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IN VITRO ANALYSIS OF ANTIMICROBIAL AND PHYTOCHEMICAL PROPERTIES OF CRUDE EXTRACTS OF SELECTED PLANTS AGAINST THE TOMATO WILT DISEASE

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ABSTRACT

The wilt disease by fungal and bacterial pathogens is one of the most devastating diseases of tomato (*Solanum lycopersicum* L.) worldwide. Chemical-based control of the wilt-causing pathogens often leads to environmental pollution and pest resistance; hence the need for alternative sustainable approaches. We evaluated the *in vitro* antimicrobial and phytochemical properties of aqueous crude extracts of roots, leaves, flowers, and barks of *Solanum incanum* L., *Laurnea cornuta*, *Tagetes minuta* L., *Ageratum conyzoides*, *Opuntia monacantha,* and *Euphorbia tirucalli* L. from Kano-Kisumu against *Erwinia chrysanthema, Ralstonia solanacearum* and *Fusarium oxysporum* isolated from diseased tomato tissues and rhizospheres. Sterile distilled water and the amoxicillin antibiotic were used as negative and positive control treatments, respectively. The experiment was carried out in diameters of zones of inhibition and levels of phytochemicals determined. *Ageratum conyzoides* and *O. monacantha* extracts were least effective against the pathogens, with means of inhibition of only up to 8.7 and 12.3 mm, respectively. *Euphorbia tirucalli* and *L. cornuta* were the most effective against the pathogens, with means of inhibition of up to 30 mm. Except for anthocyanins and anthraquinones, no significant ($P<0.05$) differences were observed for levels of other phytochemicals in different plant extracts. We concluded that plant extracts showing remarkable antimicrobial activities against the pathogens can be used to make viable formulations to combat the devastating tomato wilt disease.

Key Words: Bacterial wilt, plant extracts, *Solanum lycopersicum*

RÉSUMÉ

La maladie flétrissures causée par des agents pathogènes fongiques et bactériens est l'une des maladies les plus dévastatrices de la tomate (*Solanum lycopersicum* L.) dans le monde. La lutte chimique contre les agents pathogènes responsables du flétrissures entraîne souvent une pollution

de l'environnement et une résistance aux ravageurs; d'où la nécessité d'approches alternatives durables. Nous avons évalué *in vitro* les propriétés antimicrobiennes et phytochimiques d'extraits bruts aqueux de racines, feuilles, fleurs et écorces de *Solanum incanum* L.*, Laurnea cornuta, Tagetes minuta* L.*, Ageratum conyzoides, Opuntia monacantha* et *Euphorbia tirucalli* L. de Kano- Kisumu contre *Erwinia chrysanthema, Ralstonia solanacearum et Fusarium oxysporum* isolés de tissus et de rhizosphères de tomate malades. L'eau distillée stérile et l'antibiotique amoxicilline ont été utilisés comme traitements témoins négatifs et positifs, respectivement. L'expérience a été réalisée dans des diamètres de zones d'inhibition et des taux de composés phytochimiques déterminés. Les extraits *d'Ageratum conyzoides* et d'*O monacantha* étaient les moins efficaces contre les agents pathogènes, avec des moyens d'inhibition de seulement 8,7 et 12,3 mm, respectivement. *Euphorbia tirucalli* et L. *cornuta* se sont révélés les plus efficaces contre les agents pathogènes, avec une inhibition pouvant atteindre 30 mm. À l'exception des anthocyanes et des anthraquinones, aucune différence significative ($P < 0,05$) n'a été observée pour les concentrations d'autres composés phytochimiques dans différents extraits de plantes. Nous avons conclu que des extraits de plantes présentant des activités antimicrobiennes remarquables contre les agents pathogènes peuvent être utilisés pour élaborer des formulations viables permettant de lutter contre la maladie dévastatrice de la tomate.

Mots Clés: Flétrissure bactérienne, extraits de plantes, *Solanum lycopersicum*

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop in the world, cultivated widely in the tropics and subtropics (AVRDC, 2015; McGovernn, 2015). It provides smallholder farmers in East Africa with higher incomes per hectare than other staple crops (AVRDC, 2015). Due to its nutritive and economic importance, the tomato has become the agenda in international horticultural fora (Ayandiji and Adeniyi, 2017).

Despite its economic and nutritional importance, tomato production is affected by many diseases in sub-Saharan Africa (James *et al*., 2010; Sang *et al*., 2016). It is reported that *F. oxysporum* and other tomato pathogens are responsible for huge losses in tomato production globally (Hanaa *et al*., 2011). The bacterium, *R. solanacearum,* is classified as one of the world's important phytopathogens due to its persistence, lethality, wide host range and broad geographic distribution (Deny, 2006; Janse, 2012); and is reported to contribute to tomato yield losses of between 10-100% worldwide (Radhi *et al*., 2016).

Control of tomato pests and pathogens, just like in other crops, is largely dependent on chemical pesticides (Akkopru and Demir, 2005; Birech *et al*., 2006; Yuliar *et al*., 2015). However, this is undesirable because of development of pathogen resistance (Wagnitz, 2014), the high costs involved and the persistence and accumulation of these chemicals in the environment, as well as their effects on non-target organisms (Rai and Carpinella, 2006; Engindeniz and Ozturk, 2013; Bhattacharjee and Dey, 2014). There is need, therefore, to search for alternative ways to control and manage tomato pests and diseases.

Natural plant products seem to be a viable and appealing solution to the aforementioned problems, and many researchers worldwide are seeking to identify the effective natural products that can replace the synthetic pesticides (Kim *et al.*, 2005). It is widely known that many plants are good sources of antimicrobial agents (Obafemi *et al*., 2006), and several plants have been shown to contain bioactive constituents (Awoyinka *et al*., 2007). There is evidence that plants produce a variety of bioactive metabolites and phytochemicals, which serve as their defense mechanisms against pests (Tenover, 2006). Authors have previously observed that the potential of plants as sources of useful compounds, remains largely unexplored and screening of plants

may result in the discovery of novel compounds that could be valuable, especially in the wake of pathogen resistance (Tomoko *et al*., 2002). In this context, there is need for exploration of plant extracts as alternative pathogen control agents to combat microbial infections.

Pesticides from natural sources are often non-toxic to humans and the environment, easily degradable and do not accumulate in the environments as in the case of synthetic pesticides (Wagnitz, 2014). The use of natural products as biological control mechanisms are considered a viable alternative to synthetic products because of their efficiency (Chethana *et al*., 2012; Nashwa and Abo-Elyousr, 2012). In this study, we sought to assess the antimicrobial and phytochemical properties of crude extracts of various plant parts (the roots, barks, leaves, and flowers of *L. cornuta, A. conyzoides*, *E. tirucalli* L, *T. minuta* L, *S. incanum* L. and *O. monacantha*) of selected plants against the common tomato wilt-causing pathogens; *E. chrysanthema, R. solanacearum* and *F. oxysporum.* The objective was to evaluate the antimicrobial activities of the crude extracts of different parts of various herbs against selected tomato plant pathogens *in vitro* and thus, to identify plants or parts of plants whose extracts have the potential to be used in managing the tomato wilt disease.

MATERIALS AND METHODS

Study and sampling areas. The study was conducted at the University of Eldoret, Biotechnology laboratory and in the greenhouse located nearby. Two sampling locations; Alendu Mixed Secondary School (E 0.156693 N 34: 811165) and Migingo Primary School (E 0.156589 N 34.811310) were selected for this study.

Plant sampling, identification and handling. The sampling of plant specimens for this study was done in December, 2016 in Kano Kisumu. The fresh leaves, flowers, barks and roots of six plants (*L. cornuta, A. conyzoides*, *T. minuta* L, *S. incanum* L., and *O. monacantha*) were sampled and transported in labeled clean polyethylene bags to the Biotechnology laboratory at the University of Eldoret for further analysis. For *E. tirucalli* L., only the roots, bark and the thorny needle-like leaves were sampled since the flowers were not present at the time of sampling. In total, 23 plant specimens were collected for the study. The six plant samples were identified by a taxonomist from the School of Natural Resources, University of Eldoret as *L. cornuta, A. conyzoides*, *E. tirucalli* L, *T. minuta* L, *S. incanum* L., and *O. monacantha* (Table 1). The specimens were deposited in the University of Eldoret herbarium with the voucher numbers KSMBW/11/16/001, KSMBW/11/16/002, KSMBW/11/16/003, KSMBW/11/16/004, KSMBW/11/16/005 and KSMBW/11/16/006, respectively. Images of the different plant species which were sampled for this study are illustrated in Figure 1.

Preparation of plant extracts. Crude plant extracts were obtained following procedures previously described by Nduagu *et al*. (2008). The collected leaves, flowers, barks, and roots were cleaned with running tap water, rinsed with sterile distilled water and air-dried in the greenhouse for two weeks before being ground into fine powders using an automatic grinding machine (SM-1303FL). From each plant part, three different concentrations were formulated by weighing and suspending 1, 5 and 15 g each into 100 ml of sterile distilled water to obtain concentrations of 0.01, 0.05 and 0.15 g l^{-1}, respectively . The three different concentrations were denoted as low, medium and high, respectively; throughout the study. The suspensions were left overnight in a shaking water bath at 40 ^{o}C; and subsequently, the mixtures were filtered using sterile pieces of muslin cloth. The filtrates were stored at 4 ^{o}C to preserve them for subsequent experiments.

Pathogen isolation. The test pathogens, *E. chrysanthema*, *Ralstonia solanacearum,* and

TABLE 1. Plants used for antimicrobial and phytochemical assays

Botanical name	Common name	Family
Laurnea cornuta	Bitter Lettuce	Asteraceae/Compositae
Ageratum conyzoides	Goat weed	Asteraceae/Compositae
Euphorbia tirucalli L	Firestick plant/Pencil cactus	Eurphorbiaceae
Tagetes minuta L	Mexican Marigold	Asteraceae/Compositae
Solanum incanum L	Sodom Apple	Solanaceae
Opuntia monacantha	Prickly Pear	Cactaceae

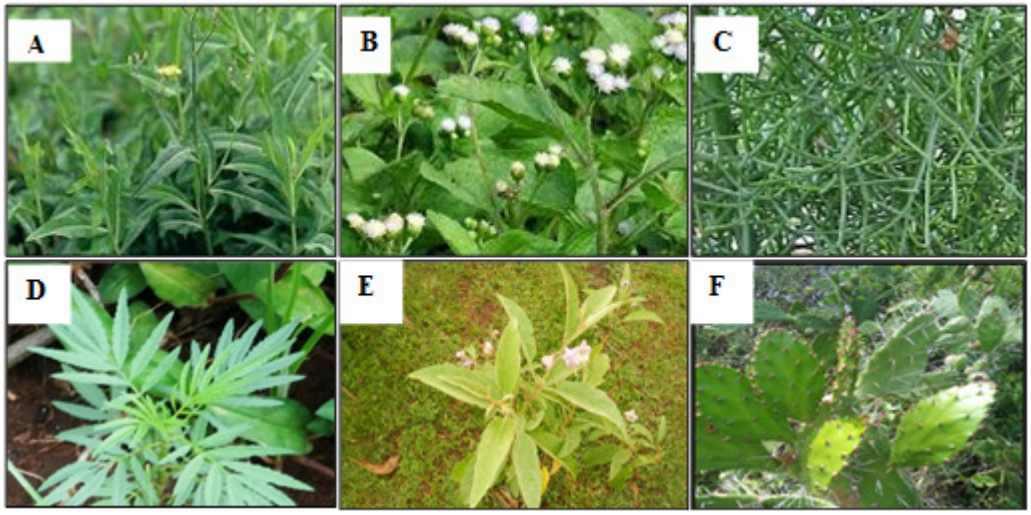

Figure 1. Images of the six plants used in the study A: *Laurnea cornuta,* B: *Ageratum conyzoides*, C: *Euphorbia tirucalli* L, D: *Tagetes minuta* L, E: *Solanum incanum* L. and F: *Opuntia monacantha.*

F. oxysporum and *Ralstonia solanacearum,* were isolated and identified from freshly sampled soil and root tissues from infected tomato plants following standard procedures as previously described by Lin *et al*. (2008); Janse (2012), and Sravani *et al*. (2014). Isolation of *F. oxysporum, R. solanacearum* and *E. chrysanthema* was done on Potato Dextrose Agar (PDA), Kelman's' Tetrazolium Chloride (TZC) agar and Crystal Violet Sodium Polypectate (CVP) agar, respectively. The *F. oxysporum* isolates were maintained on PDA slants at room temperature; while *E. chrysanthema* and *R. solanacearum* isolates were maintained on Nutrient agar slants at 37°C. Identification of *F. oxysporum* was done after incubation at 25°C for 7 days, using the single spore method (Choi *et al*., 1999), and according to morphological characteristics with the help of standard keys of identification (Agrios, 2005).

Antimicrobial assays. The Agar Well Diffusion method (Joshi *et al*., 2009; Bhalodia and Shukla, 2011), was used to perform the antimicrobial assays for the bacterial and fungal test pathogens on Nutrient Agar and Potato Dextrose agar respectively. The plates were inoculated with about 40 ml of broth cultures of the bacteria and fungi using the spread plate technique. Three wells/holes of about 9 mm diameter each, were drilled in each of the

prepared agar plates using sterile micropipette tips and equal volumes (1 µl) of the different concentrations of previously prepared plant extracts introduced into the plates, with 3 plates acting as positive controls per plant part and 1 plate of three wells acting as a negative control per plant part.

Instead of the plant extracts, three different concentrations (low, high and medium) of amoxicillin antibiotic were introduced into the wells of the three positive control plates, and sterile distilled water was introduced into the wells of each of the negative control plates. The plates were incubated in an upright position, at 37 °C for 24 hr. The diameters of zones of inhibition per plate were measured, following the period of incubation.

Phytochemical analyses. The qualitative and quantitative analyses of different phytochemicals in the different plant extracts, was performed using High-Performance Liquid Chromatography (HPLC) technique (Handa *et al*., 2008). The compounds tested for were steroids (STE), anthraquinone (ANT), alkaloids (ALKA), terpenoids (TER), saponins (SAPO), anthocyanin (ANTHO), flavonoids (FLAV), phenols-tannins (PHENO) and Ninhydrins (NINH).

Data analysis. The data were analysed using SAS version 9.1; whereby ANOVA (one way) was carried out to show statistical difference using the varying zones of inhibition between the test microbes exposed to the different extracts from *L. cornuta, A. conyzoides*, *E. tirucalli* L., *T. minuta* L, *S. incanum* L., and *O. monacantha* at 95% level of confidence. The test results were further subjected to a Tukey's post hoc test to separate the significantly different means.

RESULTS

Results on the antimicrobial activities of the crude aqueous extracts of the different plants against *F. oxysporum*, revealed that the activities of-of different extracts against this pathogen were significantly different ($P < 0.05$) at different concentrations (Table 2). No significant difference was observed for activities of *O. monacantha* bark extracts and *S. incanum* leaf and bark extracts against *F. oxysporum* across the different concentrations. Also, the root extracts of *E. tirucalli* and *L. cornuta* proved to be more potent on *F. oxysporum* than all the other root extracts, showing means of zones of inhibition of up to 13.7 and 15.7 mm, respectively. The same trend was also observed for the bark and leaf extracts of these two plants against *F. oxysporum,* with means of zones of inhibition going up to 17.3 and 20.0 mm for *L. cornuta* leaf and bark extracts, respectively. Extracts of *A. conyzoides* and *O. monacantha* were the least effective, with means of zones of inhibition ranging only between 3.7 and 11 mm (Table 2); while the most effective extracts were *E. tirucalli* and *L. cornuta*. The crude flower extracts of *O. monacantha*, *S. incanum,* and *L. cornuta* showed the greatest antimicrobial activity against *F. oxysporum* (Table 2).

Results of the effects of different concentrations of different plant extracts on *R. solanacearum* are presented in Table 3. Results revealed that *E. tirucalli, T. minuta*, *S. incanum, and L. cornuta* extracts exhibited the greatest activity against *S. solanacearum* in the descending order with the ranges of 10.3 - 34.3, 9.3 - 19.3, 9.3 - 16.0 and 8.7 - 16.0 mm, respectively. As a matter of fact, the leaf extracts of *E. tirucalli* were the most potent for *R. solanacearum;* while the crude extracts of *A. conyzoides* and *O. monacantha* were the least potent; exhibiting only means of inhibition of lows of 3.7 and 3.3 mm, respectively; and highs of 11.0 mm for both plant extracts (Table 3). Except for the leaf, flower and root extracts of *S. incanum* and the leaf and root extracts of *L. cornuta*, significant differences were observed for the activities of different plant part extracts against *R. solanacearum,* which increased significantly with increased concentrations of the extracts.

TABLE 2. Means of zones of inhibition (mm) of different concentrations of different plant extracts on *Fusarium oxysporum*

Treatment	*Ageratum conyzoides*				*Opuntia monacantha*			
	Root	Bark	Leaf	Flower	Root	Bark	Leaf	Flower
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	6.0±0.7b	19.4±2.5d	6.0±0.7b	5.6±1.1bc	5.9±0.8b	5.9±0.9b	5.3±0.8b	6.0±0.7b
Low conc.	6.7±0.6b	3.7±0.1b	4.3±0.6b	5.0±0.6bc	5.3±0.6b	6.0±0.6b	6.0±0.6bc	8.0±1.0c
Med. conc.	7.3±0.6bc	4.7±0.6c	6.0±1.0b	6.3±1.0b	6.3±0.6bc	6.7±1.0b	6.7±1.1b	9.3±0.8d
High conc.	11.7±4.7c	5.7±1.2c	6.7±0.6c	6.3±0.6c	6.7±0.5b	6.7±1.2b	7.0±0.0c	11.0±2.5bc
	Tagetes minuta				*Solanum incanum*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	8.3±0.9b	7.4±0.8b	9.0±0.7c	7.3±0.7b	8.1±0.9b	7.2±0.9b	8.7±0.5c	7.2±0.9b
Low conc.	7.7±0.6b	8.3±0.6bc	7.7±0.6bc	7.0±0.6c	8.3±0.6b	8.3±0.6c	7.0±1.0b	8.3±0.6b
Med. conc.	8.0±1.0b	8.3±3.4bc	8.3±1.2b	7.7±0.0b	8.7±0.6c	8.7±1.2c	7.3±0.6b	9.0±1.0b
High conc.	8.7±1.5c	11.0±0.6c	8.7±0.6b	8.7±1.2b	9.3 ±0.7bc	8.7±0.6c	7.0±0.0b	16.7±5.8c
	Euphorbia tirucalli				*Laurnea cornuta*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a		0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	8.0±0.0b	8.0±0.0b	8.0±0.0b		8.0±0.0b	8.3±0.5b	8.7±0.5b	8.0±0.0b
Low conc.	8.3±0.6b	9.0±1.0bc	8.0±0.0b		17.0±1.2b	14.3±2.5c	12.0±0.0c	10.7±0.7c
Med. conc.	9.7±0.8c	7.7±1.2b	10.3±2.5c		10.0±2.0c	16.3±3.7cd	11.0±2.0d	11.0±1.0c
High conc.	13.7±1.5d	12.0±3.6c	12.7±2.5c		15.7±4.0cd	20.0±0.0d	17.3±0.6e	16.3±3.2d

Means followed by the same letter(s) within each column do not differ significantly at P>0.05 (HSD)

TABLE 3. Means of zones of inhibition (mm) of different concentrations of different plant extracts on *Ralstonia solanacearum*

Treatment	*Ageratum conyzoides*				*Opuntia monacantha*			
	Root	Bark	Leaf	Flower	Root	Bark	Leaf	Flower
--VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	20.7±2.2e	17.8±3.1d	19.4±2.5d	16.9±2.3d	11.3±0.4e	18.1±2.4d	15.0±0.9e	11.6±1.5d
Low conc.	5.3±0.4c	7.3±0.2b	3.7±0.1b	9.7±0.5b	4.4±0.1b	9.3±2.1b	5.7±0.3c	4.8±0.2b
Med. conc.	8.3±0.3b	7.3±0.3c	6.3±0.2c	11.0±0.5c	6.0±0.2c	11.0±0.7c	3.3±0.3b	5.0±0.3b
High conc.	9.3±0.3d	8.7±0.2b	6.7±0.2c	11.7±0.3c	7.0±0.1d	16.0±0.5d	6.7±0.1d	6.3±0.2c
	Tagetes minuta				*Solanum incanum*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	18.1±6.1d	17.0±5.2d	19.1±4.2d	18.8±4.5c	10.6±3.8b	9.7±0.7b	10.1±0.8b	9.9±1.1bc
Low conc.	9.0±0.4b	8.7±0.6b	9.3±0.5b	9.7±1.0b	9.7±0.6b	9.3±2.1d	10.0±0.6b	9.3±0.0b
Med. conc.	10.0±0.3c	9.3±0.5c	9.3±1.1b	11.0±1.2b	10.3±0.5b	11.0±0.7c	10.0±0.0b	10.0±0.0b
High conc.	19.3±2.1d	11.7±0.8d	10.7±2.1c	11.7±0.5b	10.3±0.7b	16.0±0.5b	10.3±0.1b	10.0±0.6b
	Euphorbia tirucalli				*Laurnea cornuta*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a		0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	22.1±4.1d	18.9±3.8d	19.1±4.2c		19.3±2.5c	16.6±3.5d	15.1±0.8c	22.1±0.7e
Low conc.	12.7±2.0b	10.3±0.6b	13.3±7.3b		10.3±1.5b	9.3±2.1b	8.7±1.2b	10.0±1.7b
Med. conc.	17.3±0.7c	10.3±0.6b	16.7±2.1c		11.7±3.1b	11.0±0.7c	10.3±0.6b	10.7±2.6b
High conc.	17.3±2.5c	11.7±1.5c	34.0±3.6d		11.3±0.6b	16.0±0.5d	10.7±1.2b	12.0±1.2c

Means followed by the same letter(s) within each column do not differ significantly at P>0.05 (HSD)

Results on the antimicrobial activities of the different plant extracts against *E. chrysanthema* are presented in Table 4. The activity of the different concentrations of extracts was significantly different ($P < 0.05$). However, for the same plant, no significant ($P = 0.216$) differences appeared in antimicrobial activity against this test pathogen among the different plant parts. Crude plant extracts from *E. tirucalli* and *L. cornuta* proved to be most effective against *E. chrysanthema,* than those from other plants under study.

It was to observed that for *S. incanum* and *E. tirucalli*, crude stem extracts gave significantly higher zones of inhibition than the other plant parts; while for *O. monacantha* and *A. conyzoides*, crude flower extracts gave higher zones of inhibition of *E. chrysanthema* than crude extracts from the other plant parts (Table 4). The least effective of all the plants extracts against *E. chrysanthema* were *A. conyzoides* and *O. monacantha,* which recorded means of inhibition ranging only between 6.0 to 8.7 mm and 6.3 to 12.3 mm, respectively. Another observation was that for the different plant parts, different concentrations showed significant differences in their activities against *E. chrysanthema*. However, the root, stem and flower extracts of *A. conyzoides*, and *T. minuta*, root and leaf extracts of *S. incanum* and root extracts of *O. monacantha,* did not show significant differences in the activities of different concentrations (Table 4).

We had varying results for phytochemical levels in the different plant parts for the different plants studied (Tables 5 and 6). *Solanum incanum* samples tested positive for all phytochemicals (steroids, anthraquinone, alkaloids, terpenoids, saponins, anthocyanin, flavonoids, phenols-tannins, and Ninhydrins). Levels of Ninhydrins were the highest in this plant with a mean of 17.04 mg g^{-1}; followed by anthraquinones with a mean of 9.0 mg g^{-1}, and trailing overall was phenol-tannins with a mean of 6.56 mg g^{-1} (Table 5).

The phytochemicals that recorded the least amounts in *S. incanum* were anthocyanins and saponins, with means of only 0.05 and 0.29 mg g^{-1}, respectively. There were no significant differences ($P < 0.05$) in their quantities among the different plant parts for most of the phytochemicals, except for anthocyanins and anthraquinones where the levels were significantly different in different plant parts ($P = 0.019$ and $P = 0.021$, respectively). The levels anthraquinones in the leaves of this plant were lower than in the other plant parts, with a mean of 9.03 mg g^{-1}; while anthocyanins were more in the flowers and leaves (0.05 mg g^{-1}); but lower in its roots and stems (0.04 mg g^{-1}).

Although the flower, leaf, root and stem extracts of *A. conyzoides* contained all the phytochemical compounds tested, the levels of anthocyanins were particularly very low (average 0.035 mg g^{-1}); while the levels of Ninhydrins were the highest, with an average of 17. 33 mg g^{-1} (Table 5). The levels of steroids, alkaloids and terpenoids were almost similar across all the plant parts ranging from 2.54 to 2.56 mg g^{-1}. Except for steroids, anthocyanins and flavonoids, the levels all the other phytochemicals were not significantly different across the different plant parts ($P > 0.05$) (Table 5). Similarly, for *O. monacantha*, the levels of steroids, alkaloids, anthocyanins, and ninhydrins all turned out to be significantly different across the stem, leaves, roots, and flowers ($P < 0.005$) (Table 5). However, the levels of anthraquinones, terpenoids, saponins, flavonoids, and phenol-tannins were not significantly different across the different plant parts studied. Ninhydrins were recorded as the highest in this plant, with a mean of 16.51 mg g^{-1}; followed by anthraquinones with a mean of 9.96 mg g^{-1}; and phenol-tannins with a mean of 6.325 mg g^{-1}. The least levels of phytochemicals were recorded for anthocyanins with a mean of 0.052 mg g^{-1} and saponins mean of 0.265 mg g^{-1} (Table 5).

Results were observed for the levels of phytochemical compounds in *E. tirucalli,* with

TABLE 4. Means of zones of inhibition (mm) of different concentrations of different plant extracts on *Erwinia chrysanthema*

Treatment	*Ageratum conyzoides*				*Opuntia monacantha*			
	Root	Bark	Leaf	Flower	Root	Bark	Leaf	Flower
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	24.0±3.3c	32.6±2.7c	27.9±4.9d	33.3±2.3c	30.9±3.0c	28.7±3.9e	30.8±4.3d	29.9±3.4d
Low conc.	6.3±0.6b	7.7±1.5b	6.7±0.6c	6.0±1.0b	6.7±0.6b	6.7±1.1b	7.3±0.6c	9.0±1.0b
Med. conc.	6.3±0.6b	7.0±1.0b	6.3±0.6b	6.3±0.5b	6.3±0.6b	9.3±0.7c	6.3±0.5b	10.0±2.5c
High conc.	6.7±0.5b	8.7±1.2b	6.7±0.4c	6.7±0.6b	7.3±0.6b	11.0±0.5d	6.7±0.6bc	12.3±.0bc
	Tagetes minuta				*Solanum incanum*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	16.6±1.8c	16.1±1.6c	17.7±2.5d	16.4±1.5c	17.6±2.8	15.7±1.5d	16.6±2.1c	18.2±3.3d
Low conc.	9.7±0.4b	9.3±0.6b	9.3±0.5b	9.0±0.6b	10.3±0.6b	9.7±0.6b	9.7±0.0b	9.7±0.6b
Med. conc.	9.7±0.6b	10.3±3.2b	9.7±0.6bc	9.3±0.6b	10.7±0.5b	11.3±0.7c	10.0±0.6b	10.0±0.0bc
High conc.	10.3±0.6b	11.3±0.6b	10.0±0.0c	9.3±0.0b	12.0±2.6b	20.3±0.5e	10.3±0.6b	10.3±1.2c
	Euphorbia tirucalli				*Laurnea cornuta*			
-VE control	0.0±0.0a	0.0±0.0a	0.0±0.0a		0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
+VE control	28.1±3.1c	31.4±3.9b	30.8±4.6c		25.6±1.2c	22.7±0.9c	28.7±2.9d	26.8±4.3c
Low conc.	15.7±0.8b	24.0±6.1b	16.0±3.6b		13.7±7.0b	14.7±4.3b	15.0±5.6b	14.7±4.7b
Med. conc.	24.0±3.5c	24.3±5.2b	18.3±2.1b		18.7±5.6b	24.0±3.0c	18.7±5.2bc	21.0±3.6c
High conc.	26.7±2.0c	31.0±1.9b	28.7±0.6c		29.0±3.2c	25.3±3.0c	22.0±1.1c	26.7±3.1c

Means followed by the same letter(s) within each column do not differ significantly at P<0.05 (HSD)

TABLE 5. Levels of phytochemical compounds (mg g^{-1}) in *Solanum incanum, Ageratum conyzoides* and *Opuntia monacantha*

PL. PART	STE	ANT	ALKA	TER	SAPO	ANTHO	FLAV	PHENO	NINHY
	— — — — — — — — — *Solanum incanum* — — — — — — — — —
F	2.56a	9.03a	2.78a	2.53a	0.29a	0.05b	1.53a	6.52a	17.13a
L	2.60a	9.06ab	2.77a	2.53a	0.28a	0.05b	1.63a	6.57a	17.11a
R	2.52a	9.07b	2.76a	2.54a	0.28a	0.04a	1.50a	6.55a	17.13a
S	2.52a	9.06b	2.78a	2.54a	0.29a	0.04a	1.49a	6.61a	17.04a
Mean	2.55	9.06	2.77	2.53	0.29	0.05	1.54	6.56	17.10
CV (%)	12.5	10.1	10.3	9.4	11.1	13.5	6.7	10.6	10.4
P value	0.418	0.021*	0.090	0.419	0.361	0.019*	0.390	0.119	0.438
	— — — — — — — — — *Ageratum conyzoides* — — — — — — — — —
F	2.58b	9.04a	2.55a	2.56a	0.28a	0.04b	1.66b	6.63a	17.32a
L	2.55b	9.05a	2.56a	2.55a	0.28a	0.03ab	1.67b	6.63a	17.33a
R	2.54a	9.06a	2.57a	2.55a	0.28a	0.03a	1.49a	6.65a	17.34a
S	2.54a	9.05a	2.56a	2.55a	0.28a	0.03ab	1.60b	6.67a	17.32a
Mean	2.551	9.049	2.561	2.552	0.276	0.035	1.60	6.645	17.33
CV (%)	10.5	9.2	11.3	20.1	17.0	12.8	12.2	16.4	19.1
P value	0.027*	0.867	0.107	0.051	0.609	0.043*	0.002*	0.368	0.781
	— — — — — — — — — *Opuntia monacantha* — — — — — — — — —
F	2.86b	9.96a	2.98ab	2.25a	0.27a	0.05ab	1.19a	6.34a	16.92c
L	2.55a	9.95a	2.97ab	2.26a	0.26a	0.05a	1.17a	6.30a	16.69bc
R	2.59ab	9.97a	2.94a	2.25a	0.27a	0.05ab	1.16a	6.30a	16.34ab
S	2.85b	9.96a	2.99b	2.25a	0.27a	0.05b	1.15a	6.36a	16.12a
Mean	2.712	9.96	2.970	2.254	0.265	0.052	1.167	6.325	16.516
P value	0.016*	0.906	0.020*	0.060	0.065	0.014*	0.205	0.584	0.009**

Means followed by the same letter(s) within each column do not differ significantly at P< 0.05). F = Flower, L = Leaf, R = Root, S = Stem, STE = Steroids, ANT = Anthraquinone, ALKA = Alkaloids, TERP = Terpenoids, SAPO = Saponins, ANTHO = Anthocyanin, FLAV = Flavonoids, PHENO = Phenols-tannins, NINHY = Ninhydrins

only levels of steroids and flavonoids being significantly different across the different plant parts ($P < 0.05$) (Table 6). The levels of the rest of the phytochemical compounds were not significantly different in the different plant parts. The levels of ninhydrins, anthraquinones and phenol-tannins were the highest in all the plant parts with means of 15.02, 10.106 and 5.966 mg g^{-1}, respectively; while levels of anthocyanins and saponins were the least with means of 0.05633 and 0.2624 mg g^{-1} respectively. The levels of steroids and alkaloids were, however, almost similar in all parts of this plant.

The results recorded for *L. cornuta* phytochemical levels were very different from those recorded for all the other plants under study. Flavonoids and ninhydrins were

TABLE 6. Levels of phytochemical compounds (mg g^{-1}) in *Euphorbia tirucalli, Laurnea cornuta* and *Tagetes minuta*

PL. PART	STE	ANT	ALKA	TER	SAPO	ANTHO	FLAV	PHENO	NINHY
				Euphorbia tirucalli					
L	3.17b	10.11a	3.02a	2.24a	0.27a	0.056a	1.12b	6.23a	15.23a
R	3.14a	10.11a	3.03a	2.24a	0.25a	0.056a	1.11b	5.61a	14.85a
S	3.17b	10.11a	3.03a	2.24a	0.27a	0.057a	1.04a	6.06a	14.97a
Mean	3.16	10.106	3.0257	2.239	0.2624	0.05633	1.0889	5.966	15.02
CV (%)	10.3	10.1	12.2	10.3	6.2	13.8	21.0	4.3	12.8
P value	0.020*	0.976	0.106	0.492	0.444	0.640	0.026*	0.089	0.574
				Laurnea cornuta					
F	3.13b	10.08b	2.45a	2.48a	0.29a	0.00	0.043a	6.748	0.00
L	3.14b	10.09b	2.44a	2.45a	0.29a	0.00	0.044a	6.748	0.00
R	2.44a	9.01a	2.45a	2.54a	0.29a	0.00	0.045a	6.671	0.00
S	2.44a	9.01a	2.43a	2.54a	0.29a	0.00	0.046a	6.702	0.00
Mean	2.672	9.3676	2.4438	2.52	0.2879	0.00	0.0446	6.707	0.00
CV (%)	10.6	11.3	13.4	12.4	11.2	0.00	13.7	9.8	0.00
P value	<0.001***	<0.001***	0.100	0.490	0.145	-	0.152	0.285	-
				Tagetes minuta					
F	2.47c	9.014a	2.393a	2.56a	0.287a	0.047c	0.00	0.00	0.00
L	2.27a	9.015a	2.313a	2.57a	0.283a	0.046bc	0.00	0.00	0.00
R	2.34b	9.014a	2.328a	2.55a	0.284a	0.045ab	0.00	0.00	0.00
S	2.26a	9.016a	2.323a	2.56a	0.281a	0.043a	0.00	0.00	0.00
Mean	2.332	9.0148	2.339	2.560	0.2837	0.0453	0.00	0.00	0.00
CV (%)	11	9.7	21.7	10.8	11.8	11.6	0.00	0.00	0.00
P value	<0.001***	0.728	0.065	0.797	0.083	0.002**	-	-	-

Means followed by the same letter(s) within each column do not differ significantly at P<0.05). F = Flower, L = Leaf, R = Root, S = Stem, STE = Steroids, ANT = Anthraquinone, ALKA = Alkaloids, TERP = Terpenoids, SAPO = Saponins, ANTHO = Anthocyanin, FLAV = Flavonoids, PHENO = Phenols-tannins, NINHY = Ninhydrins

completely missing in extracts from all parts of *L. cornuta* (Table 6). For the rest of the phytochemicals, which were present in parts of this plant, significant differences were observed only for steroids and anthocyanins ($P < 0.05$). The levels of anthraquinones and phenol-tannins were the highest with means of 9.3676 and 6.707 mg g^{-1}, respectively; while levels of anthocyanins and saponins were the least with means of 0.0466 and 0.2879 mg g^{-1}, respectively (Table 6).

For *T. minuta*, flavonoids, phenol-tannins, and ninhydrins were completely lacking in all the plant parts, and only the levels of steroids and anthocyanins were significantly different across the different plant parts ($P < 0.05$) (Table 6). Similarly, only the levels of anthraquinones were high in this plant, with a mean of 9.0148 mg g^{-1}; whereas the levels of saponins and anthocyanins were very low with means of 0.28367 and 0.04533 mg g^{-1} respectively. However, the levels of steroids,

alkaloids, and terpenoids were almost similar in this plant with means of 2.332, 2.339 and 2.560 mg g^{-1} respectively (Table 6).

DISCUSSION

The investigations revealed that most of the extracts had remarkable inhibitory activities on the growth of *F. oxysporum*. The high concentrations of *L. cornuta* extracts and the flower extracts of *S. incanum* yielded the best results in terms of *in vitro* inhibition of this pathogen and were significantly higher than the means recorded from the standard antibiotic used in the study.

The extracts of *E. tirucalli* were also promising in controlling the growth of this pathogen *in vitro* (Table 2). These results concur with those of a previous study where the extracts of *E. tirucalli* were reported to exhibit excellent antimicrobial activities against laboratory isolates of *F. oxysporum* (Bhuvaneshwar *et al*., 2010). The present findings also concur with those of other studies on antifungal activities of extracts from members of the Euphorbiaceae family, where root extracts recorded larger zones of inhibition zones than bark extracts (Rajeh *et al*., 2010; Karimi *et al*., 2011), but such variations attribute to the variations in phytochemical composition can be expected for different plant species (Vallejo *et al*., 2003; Randrianalijaona *et al*., 2005). Previous investigations involving extracts of *E. tirucalli* also indicate its antimicrobial activity against various fungal strains like *Candida albicans* (Bhalodia and Shukla, 2011; Singh and Vidyasagar, 2015). The bark extracts of *E. tirucalli* are also documented to be effective against F. *oxysporum* and other fungal pathogens (Rathi *et al*., 2012; de Araújo *et al*., 2014).

Although the antifungal activities of different *A. conyzoides* extracts against *F. oxysporum* were lower than the activities of most other plants, better antifungal activities by *A. conyzoides* acetone extracts have been reported (Pal and Kumar, 2013). The antimicrobial activities of the studied plant extracts were considerably lower against this fungal pathogen as compared to the bacterial pathogens. This can be attributed to the differences in cell wall composition between bacterial and fungal organisms, since fungal cell walls contain polysaccharides and proteins which are reported to function as efflux systems that expel deleterious compounds out of their cells (Nester *et al*., 2004).

The inhibitory activities of all the tested crude plant extracts against *F. oxysporum* increased with corresponding increase in their concentrations, showing that their effectiveness depends not only on their nature but also on the quantity of bio-active ingredients they contain. The increase in concentrations of the extracts, thus implies an increase in the quantity of the active antifungal ingredients, which in turn lowered the growth of *F. oxysporum* on the agar medium. These results show that higher concentrations of these plant extracts have the potential of significantly inhibiting mycelial growth and could be lethal to this pathogen, and therefore should be investigated for the management of *Fusarium* wilt of tomato.

This study has also established the antimicrobial effects of the root, bark, leaf and flower extracts of the different plant extracts tested against the tomato wilt-causing bacterium; *S. solanacearum*. The flower extracts of *A. conyzoides* had better effectiveness against this pathogen than it did on *F. oxysporum* and *E. chrysanthema*. It should be evaluated further for its potential in the management of the tomato bacterial wilt disease. However, the root, bark and leaf extracts of *A. conyzoides*, as well as the leaf, root and flower extracts of *O. monacantha*, exhibited relatively lower antimicrobial activities against this tomato wilt-causing pathogen, showing that they may not be the best extracts with regards to the management of tomato bacterial wilt disease. Contrastingly, in a previous study (Okwori *et al*., 2006), relatively higher antimicrobial activities were

recorded for the stem, leaf and root extracts of *A. conyzoides* against different bacterial pathogens, but the tested organisms were clinical isolates and this can account for the observed differences.

Concentrations of the flower extracts of *E. tirucalli* exhibited the highest inhibitory effects against *S. solanacearum,* with means ranging from 13.3 ± 7.3 to 34.0 ± 3.6 mm. It was also noted that root extracts of *E. tirucalli* were more effective against this bacterial wilt than the root extracts of all other plants; and that even the low concentrations of these extracts yielded higher means of zones of inhibition than the high concentrations of all its other parts and of all other plant extracts.

Antibacterial activities of different *Euphorbia* species are documented (Annapurna *et al*., 2004; Sudhakar *et al*., 2006).

Although only aqueous *E. tirucalli* extracts were tested in the present study ethanolic *E. tirucalli* bark extracts are also documented to possess broad-spectrum antimicrobial activities against Gram-negative bacterial pathogens (de Araújo *et al*., 2014; Muthukumar *et al*., 2014), similar to the test pathogens in this study. These results indicate the high potential of the use of *E. tirucalli* plant extracts in the management of tomato bacterial wilt disease caused by this pathogen.

Extracts of roots, flowers, barks and leaves of *T. minuta*, *L. cornuta* and *S. incanum* had similar effects on *S. solanacearum* as portrayed in the recorded zones of inhibition, ranging from 8.7 ± 0.6 to 19.3 ± 3.1 mm. *Solanum incanum* is reported to be one of the most potent plants against microorganisms and its antimicrobial effects have been illustrated in previous studies (Britto and Senthikumar, 2001; Bari *et al*., 2010; Pavitra *et al*., 2012).

The extracts of *L. cornuta* exhibited even better inhibitory activities against *E. chrysanthema* than they did on *S. solanacearum* (Table 4). Both the medium and high concentrations of different extracts of this plant yielded means of zones of inhibition of up to 24.0 ± 3.0 and 29.0 ± 3.2 mm, respectively. Furthermore, no significant differences were observed in the antibacterial activities of the high and medium concentrations of the bark and flower extracts of this plant against E. *chrysanthema*. Generally, for this pathogen, *E. tirucalli* and *L. cornuta* extracts were more effective and yielded greater means zones of inhibition than all the other plant extracts.

Extracts of *T. minuta* and *S. incanum* exhibited moderate antibacterial activities against this pathogen, ranging from 9.0 ± 0.6 for the flower extracts of *T. minuta* to 20.3 ± 0.5 mm for the bark extracts of *S. incanum*. The root, bark, and flower extracts of *O. monacantha* and *A. conyzoides*, the root extracts of *O. monacantha* and *S. incanum*, as well as the bark extracts of *E. tirucalli* showed no significant differences in their antibacterial activities against this pathogen between the low, medium and high concentrations. Generally, *A. conyzoides* and *O. monacantha* seemed to yield the least antimicrobial activities against this particular pathogen even at the high concentration.

The crude extracts of different plant parts in this study showed varying quantities of phytochemical compounds (Tables 5 and 6). A number of phytochemicals like alkaloids, saponins and flavonoids reported to be produced by several plants (Okwu, 2004; Jaberian *et al*., 2013), were present in all plant extracts exhibiting antibacterial and antifungal activities in this study. According to Muthu *et al*., (2006) and Krishnaraju *et al*. (2007), phytochemicals are responsible for the antimicrobial activities exhibited by different plant extracts. Reports also indicate that the antimicrobial activities of plant extracts can be linked to the abundance of phytocompounds in them (Wang, 2010; Phadungkit *et al*., 2012).

Phytochemical screening of extracts of different parts of *S. incanum and A. conyzoides* revealed the presence of all tested compounds in varying quantities (Table 5).

Other studies have also confirmed the presence of alkaloids, saponins, flavonoids, tannins, and steroids in extracts of *S. incanum* (Amadi *et al*., 2010) and *A. conyzoides* (Amadi *et al*., 2002; Borkataky *et al*., 2013). In fact, these two plants portrayed the greatest quantities of flavonoids in their plant extracts, with means of 1.54 and 1.60 mg g^{-1}, respectively.

Previous studies have also reported flavonoids to have inhibitory activities and to possess antifungal and antibacterial properties against several plant pathogens, including *F. oxysporum* (Cushnie and Lamb, 2005; Galeotti *et al*., 2008). More recently, some researchers have confirmed that the bioactive components of flavonoids contain antifungal and antibacterial activities (Abdel-Ghani *et al*., 2013), as was also determined in the present study, making these two plants to be highly important for control of bacterial wilt pathogens of tomato. These results suggest that *A. conyzoides* and *S. incanum,* by virtue of containing these compounds in greater quantities in their extracts, have the potential of effectively being used in the control of the wilt-causing pathogens evaluated in this study.

Except for steroids, anthocyanins, and flavonoids, the quantities of phytochemicals in extracts from different parts of *A. conyzoides* were not significantly different ($P > 0.05$). In more recent studies, extracts of *A. conyzoides* also tested positive for alkaloids, saponins, flavonoids, phenols, tannins, and steroids (Odeleye *et al*., 2014). These results are also comparable to those obtained by other researchers, that demonstrated the presence of these phytocompounds in *A. conyzoides* (Amadi *et al*., 2002; Borkataky *et al*., 2013). More recently, it was also demonstrated that *A. conyzoides* extracts contained alkaloids, terpenoids, flavonoids, tannins, and saponins with means of 25.23, 11.80, 1.68, 3.48 and 20.73 mg g^{-1}, respectively (Ajayi *et al*., 2016). Although these means are relatively higher than what was recorded in the present study, these differences can be attributed to geographical locational differences of the plants which have been shown to impact on the types and quantities of phytocompounds (Vallejo *et al*., 2003; Badoni *et al*., 2009; Gupta *et al*., 2011).

Results of phytochemical composition and quantities in extracts of different parts of *O. monacantha* revealed that this plant contained the highest means of alkaloids, exceeded only by of extracts of *E. tirucalli* (Table 5). Literature suggests that alkaloids are potent phytochemicals in plant extracts (Kaur *et al*., 2017). Studies carried out on alkaloids extracted from a variety of medicinal plants in Nigeria, showed great antifungal activities and antimicrobial activities against both Gram-negative and Gram-negative bacteria (Garba and Okeniyi, 2012). Alkaloids are reported to exhibit great antimicrobial activity against other bacterial pathogens such as *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Maatalah *et al*., 2012), all of which are also bacterial pathogens of similar nature to those under the present study. These results imply that alkaloid-containing extracts like those of *O. monacantha* can effectively be used in inhibiting the growth of these bacterial cells responsible for the wilt disease in tomato.

This present study also established the presence of all studied phytochemicals in extracts of *E. tirucalli* plant parts, corresponding to its excellent antimicrobial activities against the tested microorganisms with zones of inhibition exceeding 14.00 mm. The results also indicated extracts of *E. tirucalli* had the highest levels of steroids, anthocyanins, and alkaloids of all the investigated plants (Table 6). Literature confirms that *E. tirucalli* extracts contain alkaloids, tannins, and phenols, all of which form the basis of its strong antimicrobial activities (Sugukumar *et al*., 2010). The presence of flavonoids and steroids as key phytoconstituents in *E. tirucalli* extracts is also reported by Ajayi *et al.* (2016), making its extracts to be highly potent to microbial cells. Flavonoids, for instance, effectively inhibit bacterial growth due to its ability to form

complexes with the extracellular cell wall proteins, thereby disrupting microbial membranes (Hemandez *et al*., 2000).

Steroids are known for their antibacterial activity by causing leakages in microbial membrane proteins (Epand *et al*., 2007). Furthermore, the phenolic compounds of *E. tirucalli* are also documented to be toxic to microorganisms and similarly, the tannins produced by it can inhibit bacteria by inactivating microbial adhesion, enzyme functions and cell-envelope proteins (Upadhyay *et al*., 2010). Except for flavonoids and steroids, no significant differences were observed in the quantities of other phytochemicals in extracts of different parts of *E. tirucalli*. These results are slightly different from previous reports concerning other species from the Euphorbiaceae family where different plant parts contain different quantities of phytoconstituents (Igbinosa *et al*., 2009; Kamba and Hassan, 2010).

Extracts from all parts of *L. cornuta* did not portray the presence of anthocyanins and ninhydrins in the present study. Nevertheless, *L. cornuta* extracts still showed remarkable antimicrobial activities against the test pathogens, implying that anthocyanins may have no antimicrobial significance but could be important as antioxidant compounds as argued previously (Yin and Chao, 2008). Thus, the observed fungal and bacterial toxic effects exhibited by the crude plant extracts of *L. cornuta* against the test pathogens in this study may have been due to other phenolic constituents of this plant.

Similarly, extracts of all parts of *T. minuta* lacked flavonoids, phenol-tannins, and ninhydrins (Table 6). Our study findings are similar to those obtained in a study carried out in Pakistan, which confirmed the presence of phytochemicals flavonoids, saponins, tannins, and alkaloids, but not phenol-tannins in *T. minuta* extracts used against Gram-positive and Gram-negative bacteria (Tahir and Khan, 2012). The present study demonstrated that *T. minuta* extracts lacked flavonoids and phenol-tannins, contradicting other studies which have observed the presence of these compounds in *T. minuta* extracts used against both Gram-positive and Gram-negative bacteria (Tereschuk *et al*., 1997; Tahir and Khan, 2012). These variations can be due to differences in geographical locations of the plant since the two studies were done in Pakistan and Argentina, respectively. Evidence suggests that geographical differences impact on phytochemical quality and quantity in plant species, thereby affecting presumed antimicrobial properties (Howard *et al*., 2002; Vallejo *et al*., 2003; Yang *et al*., 2004; Badoni *et al*., 2009; Gupta *et al*., 2011). The possible cause of these differences concerning plant phytochemicals in different geographical regions has been strongly attributed to differences in types of soil minerals (Fonseca *et al*., 2006; Borokini and Ayodele, 2012), and as such, the same plant growing in different geographical locations can have different phytochemical contents.

The absence of some phytochemicals in plant extracts has in the past been linked to the type of solvent used in the extraction (Markom *et al*., 2007). In the present study, although aqueous extracts were used, this may not be the case since the phytochemicals absent in *T. minuta* and *L. cornuta* were extractable in water for the other plants under study. Despite lacking these important antimicrobial phytochemicals, the extracts of *L. cornuta* and *T. minuta* still had appreciable levels of terpenoids, saponins, phenol-tannins, and anthocyanins, which are equally potent phytochemicals. For instance, saponins are highly antifungal (Delmas *et al*., 2000; Wang, 2010), and antibacterial (Li *et al*., 2012; Deshpande *et al*., 2013) with their antimicrobial properties, mainly attributed mainly to lysis of microbial membranes (Asl and Hosseinzadeh, 2008). This probably explains why extracts from these two plant still exhibited inhibition of *F. oxysporum, R. solanacearum and E. chrysanthema*.

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