parts tested	Eq. mg gallic acid/g dry weight	Bacterial pathogens	Bacterial growth inhibition zone (mm)*
Acacia seyal Del. var. Seyal	Leaf	172.4	*Erwinia carotovora* $_1$	14 ± 0.7^{c}
			Pseudomonas syringae pv. *syringae*	16 ± 0.5^{e}
			Ralstonia solanacearum	15 ± 0.4^{ce}
			Shigella sonnei	06 ± 0.3^{f}
			Staphylococcus epidermidis	23 ± 0.8^{g}
			Xanthomonas campestris pv. *mangiferaeindicae*$_2$	24 ± 1.1^{g}
Acacia seyal Del. var. Seyal	Root	15.46	*E. carotovora*$_1$	13 ± 0.4^{c}
			P. syringae pv. *syringae*	13 ± 0.2^{c}
			R. solanacearum	13 ± 0.6^{c}
			S. sonnei	04 ± 0.6^{a}
			S. epidermidis	18 ± 1.0^{h}
			X. campestris pv. *mangiferaeindicae*$_2$	14 ± 0.3^{c}
Achyranthus aspera L.	Leaf	7.97	*S. epidermidis*	05 ± 0.5^{a}
Achyranthus aspera L.	Root	6.74	*S. epidermidis*	07 ± 0.5^{b}
Azadirachta indica A. Juss	Leaf	41.6	*S. epidermidis*	05 ± 0.5^{a}
Dolichos oliveri Schweinf.	Leaf	24.73	*S. epidermidis*	06 ± 0.6^{ab}
Mirabilis jalapa L.[b]	Root	28.84	*E. carotovora*$_1$	18 ± 0.2^{h}
			P. syringae pv.*syringae*	10 ± 0.3^{d}
			R. solanacearum	10 ± 0.4^{d}
			S. sonnei	08 ± 1.0^{b}
			S. epidermidis	30 ± 0.4^{i}
			X. campestris pv. *mangiferaeindicae* $_1$	20 ± 0.6^{k}
			X. campestris pv. *mangiferaeindicae* $_2$	04 ± 0.4^{a}
			S. typhimurium	15 ± 0.3^{c}
Ruta chalepensis L.	Leaf	18.62	*S. epidermidis*	07 ± 0.5^{b}
Solanum incanum L.	Leaf	17.75	*E. carotovora*$_1$	05 ± 0.5^{a}
			S. epidermidis	10 ± 0.7^{dj}
	Seed	57.80	*E. carotovora*$_1$	04 ± 0.5^{a}
			S. epidermidis	15 ± 1.1^{ce}
			X. campestris pv. *mangiferaeindicae*$_2$	17 ± 0.6^{he}
Tagetes minuta L.	Leaf	36.90	*E. carotovora*$_1$	12 ± 0.3^{i}
			e pv. *syringae*	10 ± 0.6^{dj}
			earum	09 ± 1.0^{j}

Table … continued

			S. epidermidis	16 ± 0.7[ce]
			X. campestris pv. *mangiferaeindicae*$_2$	13 ± 0.8[i]
Withania somnifera L. Dunal	Leaf	11.61	*Streptococcus faecalis*	13 ± 0.5[c]
			S. epidermidis	11 ± 0.5[d]
			X. campestris pv. *mangiferaeindicae*$_2$	16 ± 0.6[e]
Control trials with chemicals:				
Tetracycline			*E. carotovora*$_1$	9 ± 0.7[j]
			E. carotovora$_2$**	7 ± 0.9[b]
			P. syringae pv.*syringae*	17 ± 1.3[eh]
			R. solanacearum	17 ± 0.8[eh]
			X. campestris pv. *mangiferaeindicae*	18 ± 1.4[eh]
			X. campestris pv. *mangiferaeindicae*$_2$	0
			*E. coli***	7 ± 0.9[f]
			S. typhimurium	11 ± 0.4[d]
			S. sonnei	9 ± 0.8[j]
			S. epidermidis	2 ± 0.4[l]
			S. faecalis	15 ± 0.7[ce]
Streptomycin			*E. carotovora*$_1$	10 ± 0.2[j]
			E. carotovora$_2$**	10 ± 0.6[dj]
			P. syringae pv.*syringae*	6 ± 0.2[f]
			R. solanacearum	0
			X. campestris pv. *mangiferaeindicae*$_2$	0
			*E. coli***	10 ± 0.9[dj]
			S. typhimurium	17 ± 1.0[eh]
			S. sonnei	2 ± 0.3[l]
			S. epidermidis	10 ± 0.7[dj]
			S. faecalis	4 ± 0.5[a]
Novobiocin			*E. carotovora*$_1$	1 ± 0.2[m]
			E. carotovora$_2$**	0
			P. syringae pv.syringae	0
			R. solanacearum	3 ± 0.3[n]
			X. campestris pv. mangiferaeindicae	5 ± 1.0[a]
			X. campestris pv. mangiferaeindicae$_2$	0
				1 ± 0.3[m]
			ium	7 ± 0.3[b]
				0
			S. epidermidis	22 ± 0.9[g]
			S. faecalis	12 ± 0.4[c]
			E. carotovora$_1$	1 ± 0.2[m]

Table … continued

Rifampicin	*E. carotovora*	2 ± 0.2^{l}
	E. carotovora$_2$**	2 ± 0.4^{l}
	P. syringae pv. syringae	4 ± 0.3^{a}
	R. solanacearum	7 ± 0.8^{b}
	X. campestris pv. mangiferaeindicae	7 ± 0.3^{b}
	X. campestris pv. *mangiferaeindicae*$_2$	0
	*E. coli***	5 ± 0.7^{a}
	S. typhimurium	14 ± 0.6^{c}
	S. sonnei	3 ± 0.3^{n}
	S. epidermidis	30 ± 0.6^{i}
	S. faecalis	10 ± 0.6^{dj}

Legend: * = Numerical data represent the means ± SE of bacterial pathogen inhibition zones. In a column, means followed by the same letter are not significantly different at the 5% level of Tukey's HSD. Strains resistant to all of the tested plant extracts are indicated only in the control trials.

** = Strains resistant to all of the plant extracts. Their inhibition indicated only in the control trials with antibiotics.

0 = No inhibition

^{b} = Maximum inhibition.

Table 5. 3 Plant extract toxicity assay against *Penicillium digitatum, Phytophthora nicotianae* and *Geotrichum candidum*

Plant species	Plant parts tested	Eq. mg gallic acid/g dry weight	Fungal pathogens inhibited by plant extracts	Fungal growth inhibition
Achyranthus aspera L.	Root	6.74	*P. nicotianae*	+
Azadirachta indica A. Juss	Leaf	41.6	*P. digitatum*	+++
Cissus quadrangularis L.	Modified leaf	10.27	*P. nicotianae*	++
Dolichos oliveri Schweinf.	Leaf	24.73	*P. digitatum*	+++
			G. candidum	+++
			P. nicotianae	++
Dolichos oliveri Schweinf.	Root	12.54	*P. nicotianae*	+++
Nicotiana tabacum L.	Stem	12.36	*P. digitatum*	+++
Nicotiana tabacum L.	Seed	11.00	*P. nicotianae*	++
Ruta chalepensis L.	Leaf	18.62	*P. digitatum*	++
Solanum incanum L.	Leaf	17.75	*P. nicotianae*	++
Solanum incanum L.	Seed	57.80	*P. digitatum*	++++
			G. candidum	++++
			P. nicotianae	+++
Solanum nigrum L.	Seed	22.58	*P. nicotianae*	+++
Tribulus terrestris L.	Leaf	17.87	*P. digitatum*	+++
			G. candidum	++
Tamaridus indica L.	Leaf	20.37	*P. digitatum*	++++
Tamarindus indica L.	Seed	44.2	*P. digitatum*	++++
			G. candidum	+++
			P. nicotianae	++++
Withania somnifera L. Dunal	Stem	6.95	*P. nicotianae*	+

Legend: * =Antimicrobial activities of plant extracts is expressed by "+" sign depending on the strength of fungal growth inhibition.
+ = Inhibition present; ++ = Strong inhibition; +++ = Very strong inhibition; ++++ = Exceptional inhibition of the fungal pathogens. Strains resistant to all of the tested plant extracts are indicated only in the control experiment (Table 2b).
* = Strain UPEcr-1; *** = Resistant strain to all of the antibiotics tested, but significantly inhibited by plant extracts.

Table 5.4 Minimum inhibitory c ns of the most efficacious plant extracts evaluated against twelve-test pathogens

Plant extracts solvents[b] and/or antibiotics[c]	Part used	Minimum inhibitory concentration values of plant extracts to twelve different test pathogens[a]		
		Bacterial food-borne pathogens	Bacterial plant pathogens	Fungal pathogens

		Ec	***Ss***	***Se***	***Sf***	***St***	***Erc***	***Ps***	***Rs***	***Xcm***	***Pd***	***Gc***	***Pn***
Acacia seyal Del. var. Seyal	leaf	NE	1:2	1: 4	NE	NE	1:3.5	1:3.5	1:3.5	1:4	NE	NE	NE
Withania somnifera L. Dunal	leaf	NE	NE	1:3	1:3.5	NE	NE	NE	NE	1:3.5	NE	NE	NE
Tagetes minuta L.	leaf	NE	NE	1:3.5	NE	NE	1:3	1:2.5	1:2.5	1:3	NE	1:2.5	NE
Dolichos oliver Schweinf	leaf	NE	NE	1:2.	NE	NE	NE	NE	NE	NE	1:3	1:2.5	1:2
Mirabilis jalapa L.	root	NE	1:2	1:5[d]	NE	1:3	1:3.5	1:2.5	1:2.5	1:3.5	NE	NE	NE
Solanum incanum L.	seed	NE	NE	1:3	NE	NE	NE	NE	NE	1:3	1:3.5	1:3	1:2.5
Tamaridus indica L.	seed	NE	NE	NE	NE	NE	NE	NE	NE	NE	1:2	1:2	1:3
Azadirachta indica A. Juss	leaf	NE	NE	1:2	NE	NE	NE	NE	NE	NE	1:2	NE	NE
Controls:													
Sterilized distilled water	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Methanol	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Acetone	-	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE
Tetracycline	-	1:2	1:2	NE	1:3	1:2.5	1:2	1:3.5	1:3.5	1:3.5	NE	NE	NE
Streptomycin	-	1:2.5	NE	1:2.5	1:2	1:3	1:2.5	1:2	NE	NE	NE	NE	NE
Novobiocin	-	NE	NE	1:4	1:2.5	1:2.5	NE	NE	NE	1:2	NE	NE	NE
Rifampicin	-	1:2	NE	1:5	1:2	1:3	NE	NE	1:2	1:2	NE	NE	NE

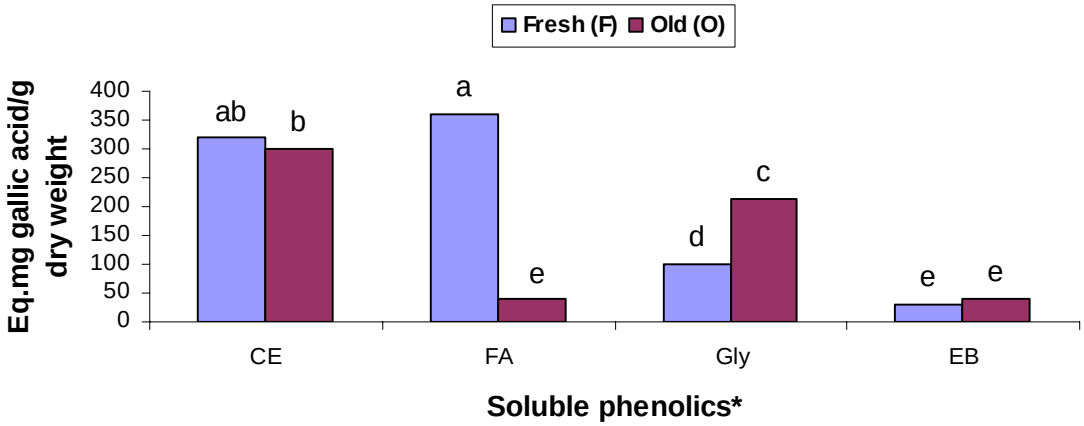
Legend: [a,] =Food-borne and plant pathogens: *Ec = Escherichia coli, Ss = Shigella sonnei, Se = Staphylococcus epidermidis, Sf = Streptococcus faecalis St = Salmonella typhimurium, Erc = Erwinia carotovora,* (UPErc-1). *Ps = Pseudomonas syringae* pv. *syringae, Rs = Ralstonia solanacearum, Xcm = Xanthomonas campestris pv. mangiferaeindicae* (UPXac-1), Pd = *Penicillium digitatum, Gc = Geotrichum candidum*, and *Pn = Phytophthora nicotianae*.

[b] = Sterilized distilled water, methanol and acetone used as negative control.

[c] =Tetracycline, Streptomycin, Novobiocin and Rifampicin as positive controls.

[d] = A plant extract and an antibiotics with higher dilution ratio of MIC efficacy, [e] = NE, not effective.

Thin layer chromatography analyses of fresh and old preparations of *A. seyal* and *W. somnifera* showed significant (P <0.05) variation in their phenolic contents (Fig. 5.1 and 5.2). Fresh preparations of *A. seyal* extracts showed significantly (P <0.05) higher concentrations of free acid (FA) and glycoside (Gly) phenolics than the old preparations (Fig. 5. 1). All fresh preparations of *W. somnifera* extracts showed high concentrations of crude extract (CE), free acids (FA), glycosides (Gly) and ester bound (EB) phenolics content unlike the old preparations (Fig. 5.2). The TLC analyses of *A. seyal* and *W. somnifera* showed the presence of high concentration of Gallic, Ferulic and Syringic acid compounds as a principal component of the phenolic compounds (Fig. 5.3).



Legend: *= Quantification of total soluble phenolic compounds in CE= crude extract, FA= free acid, Gly.= glycoside, and EB= ester bound compounds. Bars with similar letters are not significantly different at Fisher's protected LSD (P <0.05) analysis and t-grouping.

Fig. 5.1. Quantification of total soluble phenolic compounds in fresh and old preparations of *Acacia seyal* extracts.

Table 5.5 Chromatography analysis (R_f values) of plant extracts in selected thin layer chromatography solvent systems

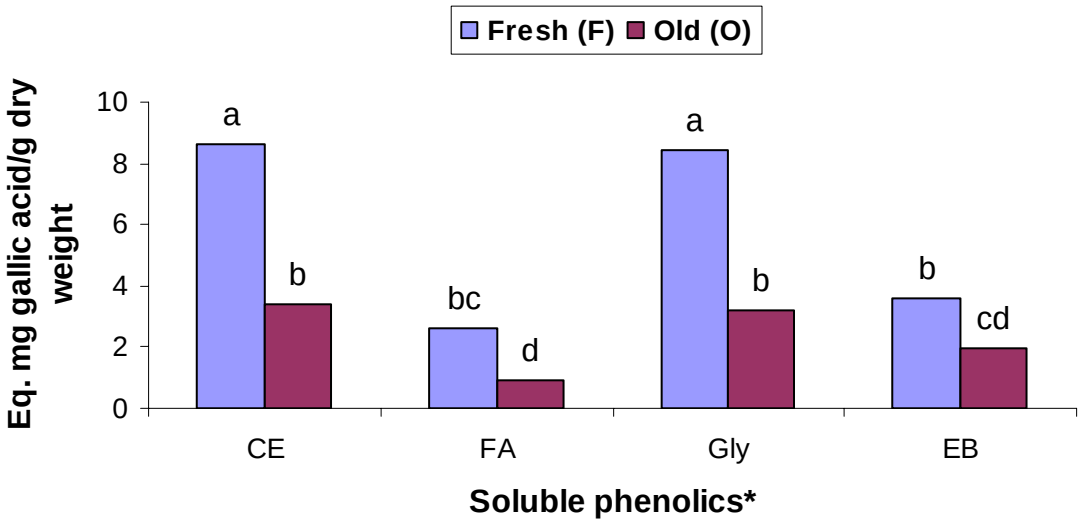
Separation solvent system	R_f values of plant extract compounds Plant material code[a]							
	H_1	I_1	K_1	Q	V_1	X_2	Z	ZA
Toluene/ ethyl acetate (1:1)*	nd	nd	nd	0.27g, 0.33	0.05, 0.4	nd	nd	0.39
Chloroform/ ethanol/ ethyl acetate/ acetone/ water (50:20:20:5:3.5)	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl acetate/ formic acid/ water (3:1:3)	nd	nd	nd	nd	nd	nd	nd	nd
Butanol/ ethanol/ water (5:1:2)*	0.03a, 0.09b, 0.16c, 0.23d, 0.28e, 0.3, 0.34h, 0.36, 0.42j, 0.55k, 0.69, 0.78L	nd	0.03a, 0.08m, 0.14, 0.2p, 0.23d, 0.29f, 0.34h, 0.41, 0.46, 0.53, 0.66n, 0.78L	0.02, 0.07, 0.1, 0.16c, 0.22, 0.32i, 0.45, 0.55k, 0.62, 0.73	0.03a, 0.09b, 0.15, 0.21, 0.32i, 0.42j, 0.52, 0.6, 0.68, 0.78L	0.11o, 0.15, 0.16c, 0.2p	0.03a, 0.11o, 0.19, 0.28e	0.08m, 0.12, 0.2p, 0.27g
Toluene/ acetic acid (4:1)	nd	nd	nd	nd	nd	nd	nd	nd
Chloroform/ ethyl acetate/ formic acid (5:4:1)	nd	nd	nd	nd	nd	nd	nd	nd
Butanol/ acetic acid/ water (6:1:2)	nd	nd	nd	nd	nd	nd	nd	nd
Acetic acid (10%)	nd	nd	nd	nd	nd	nd	nd	nd
Methanol/ butanol/ ethyl acetate/ dichloromethane (1:1:1:1)	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl acetate/ acetic acid/ water (3:1:3)	nd	nd	nd	nd	nd	nd	nd	nd
Ethyl acetate/ acetic acid/ formic acid/ water (50:5.5:5.5:13)*	0.54, 0.66n, 0.74	0.63	0.76	0.65, 0.84, 0.92	0.29f, 0.42j, 0.55k, 0.66n, 0.75	nd	nd	0.93
Chloroform/ acetone/ formic acid (9:2:1)	nd	nd	nd	nd	nd	nd	nd	nd

Legend: [a] = Ratio of front compound migration on TLC in a separation solvent system. The R_f values followed by similar letters may indicate similar compounds in each plant extract.

* = Selected solvent systems for high separation of plant extract compounds to determine the R_f value of bands on the chromatogram developed.

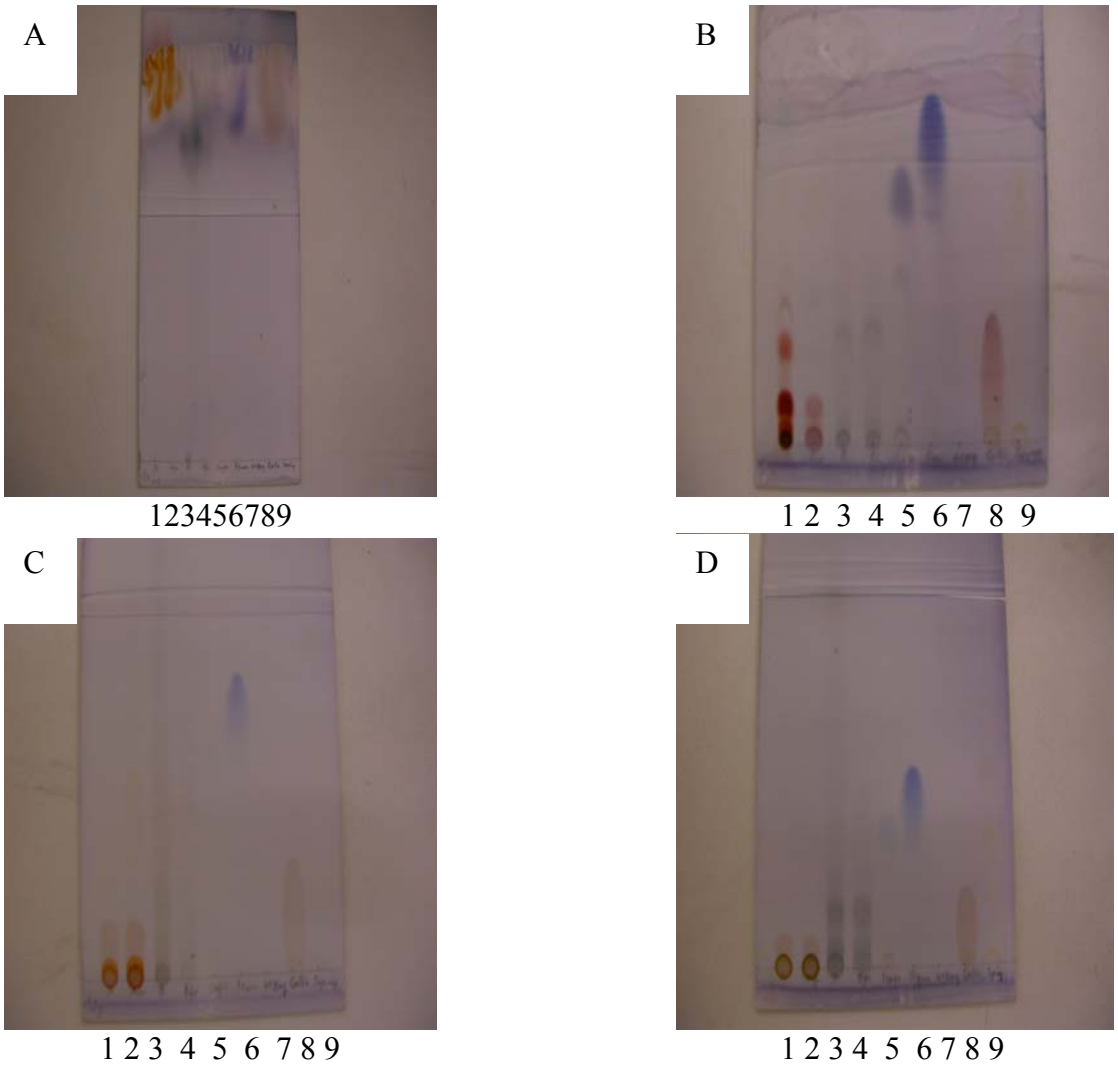
H_1 = *Withania somnifera* L. Dunal; I_1, *Acacia seyal* Del. var. Seyal; K_1, *Dolichos oliveri* Schweinf; Q, *Tagetes minuta* L.; V_1, *Solanum incanum* L.; X_2, *Tamardius indica* L.; Z =*Mirabilis jalapa* L.; *Azadirachta indica* L.

nd = Not determined.



Legend: * = For description refer to figure 5.1.

Fig. 5.2. Quantification of total soluble phenolic compounds in fresh and old preparations of *W. somnifera* extracts.



Legend: The labelled TLC plates are described as follows: A = crude extract (CE), B=free acid (FA), C= glycoside (Gly) and D = ester bound (EB) phenolics. Each plate lane number represented [1= *A. seyal* (fresh) extract, 2 = *A. seyal* (old) extract, 3 = *W. somnifera* (fresh) extract, 4 = *W. somnifera* (old) extract, and standard chemicals [5 = iso-ferulic, 6 = *p*-coumaric, 7 = 4H benzoic acid, 8 = gallic acid and 9 = synergic acid] as a reference compounds, respectively.

Fig. 5. 3. Thin layer chromatography of fresh and old preparations of *Acacia seyal* and *Withania somnifera* leaf extracts.

5.3.5 High performance liquid chromatography of fresh and old plant extracts

High performance liquid chromatography separation, identification and quantification of fresh and old extracts of *Acacia seyal* are depicted in table 5.6–5.9 and *Withania somnifera* in table 5.10–

5.13, respectively. Except the concentration of glycoside and ester bound phenolic compounds of *Acacia seyal* and *W. somnifera* extracts, no significant (P <0.05) variation was observed in CE, FA, and EB phenolics concentrations of fresh and old extract preparations of both plants. *Acacia seyal* extracts exhibited a diverse group of phenolic compounds [gallic acid, 3, 4 Dihydroxy benzoic acid, ferulic acid, caffeic acid, *p*-coumaric acid and salicylic acid]. Higher concentrations of gallic acid were obtained from both fresh (758.05 mg/ml) and old (948.73 mg/ml) preparations of *A. seyal* extracts (Table 5.9).

Table 5.6 Fresh and six month old preparations of *Acacia seyal* crude extract active compounds separation, identification and quantification using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)	Retention time	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)
A	4.16o ± 0.01	22.36j ± 0.00	2.03j ± 0. 00	340	nd	nd	4.12o± 0.01	25.42k ± 0.01	1.94j ± 0.01	280	nd	nd
B	4.81n± 0.01	13.41l± 0.01	1.64k ± 0.01	280	nd	nd	6.13n ± 0.02	6.08mn ± 0.02	0.61l ± 0.02	280	Gallic acid	1.21 ± 0.01
C	6.22m ± 0.02	3.73o ± 0.01	0.54m ± 0.01	280	Gallic acid	1.01± 0.01	11.22m ± 0.01	3.96n ± 0.02	0.33n ± 0.01	280	3,4D	1.55 ± 0.01
D	11.23l ± 0.01	5.62n ± 0.01	0.44n ± 0.01	280	3,4 D	2.21 ± 0.01	15.87l ± 0.01	183.18d ± 0.01	22.66d ± 0.01	280	nd	nd
E	15.9k ± 0.01	33.02i ± 0.02	3.31h± 0.00	280	nd	nd	16.24k± 0.01	128.27g ± 0.14	16.07g ± 0.02	280	nd	nd
F	16.27j ± 0.01	138.2f ± 0.02	16.79d ± 0.01	280	nd	nd	24.16j ± 0.01	151.18f ± 0.01	17.09e ± 0.01	280	nd	nd
G	24.24i ± 0.01	155.02e ± 0.01	17.37c ± 0.00	280	nd	nd	26.34i ± 0.01	56.12i ± 0.02	3.52h ± 0.01	340	nd	nd
H	26.51h± 0.01	55.93h ± 0.01	3.47g ± 0.01	280	nd	nd	27.98h ± 0.01	6.44m ± 0.02	0.36m ± 0.01	280	Ferulic acid	1.11 ± 0.01
I	27.54g ± 0.01	19.12k ± 0.01	0.67l ± 0.01	325	Ferulic acid	0.91 ± 0.01	28.62g ± 0.01	877.92a ± 0.02	86.1a ± 0.02	280	nd	nd
J	28.73f ± 0.01	682.9a ± 0.02	65.78a ± 0.01	280	nd	nd	30.18f ± 0.01	178.53e ± 0.02	16.09f ± 0.02	280	nd	nd
K	30.32e ± 0.01	178.15d ± 0.01	16.62e ± 0.00	280	nd	nd	32.08e ± 0.01	8.42lm ± 0.02	0.67k ± 0.01	280	nd	nd
L	35.44d ± 0.01	216.7b ± 0.01	18.33b ± 0.00	280	nd	nd	35.28d ± 0.01	351.95b ± 0.07	29.23b ± 0.01	280	nd	nd
M	36.57c ± 0.17	68.88g ± 0.01	2.06i ± 0.01	280	nd	nd	36.47c ± 0.01	105.64h ± 0.02	2.40i ± 0.01	280	nd	nd
N	38.61b± 0.01	11.96m ± 0.01	0.53m ± 0.01	280	Salicylic acid	6.25 ± 0.01	38.38b ± 0.01	10.41l ± 0.49	0.38m ± 0.02	280	Salicylic acid	6.31 ± 0.02
O	41.18a ± 0.01	208.04c ± 0.01	16.31f ± 0.00	280	nd	nd	40.98a ± 0.01	326.26c ± 0.02	25.66c ± 0.02	280	nd	nd

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.7 Fresh and six month old preparations of *Acacia seyal* leaf extracts free acid active ingredients separation, identification and quantification of phenolic compounds using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)
A	3.54a ± 0.01	61.63o ± 0.01	4.83j ± 0.04	280	nd	nd	3.54w ± 0.02	67.99v ± 0.04	4.97o ± 0.02	280	nd	nd
B	6.34b ± 0.02	644.25a ± 0.04	124.65a ± 0.02	280	Gallic acid	62.46a ± 0.03	6.11v ± 0.02	79.08t ± 0.02	3.8t ± 0.02	280	Gallic acid	60.63
C	11.51c ± 0.02	40.02v ± 0.02	1.75t ± 0.02	280	3,4 D	2.33d ± 0.03	11.12u ± 0.02	135.31m ± 0.02	6.27h ± 0.02	280	3,4 D	5.37
D	12.43d ± 0.01	19.55x ± 0.02	2.18s ± 0.01	325	nd	nd	12.05t ± 0.02	171.38k ± 0.02	5.79l ± 0.02	280	nd	nd
E	15.26e ± 0.02	102.44j ± 0.01	8.33g ± 0.01	280	nd	nd	15.27s ± 0.02	92.76r ± 0.02	6.47g ± 0.02	280	nd	nd
F	15.91f ± 0.03	465.12c ± 0.01	29.09c ± 0.02	280	nd	nd	15.37s ± 0.04	829.47a ± 0.02	77.14a ± 0.02	280	nd	nd
G	16.26g ± 0.01	292.22d ± 0.02	19.62d ± 0.02	280	nd	nd	16.26r ± 0.02	223.43g ± 0.02	15.89c ± 0.02	280	nd	nd
H	16.36h ± 0.03	545.45b ± 0.02	41.57b ± 0.02	280	nd	nd	16.65q ± 0.02	260.43d ± 0.02	18.78b ± 0.02	280	nd	nd
I	18.19i ± 0.02	44.21s ± 0.02	2.75q ± 0.02	280	nd	nd	17.92p ± 0.02	61.17x ± 0.02	5.87k ± 0.02	280	Nd	nd
J	19.32j ± 0.02	60.85p ± 0.02	2.64r ± 0.01	280	Caffeic acid	1.39e ± 0.02	18.59o ± 0.02	67.04w ± 0.02	4.38s ± 0.02	325	Caffeic acid	280 ± 0.02
K	21.73k ± 0.01	183.29g ± 0.02	4.57l ± 0.02	280	nd	nd	21.86n ± 0.02	84.68s ± 0.02	4.82p ± 0.02	280	nd	nd
L	24.18l ± 0.02	85.13l ± 0.01	4.32m ± 0.02	280	nd	nd	24.21m ± 0.02	233.64f ± 0.02	5.64m ± 0.02	280	nd	nd
M	25.85m ± 0.02	38.65w ± 0.01	3.04o ± 0.02	325	P-coumaric acid	5.74c ± 0.04	25.68l ± 0.02	75.47u ± 0.02	5.38n ± 0.02	325	P-coumaric acid	3.56 ± 0.03
N	26.25n ± 0.02	154.12h ± 0.02	7.21h ± 0.02	280	nd	nd	26.03k ± 0.02	131.83o ± 0.02	7.64f ± 0.02	280	nd	nd
O	27.02o ± 0.02	185.61f ± 0.02	8.95f ± 0.02	325	Ferulic acid	1.12f ± 0.01	27.04j ± 0.02	187.75j ± 0.02	10.26e ± 0.02	325	Ferulic acid	4.2 ± 0.02
P	27.57p ± 0.02	42.47u ± 0.02	5.27i ± 0.01	280	nd	nd	27.59i ± 0.02	390.56b ± 0.02	13.13d ± 0.02	280	nd	nd
Q	28.62q ± 0.02	224.82e ± 0.01	19.38e ± 0.03	280	nd	nd	29.21h ±0.02	105.09q ± 0.02	5.81l ± 0.02	280	nd	nd
R	29.55r ± 0.02	80.82n ± 0.02	2.75q ± 0.02	280	nd	nd	30.72g ± 0.02	129.31p ± 0.02	4.74q ± 0.02	280	nd	nd
S	32.09s ± 0.02	46.03r ± 0.02	2.17s ± 0.02	280	nd	nd	32.14f ± 0.02	133.21n ± 0.02	6.11i ± 0.02	280	nd	nd
T	35.27t ± 0.01	87.29k ± 0.02	4.64k ± 0.03	280	nd	nd	35.33e ± 0.03	257.78e ± 0.02	5.93j ± 0.02	280	nd	nd
U	36.34u ± 0.02	55.72q ± 0.02	2.84p ± 0.02	340	nd	nd	36.4d ± 0.02	215.42h ± 0.02	5.93j ± 0.02	280	nd	nd
V	38.11v ± 0.03	42.83t ± 0.01	1.15u ± 0.01	280	Salicylic acid	6.23b ± 0.03	38.98c ± 0.01	208.44i ± 0.02	3.65v ± 0.03	280	Salicylic acid	2.58
W	40.97w ± 0.03	82.21m ± 0.02	2.64r ± 0.03	280	nd	nd	41.06b ± 0.02	314.35c ± 0.02	4.69r ± 0.02	280	nd	nd
X	45.43x ± 0.01	104.83i ± 0.02	3.66n ± 0.02	280	nd	nd	45.82a ± 0.02	148.29l ± 0.02	3.72u ± 0.02	280	Nd	nd

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.8 Fresh and six month old preparations of *Acacia seyal* leaf extract glycoside active ingredients separation, identification and quantification using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)
A	6.1t± 0.02	7.74t ± 0.02	1.08t ± 0.02	280	nd	nd	4.3j ± 0.01	244.95d ± 0.04	4.74f ± 0.02	280	nd	nd
B	11.21s ± 0.02	42.6s1± 0.02	2.84r ± 0.01	280	3,4 D	1.67d ± 0.02	6.03i ± 0.02	20.46j ± 0.03	1.34j ± 0.02	280	Gallic acid	23.74 ± 0.01
C	15.89r ± 0.02	812.95i ± 0.02	86.56f ± 0.03	280	nd	nd	15.92h ± 0.02	1694.77a ± 0.02	209.18a ± 0.02	280	nd	nd
D	16.23q ± 0.02	625.46j ± 0.02	66.94h ± 0.03	280	nd	nd	-	-	-	-	-	-
E	16.63p ± 0.02	331.85o ± 0.01	29.14l ± 0.04	280	nd	nd	-	-	-	-	-	-
F	19.16o ± 0.02	51.14r ± 0.02	2.64s ± 0.03	280	Caffeic acid	6.36b ± 0.02	-	-	-	-	-	-
G	21.99n ± 0.02	53.24q ± 0.02	4.57q ± 0.02	280	nd	nd	-		-	-	-	-
H	24.15m ± 0.02	1135.75g ± 0.03	121.19d ± 0.01	280	nd	nd	-	-	-	-	-	-
I	25.28l ± 0.02	418.91n ± 0.02	21.77o ± 0.02	280	P-coumaric acid	4.73c ± 0.03	-	-	-	-	-	-
J	25.75k ± 0.02	484.16l ± 0.01	32.13k ± 0.01	280	nd	nd	-	-	-	-	-	-
K	26.35j ± 0.03	603.09k ± 0.02	39.22j ± 0.02	340	nd	nd	26.06g ± 0.02	61.81f± 0.04	5.87e ± 0.01	280	Nd	nd
L	27.63i ± 0.03	239.79p ± 0.02	16.64p ± 0.02	280	Ferulic acid	1.06e ± 0.06	-	-	-	-	-	-
M	28.53h ± 0.04	13958.32a ± 0.02	1230.15a ± 0.03	280	nd	nd	28.69f ± 0.02	1420.74b ± 0.02	144.65b ± 0.03	280	nd	nd
N	30.16g ± 0.02	1229.36f ± 0.02	99.92e ± 0.02	280	nd	nd	30.28e ± 0.03	28.67i ± 0.02	2.65h ± 0.02	280	nd	nd
O	35.18f ± 0.03	13621.36b ± 0.02	954.11b ± 0.02	280	nd	nd	32.19d ± 0.02	52.31g ± 0.02	4.37g ± 0.03	280	nd	nd
P	36.43e ± 0.01	4644.51d ± 0.02	84.41g ± 0.02	280	nd	nd	35.39c ± 0.02	259.29c ± 0.02	21.45c ± 0.04	280	nd	nd
Q	38.15d ± 0.02	430.45m ± 0.02	25.69m ± 0.02	280	Salicylic acid	13.17a ± 0.02	36.59b ± 0.01	44.34h ± 0.03	1.43i ± 0.03	280	nd	nd
R	40.92c ± 0.02	9982.26c ± 0.06	656.39c ± 0.02	280	nd	nd	41.11a ± 0.02	177.11e ± 0.03	14.44d ± 0.02	280	nd	nd
S	43.08b ± 0.01	2016.62e ± 0.05	48.79i ± 0.02	280	nd	nd	-	-	-	-	-	-
T	47.23a ± 0.02	1130.93h ± 0.02	24.84n ± 0.03	280	nd	nd	-	-	-	-	-	-

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.9 Fresh and six month old preparations of *Acacia seyal* leaf extracts ester bound phenolic compounds active ingredients separation, identification and quantification using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)
A	6.35o ± 0.02	6052.87a ± 0.02	1131.75a ± 0.03	280	Gallic acid	758.05a ± 0.03	6.34o ± 0.02	7497.46a ± 0.02	1390.06a ± 0.02	280	Gallic acid	948.73 ± 0.93
B	11.09n ± 0.02	4.33o± 0.02	0.32o± 0.01	280	3, 4 D	1.68d ± 0.01	11.09n ± 0.01	3.54o ± 0.02	0.37o ± 0.02	325	3, 4 D	1.35 ± 0.04
C	15.90m ± 0.02	404.57g ± 0.03	33.51g ± 0.01	280	nd	nd	15.87m ± 0.02	2313.66b ± 0.03	270.64b ± 0.02	280	nd	nd
D	16.64l ± 0.02	608.59e ± 0.02	54.1e ± 0.02	280	nd	nd	16.24l ± 0.02	214.71k± 0.02	17.07k ± 0.02	280	nd	nd
E	19.91k± 0.02	25.53n ± 0.02	2.88 m± 0.02	340	Caffeic acid	9.23b ± 0.02	18.84k ± 0.02	8.78 n± 0.02	0.85n ± 0.02	340	Caffeic acid	9.91 ± 0.03
F	24.16j ± 0.02	192.48l ± 0.02	21.32i ± 0.02	280	nd	nd	24.16j ± 0.02	280.86j ± 0.02	28.12g ± 0.02	280	nd	nd
G	25.79i ± 0.02	270.20h ± 0.02	22.97h ± 0.02	280	nd	nd	26.34i ± 0.03	31.12m ± 0.02	1.64m ± 0.02	280	nd	nd
H	27.61h ± 0.02	39.83m ± 0.01	2.66 n± 0.02	340	Ferulic acid	0.72e ± 0.55	27.55h ± 0.02	78.61l ± 0.03	5.29l ± 0.02	340	Ferulic acid	1.16 ± 0.12
I	28.61g ± 0.01	1027.02c ± 0.02	98.8c ± 0.01	280	nd	nd	28.60g ± 0.02	1330.92d ± 0.03	124.58d ± 0.02	280	nd	nd
J	30.16f ± 0.01	201.05k ± 0.03	18.31j ± 0.02	280	nd	nd	30.16f ± 0.02	281.15i ± 0.03	24.06i ± 0.03	280	nd	nd
K	31.74e ± 0.02	568.67f ± 0.02	48.26f ± 0.02	280	nd	nd	31.73e ± 0.02	679.64f ± 0.02	59.34f ± 0.02	280	nd	nd
L	35.24d ± 0.02	1333.35b ± 0.02	108.52b ± 0.02	280	nd	nd	35.24d ± 0.02	1834.86c ± 0.02	148.03c ± 0.03	280	nd	nd
M	38.72c ±0.02	246.65i ± 0.03	17.64k ± 0.02	280	Salicylic acid	3.04c ± 0.03	38.74c ± 0.02	347.67h ± 0.02	24.34h ± 0.03	280	Salicylic acid	4.11 ± 0.02
N	40.93b ± 0.01	850.33d ± 0.03	61.67d ± 0.02	280	nd	nd	40.96b ± 0.02	1172.71e ± 0.03	83.86e± 0.03	280	nd	nd
O	43.92a ± 0.02	238.06j ± 0.02	12.97l ± 0.02	280	nd	nd	43.93a ± 0.02	381.36g ± 0.02	17.67j ± 0.02	280	nd	nd

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.10 Identification and quantification of fresh and six months old crude extracts of *Withania somnifera* using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)
A	2.09g ± 0.03	2.39g± 0.02	0.46e ± 0.03	280	nd	nd	3.79e ± 0.02	57.19a ± 0.02	1.87a ± 0.02	280	nd	nd
B	3.76f ± 0.02	41.49b ± 0.02	1.85b ± 0.04	280	nd	nd	9.10d ± 0.02	1.69d ± 0.02	0.26d ± 0.02	280	nd	nd
C	5.31e ± 0.02	198.65a ± 0.03	30.89a ± 0.02	280	nd	nd	-	-	-	-	-	-
D	6.36d ± 0.02	2.54f ± 0.04	0.48e ± 0.03	280	nd	nd	-	-	-	-	-	-
E	26.36c ± 0.02	5.54d ± 0.04	0.69c ± 0.02	280	nd	nd	28.72c ± 0.02	3.66c ± 0.02	0.31c ± 0.02	280	nd	nd
F	35.29b ± 0.03	5.44e ± 004	0.35f ± 0.04	280	nd	nd	35.65b ± 0.02	11.21b± 0.02	0.40b ± 0.02	280	nd	nd
G	40.96a ± 0.02	18.94c ± 0.02	0.56d ± 0.03	280	nd	nd	41.16a ± 0.02	3.65c ± 0.02	0.30c ± 0.01	280	nd	nd

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.11 Identification and quantification of free acid by high performance liquid chromatography from fresh and six months old crude extracts of *Withania somnifera*

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)
A	3.52k ± 0.02	52.28d ± 0.02	4.56c ± 0.02	280	nd	nd	3.53j ± 0.02	50.28d ± 0.02	4.54c ± 0.02	280	nd	nd
B	5.29j ± 02	11.31h± 0.02	1.82f ± 0.02	280	nd	nd	6.34i ± 0.02	2.00j ± 0.02	0.39g ± 0.02	280	nd	nd
C	11.32i ± 0.03	3.56k ± 0.02	0.44i ± 0.02	280	3, 4 D	1.39 ± 0.01	11.13h ± 0.01	5.36h ± 0.02	0.53f ± 0.02	280	3, 4 D	2.01± 0.02
D	20.86h ± 0.02	672.16a ± 0.03	5.21b ± 0.03	280	nd	nd	18.18g ± 0.02	512.59a ± 0.02	5.19b ± 0.02	280	nd	nd
E	23.54g ± 0.03	77.17c ± 0.02	2.12d ± 0.02	280	nd	nd	20.87f ± 0.02	494.57b ± 0.02	5.42a ± 0.02	280	nd	nd
F	27.43f ± 0.02	99.39b ± 0.02	9.42a ± 0.02	325	nd	nd	23.01e ± 0.02	72.92c ± 0.02	2.99d ± 0.02	280	nd	nd
G	29.25e ± 0.01	28.43e ± 0.01	1.95e ± 0.02	280	nd	nd	27.78d ± 0.03	2.38i ± 0.02	0.25i ± 0.02	325	nd	nd
H	33.19d ± 0.3	5.31j ± 0.02	0.35j ± 0.01	280	nd	nd	32.06c ± 0.03	5.64g ± 0.02	0.29h ± 0.02	280	nd	nd
I	38.37c ± 0.02	6.37i ± 0.02	0.45i ± 0.02	280	Salicylic acid	6.09 ± 0.02	38.37b ± 0.02	18.31e ± 0.01	0.31h ± 0.02	280	Salicylic acid	6.91± 0.02
J	39.79b ± 0.02	11.67g ± 0.02	0.94g ± 0.01	280	nd	nd	39.78a ± 0.02	10.55f ± 0.02	0.74e ± 0.01	280	nd	nd
K	43.55a ± 0.02	15.41f ± 0.02	0.78h ± 0.02	280	nd	nd	-	-	-	-	-	-

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.12 Fresh and six months old preparations of *Withania somnifera* extract glycoside phenolic compounds active ingredients separation, identification and quantification using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ml)
A	4.04g ± 0.02	129.97b ± 0.01	2.33a ± 0.01	340	nd	nd	3.7 ± 0.01	71.83 ± 0.02	2.68 ± 0.01	340	nd	nd
B	6.05f ± 0.02	15.29d ± 0.02	2.12b ± 0.03	280	nd	nd	5.11 ± 0.01	24.58 ± 0.02	1.52 ± 0.02	280	nd	nd
C	11.21e± 0.01	2.08g ± 0.02	0.31f ± 0.02	280	3, 4 D	8.09	10.97 ± 0.02	3.74 ± 0.01	0.45 ± 0.03	280	3, 4 D	1.46 ± 0.02
D	19.24d ± 0.03	3.75f ± 0.02	0.43g ± 0.01	325	Caffeic acid	5.66	-	-	-	-	nd	nd
E	35.63c ± 0.02	24.02c ± 0.02	0.77d ± 0.01	280	nd	nd	-	-	-	-	nd	nd
F	37.37b ± 0.02	164.21a ± 0.02	1.26c ± 0.02	280	nd	nd	36.92 ± 0.03	7.77 ± 0.02	0.57 ± 0.01	280	nd	nd
G	40.15a ± 0.03	10.59e ± 0.02	0.59e ± 0.02	280	nd	nd	39.75 ± 0.02	8.19 ± 0.02	0.56 ± 0.02	280	Salicylic acid	5.86 ± 0.03

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

Table 5.13 Fresh and six months old preparations of *Withania somnifera* extracts ester bound phenolic compounds active ingredients separation, identification and quantification using high performance liquid chromatography

HPLC peak (code)	Crude extract preparations											
	Fresh						Six months old					
	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)	Retention time (RT)	Area of the peak	Height of the peak	Max. absorption (nm)	Compound name	[ ] (mg/ ml)
A	3.52k ± 0.01	39.44a ± 0.02	4.20a ± 0.01	340	nd	nd	3.53i ± 0.02	41.56a ± 0.02	4.43a± 0.02	280	nd	nd
B	6.32j ± 0.02	1.88k ± 0.02	0.35f ± 0.02	280	Gallic acid	0.00	6.36h ± 0.03	22.76b ± 0.02	4.44a ± 0.02	280	Gallic acid	0.00
C	7.87i ± 0.02	6.53h ± 0.01	1.07c ± 0.02	280	nd	nd	7.87g ± 0.02	7.61e ± 0.01	1.26b ± 0.01	280	nd	nd
D	11.08h ± 0.01	9.63f ± 0.02	1.08c ± 0.01	280	3, 4 D	3.81c± 0.01	11.08f ± 0.01	11.34d ± 0.01	1.21c ± 0.01	280	3, 4 D	2.90a ± 0.03
E	18.19g ± 0.02	9.75e ±0.02	1.07c ± 0.02	280	nd	nd	18.22e ± 0.01	5.63h ± 0.02	0.61d ±0.02	280	Vanilic acid	1.28b ± 0.02
F	19.01f ± 0.01	12.43d ± 0.02	1.46b ± 0.02	325	Caffeic acid	9.54a ±0.02	-	-	-		-	-
G	25.29e ± 0.02	8.49g ± 0.02	0.86d ± 0.02	280	nd	nd	-	-	-		-	-
H	27.84d ± 0.01	1.92j ± 0.02	0.19g ± 0.02	340	Ferulic acid	0.00	27.51d± 0.02	4.95i ± 0.01	0.44e ± 0.02	325	Ferulic acid	0.00
I	33.13c ± 0.02	16.89b ± 0.02	0.75e ± 0.03	340	nd	nd	31.92c ± 0.02	7.44f ±0.02	0.40f ± 0.02	280	nd	nd
J	39.81b ± 0.02	13.5c ± 0.02	1.02c ± 0.06	280	Salicylic acid	6.42b ± 0.02	35.97b ± 0.02	12.82c ± 0.02	0.38f ± 0.02	280	nd	nd
K	41.91a ± 0.01	3.96i ± 0.02	0.29f ± 0.01	325	nd	nd	41.19a ± 0.01	6.44g ± 0.02	0.44e± 0.01	280	nd	nd

Legend: In each column, means with the same letter are not significantly different. Unidentified chemical compounds in the phenolics family are designated by (nd) = not determined.

5.4 DISCUSSION

Plants have great potential to synthesize aromatic substances, most of which are phenolics and their oxygen-substituted derivatives (Cowan, 1999). The search for potential ethnobotanical compounds from plant material requires intensive *in vitro* screening of plant extracts. In this study, 37 extracts from 23 plant species collected from three citrus growing regions of Ethiopia were screened for their antimicrobial activity. Twenty-one plant extracts from 13 species (56%) showed some degree of antimicrobial activity to at least one of the pathogens challenged. Seven of these species [*A. aspera*, *T. terrestris*, *W. somnifera*, *A. seyal*, *D. oliver*, *C. quadrangularis* and *M. jalapa*] were, to our knowledge, not previously reported for their ethnobotanical potential. According to Rojas et al. (2003), this report indicates the high therapeutic potential of tropical flora where numerous species are yet to be documented and investigated.

Some plant extracts demonstrated strong selective antifungal and antibacterial activities, which may indicate their potential as antimicrobial products. *In vitro* tests showed eight of these extracts [leaf extracts of *D. oliveri*, *T. minuta*, *R. chalepensis*, *S. incanum* and *A. indica*; seed extracts of *S. incanum* and root extracts of *A. aspera* and *A. seyal*] demonstrated antimicrobial activity to both fungal and bacterial pathogens. A further nine [leaf extracts of *T. terrestris* and *T. indica*; stem extracts of *N. tabacum* and *W. somnifera* and *C. quadrangularis*; seed extracts of *S. nigrum*, *N. tabacum* and *T. indica* and root extracts of *D. oliver*] exhibited selective antifungal activity only, and four [leaf extracts of *A. aspera*, *W. somnifera*, *A. seyal* and root extracts of *M. jalapa*] showed selective antibacterial activity. The plant extracts tested in this study were highly effective against the Gram-positive bacterium *S. epidermidis* compared to the Gram-negative bacteria. Differences in the antimicrobial effect of the plant extracts tested against Gram-positive and Gram-negative bacteria may be due to differences in permeability barriers. Similar reports indicate susceptibility of the Gram-positive bacterium *S. epidermidis* to other plant extracts such as *Cordia curassavica, Lantana achyranthifolia* and *Lippia graveolens* (Hernandez et al., 2003) and seed extracts of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004). In this study, the inhibition halo formed by the root extract of *M. jalapa* showed high inhibitory activity against *S. epidermidis*. The inhibitory activity found in this study was more pronounced than that reported by Hernandez et al. (2003) when he evaluated certain plants for their antimicrobial activities against several bacterial pathogens. The inhibitory effect of *M. jalapa* was at a similar level of effectiveness as Rifampicin.

The antimicrobial activity of plant extracts depends on the type and amount of phenolics present in the plant tissue and the pathogen's inherent resistance (Martini et al., 2004). Quantitative

information obtained from the Folin-Ciocalteu method provides information about the amount of soluble phenolics in the plant extract. *A. seyal*, unlike any other plant extract tested, had a high content of equivalent mg Gallic acid/g dry weight both in fresh and old extract preparations. The result is also supported by HPLC analyses due to the presence of high concentration of gallic acid, para-coumaric acid, ferulic acid, caffeic acid, 3,4 dihydroxy benzoic acid and salicylic acid. This may attribute to its strong antimicrobial activity as determined when oxidized to natural aromatic polymer compounds (cinnamic acid derivatives) to inhibit auto-oxidation of oils and fats in the host tissue (Cowan, 1999). It could be due to better extraction by the methanolic solvent system as compared to water (data not indicated here) (Ozkan et al., 2004).

Although there was a significant (P <0.05) difference in the phenolic concentration of fresh and old preparations of *W. somnifera* extracts, the total phenolic concentration was very low unlike *A. seyal* extracts. A result from HPLC analysis has also supported this fact that phenolic compounds are present at very low concentrations. According to Rahman et al. (1991), the majority of compounds in *W. somnifera* extracts are withanolides, glycowithanolides with a very low proportion of alkaloids (0.2%). These results validate the importance of other compounds in plant extracts antipathogenic activity.

The R_f value of the selected plant extracts depicted on the TLC chromatogram correspond with the value of different phenolics that may be involved in the antipathogenic activity of the plant material (Block et al., 1958; Smith, 1960). Plant extracts that exhibit broad-spectrum *in vitro* activity against microbial pathogens, i.e. *W. somnifera*, *A. seyal* and *M. jalapa*, showed no visible band formation in one or more of the separation solvent systems under 254 or 366 nm. According to Harborne (1964), measurements of the ultraviolet absorption spectrum may be affected by etherification and/ or glycosylation of the hydroxyl group to detect phenolics under given UV light spectra. Alternatively, this could be an indication for the presence of protein conjugated antimicrobial compounds with non-specific and/ or synergistic interactions in the system (Cowan, 1999).

The MIC value of the eight plant extracts selected in this study ranged between 1:1 and 1:5 indicating the strength of their active compounds. According to Cruickshank and Perrin (1964), toxic phenolic compounds present in such low concentrations may have a stimulatory effect on pathogen growth. In this study, some plant extracts were ineffective against some of the test pathogens used. Amongst these, *E. coli* and one strain of *E. carotovora* (UPErc-2) proved highly resistant to all plant extracts tested. This characteristic may be attributed to their similar replication

origin, being under the same family, Enterobacteriaceae (Takeda et al., 1982). Similar results were reported for *E. coli* by Hernandez et al. (2003), which described possible development of resistance by the bacteria. To our knowledge, resistance development by *E. carotovora*$_2$ has not been reported in previous studies. On the other hand, the *X. campestris*$_2$, which showed resistance to all antibiotics tested, was significantly inhibited by *A. seyal*, *W. somnifera*, *T. minuta* and *M. jalapa*. To our knowledge this is also the first report of antimicrobial activity of these plant extracts against the pathogen. Although the dilution ratio and antimicrobial efficacy varies from one plant to another, about 65% of the plant extracts were found effective against several bacterial strains screened.

Preliminary *in vivo* tests with some selected plant extracts showed remarkable control of fruit decay due to *P. digitatum* in South Africa (data not included in this study), which may indicate the promising potential of the plant extracts for postharvest disease control, especially for the citrus industry. Future research advances on this aspect is important to determine the active chemical compounds of these plant extracts for commercial use.

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