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In vitro antimicrobial assay of selected medicinal plants against medically important plant and food-borne pathogens

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Abstract
The traditional use of plant materials for treatment of human ailments dates back to prehistoric times. In this study, the antipathogenic activity of 37 extracts of 23 plant species, which originally were used to control citrus pre-harvest diseases and human aliments are reported. An *in vitro* screening for antimicrobial activity was conducted against seven plant pathogens and five food-borne pathogens. In total, 21 extracts from 13 plant species exhibited some degree of antimicrobial activity to at least one pathogen. Of these, six species, i.e. *Achyranthes aspera***,** *Tribulus terrestris*, *Acacia seyal*, *Dolichos oliveri*, *Cissus quadrangularis* and *Mirabilis jalapa* are species with no known previous reports of antimicrobial activity against the tested pathogens. The minimum inhibitory concentration (MIC) of eight selected plant extracts were found to be between 1:2 and 1:5 (v: v). 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. Further elucidation of the active ingredients of these plant extracts is commendable for practical utility and control of resistance development.

Keywords: Antimicrobial activity, Antifungal activity, Antibacterial activity, Plant extracts, Plant pathogen, Human pathogens.

Introduction
Plants are indispensable sources of medicinal importance used in both Western type pharmaceutical products and local medicinal preparations. The traditional use of plant materials for treatment of human ailments dates back to prehistoric times (Cowan, 1999) [10]. According to the World Health Organization, 80% of the world's population relies on traditional medicines to meet their daily health requirements (World Health Organization, 2009) [40]. However, from the estimated 250 000 species of higher plants described to date, only 5-15% have been studied for their potential therapeutic value (Steep, 2004; Goel and Sharma, 2014) [34, 17].
Ethiopia is a tropical country with a high floral diversity and endemism (Brenan, 1978) [8]. According to Teweldebirhan, there are about 7 000 species of higher plants in Ethiopia, of which 12% are endemic (Tewoldebirhan, 1991) [36]. More than 80% of the Ethiopian population depends on traditional remedies (Dawit and Ahadu, 1993) [14], derived mainly (95%) from plant material (Dawit, 1986) [13]. 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.
The use of plant derived antimicrobial agents have great potential in reducing the dependence on synthetic antibiotics and minimizing the chance for development of antibiotics resistance in food and other spoilage microorganisms. Several researchers have studied ethnobotanical (Giday, 2001; Desissa and Binggeli, 2002; Hernandez *et al*., 2003; Abiyot *et al*., 2006; Tigist *et al*., 2006; Abera, 2014) [16, 15, 19, 3, 38, 2], phytochemical (Abegaz and Woldu, 1991; Dagne and Abate, 1995) [1, 12] and antimicrobial activities (Habtemariam *et al*., 1993; Mammed, 2002) [18, 22] of a variety of medicinal plants. The antimicrobial activity of plant oils and extracts has formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies (Regnier *et al*., 2012; Aumeeruddy-Elalfi *et al*., 2015) [29, 4]. Plant-derived antioxidant compounds may potentiate the body's antioxidant and anti-inflammatory defense mechanisms or act as antioxidants (Sagnia *et al*., 2014) [31]. Such products have proved to be effective against a range of human and foodborne pathogens.

In this work we report 37 extracts of 23 medicinal plants from three citrus growing regions in Ethiopia for their antimicrobial activity against major plant and food-borne pathogens.

Materials and Methods

Plant material

Twenty-three cultivated and wild medicinal plant spp. were collected from three citrus growing areas in Ethiopia namely, Somalia, Oromia and Amhara Regional States (Table 1). 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 Haromaya University, Ethiopia, for identification. Identities of plant spp. were confirmed by Dr. Lisanework Nigatu from the Department of Plant Science, and voucher specimens were deposited. 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 in the dark until further use. Samples were brought to the Plant Pathology Laboratories, University of Pretoria, South Africa according to quarantine handling and processing procedures (Plant material import permitP0017192, the Department of Agriculture, Forestry and Fisheries. Plant destruction protocols were followed during and after processing of samples at this ISO 17025 accredited facility (South African National Accreditation System No. T0148). Plant rights and traditional knowledge are protected within the University's ethical criteria (UP requirements Code of Ethics for Research Rt 429/99) and were followed accordingly.

Plant material extraction

Two solvents, i.e. methanol/acetone/water (7:7:1 v:v) (Regnier and Macheix, 1996) [30] and distilled water alone (Bautista-Banos *et al*., 2003) [6] 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 briskly using a VM-300 vortex mixer (Labotec, Johannesburg, South Africa.) and placed on a rotary shaker (Stuart Scientific, United Kingdom) at 170 rpm for an hour. Samples were centrifuged in a micro-centrifuge (Sigma, Germany) at 5000 x g for 10 min. The third extraction was placed overnight on the rotary shaker (Sigma) and centrifuged. 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 sterilized through a hypodermic syringe filter (0. 22 µm pore size) into sterilized containers. Suspensions were either used immediately or kept at -4 ºC for later use. The phenolic content of each plant extract was determined using Folin Ciocalteu method (Bray and Thorpe, 1954) [7].

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 collected 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 (STD1) (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 spore concentration of 10^4 spores ml^{-1} was prepared using a haemacytometer and a preparation containing 10^4 spores ml^{-1}. 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 for 24 h at 25 ºC were determined using a Petroff-Hausser counting chamber. A standardized concentration of 10^8 cells ml^{-1} was used in all subsequent tests.

In vitro antimicrobial assay

Two assay techniques, i.e. the agar plate (Thornberry, 1950) [37] with some modification and agar well diffusion assay (Barbour *et al*., 2004) [5] were used to evaluate the antimicrobial activity of plant extracts against fungal and bacterial pathogens.

Agar plate technique

Plant extract amended agar plates were used. This method was selected to screen plant extracts for their efficacy against the fungal pathogens *P. digitatum*, *G. candidum* and *P. nicotianae*. Aliquots of 9 ml PDA were made up in test tubes, autoclaved and cooled down to 50 ºC, after which one ml of the plant extract was added aseptically, poured into a Petri plate (90 mm diameter) and swirled to cover the base. Fungal agar blocks (3 x 3 mm) cut from the cultures prepared 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[24] 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.

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}. Aliquots of 100 µl were spread evenly onto individual STD1 agar plates. On each plate, four equidistant wells were made in the agar with a 0.5 mm diameter sterilized 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, Johannesburg) (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 sterilized 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.

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) [5]. Eight plant extracts that showed a wide range of antimicrobial activity were used for further tests. One ml of each plant extract 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 inocula. 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 sterilized distilled water were regarded as negative controls, whereas antibiotics were incorporated as positive controls. Experiments were performed in triplicate.

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 test at $P < 0.05$ level of significance.

Results and Discussion

Twenty-one plant extracts from 13 species (56%) showed some degree of antimicrobial activity to at least one of the pathogens challenged (Table 1 and 2). Six of these species (*A. aspera*, *T. terrestris*, *A. seyal*, *D. oliveri*, *C. quadrangularis* and *M. jalapa*) were, to our knowledge, not previously reported for their ethnobotanical potential. According to (Goel and Sharma, 2014) [17], this report indicates the high therapeutic potential of tropical flora where numerous species are yet to be documented and investigated.

Eight of the extracts showed broad-spectrum activity against both fungal and bacterial pathogens (Table 1 and 2). The most effective plant species regarding antimicrobial activity were found in Hursso, Somalia Regional State. Plant leaves were found to be more inhibitory (44.2%), followed by stem (27.9%) and root (14%) extracts. 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 pathogens challenged. The bacterial inhibition zones were in the range of 4-30 mm. Maximum inhibition was detected with *M. jalapa* against *S. epidermidis*, which showed better efficacy compared to other plant materials (Aumeeruddy-Elalfi *et al*., 2015) [4]. The latter pathogen was found to be the most susceptible to over 80% of plant extracts evaluated (Table 1). Similar susceptibility results were reported on *Staphylococcus spp* when lichen preparations were applied (Indhumathi and Mohandass, 2014) [20]. Two species of bacterial pathogens [*E. carotovora*$_1$ and *E. coli*] showed resistance to all 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$_1$ was not affected by streptomycin. Similarly, *R. solanacearum* showed resistance to streptomycin, whereas *E. carotovora*$_2$, *P. syringae* pv. *syringae* and *S. sonnei* were resistant to novobiocin (Table 2). Sterilized distilled water, methanol and acetone did not have any inhibitory effect on the pathogens.

Some plant extracts demonstrated strong selective antifungal and antibacterial activities, which may indicate their potential as antimicrobial products. *In vitro* tests showed that 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 with exceptional antifungal activity of *S. incanum* L. (Table 1 and 2). Similar results have also indicated the broad spectra nature of *S. incanum* against bacterial and fungal pathogens (Indhumathi and Mohandass, 2014; Mwonjoria *et al*., 2014) [20, 24], anticancer, antipyretic, antinociceptive, hypoglycemic and an orexic effects (Barbour *et al*., 2004) [5]. A further nine [leaf extracts of *T. terrestris* and *T. indica*; stem extracts of *N. tabacum*, *W. somnifera* and *C. quadrangularis*; seed extracts of *S. nigrum*, *N. tabacum* and *T. indica* and root extracts of *D. oliveri*] exhibited selective antifungal activity.

Table 1: 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}
Achyranthes aspera L.	Leaf	7.97	*S. epidermidis*	05 ± 0.5^{a}
Achyranthes 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]
Solanum incanum L.	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]
			P. syringae pv. *syringae*	10 ± 0.6[dj]
			R. solanacearum	09 ± 1.0[j]
			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			*Erwinia carotovora*$_1$	9 ± 0.7[j]
			E. carotovora$_2$**	7 ± 0.9[b]
			Pseudomonas syringae pv. *syringae*	17 ± 1.3[eh]
			Ralstonia solanacearum	17 ± 0.8[eh]
			Xanthomonas campestris pv. *mangiferaeindicae*	18 ± 1.4[eh]
			X. campestris pv. *Mangiferaeindicae*$_2$	0
			*Escherichia coli***	7 ± 0.9[f]
			Salmonella typhimurium	11 ± 0.4[d]
			Shigella sonnei	9 ± 0.8[j]
			Staphylococcus epidermidis	2 ± 0.4[l]
			Strepotcoccus 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
			*E. coli***	1 ± 0.3[m]
			S. typhimurium	7 ± 0.3[b]
			S. sonnei	0
			S. epidermidis	22 ± 0.9[g]
			S. faecalis	12 ± 0.4[c]
			E. carotovora$_1$	1 ± 0.2[m]
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 2: 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*
Achyranthes 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	++
Tamarindus 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.
*** = Resistant strain to all of the antibiotics tested, but significantly inhibited by plant extracts.

Table 3: Minimum inhibitory concentrations 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 oliveri 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
Tamarindus 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. ne = not effective.

Exceptional performance was also exhibited by *T. Indica* like *S. incanum* when compared to the activity of other plant extracts against the tested fungal pathogens. Though, the health related effects of *T. Indica* has been reviewed and indicated by many workers (Nwodo *et al*., 2011; Kuru, 2014) [25, 21], its effect against selected plant fungal pathogens (*P. Digitatum* and *P. nicotianae*) is reported in this study. On the other hand, four [leaf extracts of *A. aspera*, *W. somnifera*, *A. seyal* and root extracts of *M. jalapa*] shown selective antibacterial activity (Vidya *et al*., 2011) [39].

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 (Silva *et al*., 2009; Ramesh and Mahalakshmi, 2014; Ramos and Gomes, 2014) [32, 27, 28]. Similar reports indicate the susceptibility of the Gram-positive bacterium, *Staphylococcus spp* to lichen (*Cladonia verticillaris*) preparation (Ramos and Gomes, 2014) [28], other plant extracts such as *Cordia curassavica, Lantana achyranthifolia* and *Lippia* graveolens (Silva *et al*., 2009) [32] and seed extracts of *Syzygium jambolanum* (Chandrasekaran and Venkatesalu, 2004) [9].

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 Martini *et al*. (2004) [23] when certain plants were evaluated 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 secondary metabolites present in the plant tissue (Regnier and Macheix, 1996; Stankovi´c *et al*., 2015) [30, 33], and the pathogen's inherent resistance (Ozkan *et al*., 2004) [26]. Quantitative information obtained from the Folin-Ciocalteu method may provide information about the active ingredients of soluble phenolics in the plant extract. *Acacia seyal*, unlike any other plant extracts tested, had a high content of equivalent mg Gallic acid/g dry weight. 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; Stankovi´c *et al*., 2015) [10, 33]. Or, it could be due to better extraction by the methanolic solvent system as compared to water (data not indicated here) (Cruickshank and Perrin, 1964) [11].

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 (Table 2). According to (Cruickshank and Perrin, 1964) [11], toxic phenolic compounds present in such low concentrations may have a stimulatory effect on pathogen growth at low concentrations. 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, belonging to the same family, *Enterobacteriaceae* (Takeda *et al*., 1982) [35]. Similar results were reported for *E. coli* by Ozakan *et al*. (2004) [26], which described possible development of resistance by the bacteria. To our knowledge, resistance development by *E. carotovora* has not been reported in previous studies. On the other hand, the *X. campestris* pv. *mangiferaeindicae* (isolate UPXac-2), which showed resistance to all antibiotics tested, was significantly inhibited by *A. seyal*, *W. somnifera*, *T. minuta* and *M. jalapa*. This is also the first report to our knowledge of antimicrobial activity of these plant extracts against the pathogens tested. 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 (data not included in this report), which may indicate promising potential for postharvest disease control, especially for the citrus industry. Future research will be focusing on this aspect and determining the active chemical compounds involved.

Conclusion

In this study, it was found that some of the plant extracts tested was highly effective against the Gram-positive bacterium *S. epidermidis* compared to the Gram-negative bacteria. Despite the development of resistance by strains of *E. coli* and *E. carotovora* (UPErc-2) to all plant extracts tested, the application of *A. seyal*, *W. somnifera*, *T. minuta* and *M. jalapa* extracts was found to be effective against the *X. campestris* pv. *mangiferaeindicae* (isolate UPXac-2), which showed resistance to all antibiotics tested. Therefore, the search and verification of the active ingredients from these plant extracts would provide a solution to control the development of resistant in plant and animal pathogens.

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