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Phytochemical analysis and antifungal activity of *Tithonia diversifolia* and *Kigelia africana* extracts against *Fusarium oxysporum* in tomato

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Fusarium wilt can cause severe losses in many vegetables and flowers, field crops and plantation crops. The main method for controlling Fusarium wilt is through chemical methods which have the disadvantage of polluting the environment and are expensive. Thus, there is a need for more environmentally friendly and cheaper techniques for controlling Fusarium Wilt. This study screened the phytochemical compounds of two plant extracts; *Tithonia diversifolia* and *Kigelia africana* and assessed their potency in controlling plant fungal pathogen *F. oxysporum*, which causes fusarium wilt in tomatoes. The phytochemical analysis revealed that the two plant extracts contained saponins, tannins, terpenoids, flavonoids, glycosides and phenolics except alkaloids and steroids which were only found in *T. diversifolia*. The mean inhibitory zones ranged from 7.93 to 10.44 mm for *T. diversifolia* at 25 to 100 g/l. The mean inhibitory zones for *K. Africana* ranged from 12.07 to 15.56 mm at 25 to 100 g/l. *K. Africana* extracts had the highest inhibitory (antimicrobial) activity. Combining both extracts was more effective compared to the single extract. The effect of the combined plant extract and positive control was statistically significant ($p < 0.05$). This study provides scientific evidence that *K. africana* and *T. diversifolia* extract possess antifungal activity and can be used as a broad-spectrum in managing microbial diseases.

Key words: Plant extracts, phytochemical screening, antimicrobial and antifungal properties, *Fusarium oxysporum.*

INTRODUCTION

Soil-borne pathogens escape competition with other microorganisms by penetrating the host plant roots (Haas and Defago, 2005). Plants infected by soil-borne pathogens suffer from various symptoms such as wilt, root rot and damping-off of seedlings. Main agricultural soil-borne fungal diseases include Fusarium wilt (*Fusarium oxysporum* Schlechtthe), Verticillium wilt (*Verticillium dahliae*) and damping-off diseases (Ampt et

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al., 2019). Fusarium wilt can cause severe losses in many vegetables and flowers, field crops and plantation crops (Michielse and Rep, 2009). In tomato, two distinct forms of the pathogen are found and can cause either vascular wilt or a crown and root rot and both occur throughout most tomato growing areas (Larkin and Fravel, 1998). Use of Fusarium resistant tomato cultivars can provide some degree of control of these diseases but the occurrence and development of new pathogenic races is a continuing problem (Jones et al., 1991). Another problem is that *F. oxysporum* can persist in the soil for a very long time and is spread by insects, water and garden equipment (Agrios, 2005). Therefore, increasing interest in the introduction of bio-control agents for managing soil-borne pathogens mainly due to the non-target effects of synthetic pesticides (Cook, 1993).

Kenya is one of sub-Saharan Africa's top tomato producers and grows more than 400,000 tonnes of the fruit every year, which corresponds to 14% of the whole vegetable harvest and 7% of its total horticultural production (Government of Kenya (GoK), 2016). Tomatoes are either grown on fields or in greenhouses. Production in the field accounts for 95% while greenhouse production accounts for 5% of the entire tomato production in Kenya (Food and Agriculture Organization [FAO], 2018). Tomatoes are also a source of income to the small-holder farmers hence contributing to food security and economic development (Ongeri, 2014). However, commercial farming of this important crop is under threat from pests and diseases (Berrueta et al., 2012), mainly Fusarium wilt caused by the fungus *Fusarium oxysporum* (Ajilogba and Babalola, 2013) causing significant yield losses to farmers.

Farmers prefer the use of synthetic fungicides or cultural practices to control Fusarium wilt disease in tomato (Mwangi et al*.,* 2015). However, cultural practices such as crop rotation and shifting cultivation are no longer effective due to insufficient land for cultivation (Bawa, 2016). Furthermore, commercial fungicides are unaffordable to many poor farmers and excessive use is environmentally toxic and can lead to the development of more resistant pathogen strains (Aktar et al., 2009; Njoroge, 2014). Due to these limitations to control Fusarium wilt disease, there is a need to seek alternative control methods of biological origin for the management of the disease. Plant secondary metabolites pose possible alternatives because they are environmentally friendly and have minimal effect on non-target soil microorganisms (Malkhan et al., 2012; Njoroge, 2014).

In this study, two plants, *Tithonia diversifolia* (Hemsl.) A. Gray and *Kigelia africana* (Lam.) Benth was screened for bioactive compounds and assessed for their potency in controlling plant fungal pathogen *F. oxysporum* that causes Fusarium wilt in tomatoes. Both plants have antimicrobial substances that have been successfully demonstrated against food and human pathogens (John-Dewole et al., 2013; Saini et al., 2009; Rejeki and Addy, 2017).

MATERIALS AND METHODS

Collection and preparation of plant samples

Plant samples of *T. diversifolia* (leaves) and *K. africana* (fruits) were collected from the field. They were washed and dried under shade until all the water molecules evaporated and plants became well dried for grinding. About 100 g of the dried sample was chopped and grounded into a powder. The sample was then mixed with 100 ml of distilled water to make a solution. The water extract was incubated at room temperature for 36 h and then filtered. The extract was then stored in airtight containers in the refrigerator for phytochemical analysis.

Phytochemical analysis of crude extract

All crude extracts were screened for phytochemical contents using standard procedures (Parekh and Chanda, 2008; Yadav and Agarwala, 2011). They were tested for tannins using 5 ml of extract dissolved in 1 ml of distilled water, filtered and then added 2 ml of Iron (III) Oxide. They were also tested for alkaloids (Wagner test), terpenoids (Salkowski test), flavonoids (Sodium hydroxide test), glycosides (Keller-Kilian test) and saponins (Froth test). Phenolics were tested by adding 1ml of ferric chloride solution to 2ml of the plant extract.

Collection and identification of *Fusarium oxysporum*

Sites with Fusarium wilt symptoms were identified in a tomato plantation. Soil samples were collected at the rhizosphere of the tomato plant. The samples were stored in sterile containers containing sterile distilled water ready for *F. oxysporum* isolation. They were inoculated into Petri dishes containing Potato Dextrose Agar (PDA) medium and incubated at 28°C for 7 days. After growth, the fungus was sub-cultured to obtain a pure culture. Identification and isolation of *F. oxysporum* were done according to De Carolis et al. (2012) by determining the colony colour and investigating the presence and shape of the macroconidia, microconidia and chlamydospores. Morphological characteristics were studied using light microscopy.

Antifungal activity test of *F. oxysporum*

The antifungal activity of the plant extracts was determined using the disc diffusion assay according to Benkeblia (2004). Plates of PDA media were prepared. Sterile filter papers (discs) of 6 mm in diameter were impregnated with 2 ml of the plant extract at the concentration of 25, 50 and 100 g/ml and placed on the culture medium. For controls, discs were saturated with pure water extracts. After 30 min, plates were inverted and incubated at 28°C for seven days to allow growth. Antifungal activity was evaluated by measuring the zone of inhibition against the test organisms and expressed in millimeters. The combined effect of the extract was determined by combining the two plant extracts in the ratio of 0:30, 10:20, 30:30, 20:10 and 30:0 ml of *K. africana* and *T. diversifolia*, respectively. A commercial fungicide was used as a control. Preparation of antimicrobial test disc was replicated three times for each test.

Statistical analysis

Statistical Analysis Software (SAS) version 9.4 was used to analyze data on the zone of inhibition. Two -way analysis of variance

Table 1. Phytochemical screening of the *Tithonia diversifolia* (leaf) and *Kigelia africana* (fruit) plant extracts.

Phytochemical present	Plants crude extracts	
	***Tithonia diversifolia* (Leaf)**	***Kigelia africana* (Fruit)**
Saponins	+	+
Tannins	+	+
Alkaloids	+	-
Terpenoids	+	+
Flavonoids	+	+
Glycosides	+	+
Phenolics	+	+
Steroids	+	-

+, Presence of the phytochemical; -, absence of the phytochemical.

Figure 1. *Fusarium oxysporum* growing in PDA media showing white mycelia, with a cotton appearance and pink colour on the reverse side of the plates.

(ANOVA) was carried out to determine variation among the zones of inhibition at α=0.05. Least Significance Difference was used to determine the difference among the means.

RESULTS

Phytochemical screening of the plant extracts

Several phytochemical compounds of the plant extracts were identified for both plants (Table 1). These were saponins, tannins, alkaloids, terpenoids, flavonoids, glycosides, phenolics and steroids. However, steroids and alkaloids were only found in *T. diversifolia* leaf extract and not in *K. africana* fruit.

Identification of *F. oxysporum*

F. oxysporum grown on the PDA media produced white mycelia, with a cotton appearance that was pink in colour on the underside of the plate (Figure 1). The microscopic features observed during identification were septations and shapes of conidia and chlamydospores. Masses of conidiophores were produced in the culture. The microconidia were abundant and kidney-shaped. The macroconidia were also abundant, slightly curved (sickle cell shape) with septations.

Antimicrobial activity of the plant extracts

The antifungal activities of *T. diversifolia* and *K. africana* aqueous extracts against *F.oxysporum* were demonstrated by observable zones of inhibition (Figure 2). The mean inhibitory zones were highest at 100g/l in both plants, although *K. africana* extract portrayed the highest inhibitory activity (Table 2).

A combined extract of the two plants produced a slightly more antimicrobial activity against *F. oxysporum* compared to when the extract was used singly. The effect of the plant extract and the positive control was statistically significant ($p < 0.05$). The mean zone of inhibition of the plant extracts ratios ranged from 17.25 to

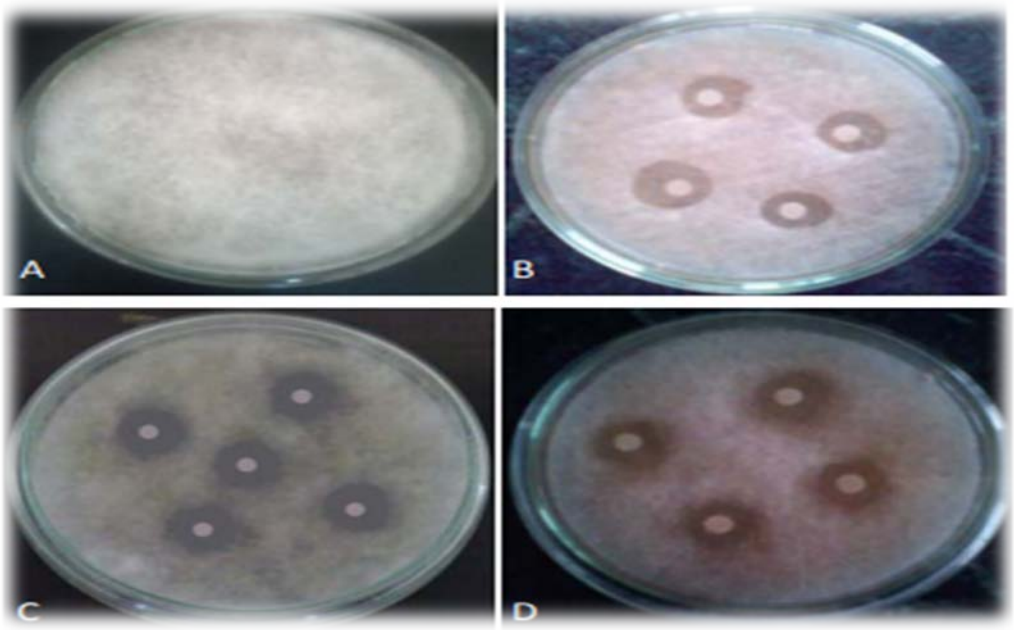

Figure 2. (A) A disc containing water as the control; **(B)** A disc containing *Kigelia africana* plant extract; (C and D) discs containing *Tithonia diversifolia* plant extract.

Table 2. Mean inhibitory zones of *Kigelia africana* and *Tithonia diversifolia* in different concentrations.

Treatment concentration (g/l)	Plant	Zone of inhibition (mm)
100	*Kigelia africana*	15.56
	Tithonia diversifolia	10.44
50	*Kigelia africana*	13.30
	Tithonia diversifolia	9.63
25	*Kigelia africana*	12.07
	Tithonia diversifolia	7.93

9.45 mm (Table 3). The highest mean of inhibition was experienced in the combined extract where both plants had the same ratio (C3). Treatments, where *K. africana* had the highest concentration, produced the highest zone of inhibition (C5) as compared to the treatment where *T. diversifolia* was the highest (C1). However, there was a significant difference between the control and the combined plant extracts.

DISCUSSION

Phytochemical analysis of the plant extract

This study revealed the presence of saponins, tannins, alkaloids, terpenoids, flavonoids, glycosides, phenolics and steroids in the aqueous extracts of *K. africana* and *T. diversifolia*. These are bioactive compounds with antimicrobial activity and they could have been attributed to the ability in inhibiting growth of *F. oxysporum*. Alkaloids, tannins, flavonoids and saponins display several biological properties and have been reported to have antimicrobial activity against several pathogenic microorganisms (Avato et al., 2006; John-Dewole and Oni, 2013; Rejeki and Addy, 2017). Terpenoids and phenolics on the other hand are essential oils that inhibit microbial oxygen uptake and oxidative phosphorylation (Griffin et al., 1999). Terpenoids have been isolated and tested against pathogenic bacteria and fungi and showed a high range of inhibition zones (Singh and Singh, 2003).

Kigelia africana and *T. Diversifolia* are widely distributed in nature, easily accessible and have the ability to inhibit

Table 3. Zones of inhibition of *F. oxysporum* when exposed to different ratios of the combined plant extracts of *K. africana* and *T. diversifolia.*

Treatment code	Combined ratio (ml) (*K. africana: T. diversifolia*)	Mean zone of inhibition (mm)
P1	Control	23.78
C1	0:30	9.45
C2	10:20	15.00
C3	30:30	17.25
C4	20:10	14.08
C5	30:0	16.75
	LSD	0.569
	Mean	15.83951
	CV	4.293522

the growth of pathogens due to their bioactive compounds (Rejeki and Addy, 2017; John-Dewole and Oni, 2013). They also have medicinal value and have been among many medicinal plants tested for antimicrobial activity (Neela et al., 2014). However, the majority of the studies have been conducted on human bacterial pathogens. For instance, Fasola and Iyamah (2014) found the presence of terpenoids, flavonoids, tannins, alkaloids and saponins in *Tithonia diversifolia* when used to treat malaria. Similarly, Muyenga et al. (2015) confirmed that *K. africana* extract contains terpenoids, flavonoids, tannins and saponins that were used to treat various human bacterial diseases. Furthermore, leaf extracts of *T. diversifolia* have been reported to have phytochemical and antimicrobial activities of pharmaceutical importance (John-Dewole and Oni, 2013; Ogoti et al., 2015). Besides, Odeyemi et al. (2014) revealed the presence of alkaloids, phenol, flavonoids, sesquiterpene, monoterpenes and diterpenes in the leaf extract of *T. Diversifolia* against common environmental pathogenic bacteria. This study indicates that these two plants *K. africana* and *T. diversifolia* possess antifungal activity and can be used as broad-spectrum fungicides against *F. oxysporum*. As compared to *T. diversifolia*, extracts of *K.africana* did not show the presence of alkaloids and steroids. Similar studies indicate that the fruit extract of *K. africana* contains a high amount of flavonoid and tannin content, with very low content on alkaloid compared to other plants (Rejeki and Addy, 2017; Abdulkadir et al., 2015). The absence of alkaloids in fruits has been reported by Bondjengo et al. (2017) and is attributed to differences in plant species or type of method used in extraction.

Identification of *Fusarium oxysporum*

Microscopic characteristics observed in this study such as the presence of oval non-septate microconidia and macroconidia with a slight curvature, septate, pointed apical cell and pink in colour confirm that the isolates were of *Fusarium oxysporum.* This supplements similar studies on the characterization of the fungal pathogen (Shobha and Kumudini, 2012; El Kichaoui, 2016; Bedasa, 2018).

Antimicrobial activities of the plant extract

As the concentration of *K. africana* extract increased, the mean mycelia diameter decreased (the inhibition zone increased). This supports similar research findings on the anti-fungal activity of *K. africana* against *F. oxysporum* (Itonga, 2011; Zofou et al., 2013; Rejeki and Addy, 2017). *Kigelia africana* extracts are reported to induce the disruption in fungal cell metabolism, increased permeability of fungal plasma membrane and destruction of the conidial wall structure (Al-Mujammaa'e, 2008). Organic extract of the fruit contains unique phytochemical constituents such as naphthoquinones, p- coumaric acid, ferulic acid, phenylpropanoids, kigelinone, dehydro α lapachone and lapachol, β-sitosterol, 3-dimethyl kigelin, ferulic acid and iridoids that toxic to plant fungal pathogen (Chenia, 2013; Bello et al., 2016; Chinsembu, 2019), although they were not identified in this study probably due to the use of aqueous extract alone. Water extract is not very effective on some microorganisms (Itoandon et al., 2012) because only a few phytochemicals can dissolve in water due to its low polarity (Gupta et al., 2010).

The current study revealed that an increase in the concentration of *T. diversifolia* leaf extracts reduced the mycelia growth of *F. oxysporum*. Leaf extracts of *T. diversifolia* are toxic to *F. oxysporum* and inhibit mycelial growth and spore germination (Enyiukwu et al., 2014; Adekunle, 2005). The plant has a promising broad-spectrum antimicrobial activity that has been attributed to various compounds such as aradiol, squalene and a mixture of stigmasterol and sitosterol (Tagne et al., 2018). Combined plant extracts of *K. africana* fruit and *T.*

diversifolia were more effective to inhibit the growth of *F. oxysporum* as compared to the single extract. This is because a combination of plant extract ratios increases the antimicrobial activity of the extract compared to when the plant extract is used singly (Akila et al., 2011; Mubarak et al., 2011; Bassolé and Juliani, 2012)

CONCLUSION AND RECOMMENDATION

This study investigated the extracts of *K. africana* fruit and *T. diversifolia* leaf ability to inhibit the growth of *F. oxysporum* which causes Fusarium wilt in Tomatoes. The phytochemical analysis revealed that the two plant extracts contained saponins, tannins, terpenoids, flavonoids, glycosides and phenolics except for alkaloids and steroids which were only found in *T. diversifolia*. The mean inhibitory zones ranged from 7.93 to 10.44 mm for T*. diversifolia* at 25 to 100 g/l. The mean inhibitory zones for *K. Africana* ranged from 12.07 to 15.56 mm at 25 to 100 g/l*. K. Africana* extracts had the highest inhibitory (antimicrobial) activity. Combining both extracts was more effective as compared to the single extract and this could be attributed to the synergistic effects of combining the two extracts. These plants may provide an effective measure of managing Fusarium wilt disease of tomatoes and could play an integral part in integrated pest management. However, there is a need for more studies on the isolation of the compound responsible for antifungal activity and ex-vitro experiments to test the efficacy of these plant extracts on the field. This study provides scientific evidence that *K. africana* and *T. diversifolia* extract possess antifungal activity and can be used as a broad-spectrum in managing microbial diseases.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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