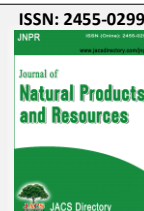




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Effects of Solvent Extracted Bioactive Compounds from the Bark, Roots and Leaves of *Croton jatrophioides* on Tomato Wilt Disease (*Fusarium oxysporum f. sp. lycopersici*)

A. Kinyoda¹, M. Mghalu², P. Guyo³, S. Muti^{1,*}

¹Department of Crop Sciences, Pwani University, P.O Box 195-80108, Kilifi, Kenya.

²Department of Biological Sciences, Pwani University, P.O Box 195-80108, Kilifi, Kenya.

³Department of Chemistry, Pwani University, P.O Box 195-80108, Kilifi, Kenya.



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ABSTRACT

Fusarium wilt of tomatoes, caused by *Fusarium oxysporum*, is a major and most prevalent soil borne disease in tomato plants both in the field and green house. The economic impact of this disease cannot be underestimated. All recommended control strategies have proved to be ineffective in controlling the pathogen. Studies have shown that *Croton jatrophioides* has medicinal activity against the phytopathogens. This study was conducted to evaluate the efficacy of extracts from *Croton jatrophioides* to suppress *Fusarium oxysporum*. Specific objectives were to extract bioactive compounds from *C. jatrophioides* for use against *F. oxysporum*; to determine the minimum inhibition concentration of crude extract that could elucidate response against *F. oxysporum*; to test *in-vitro* the efficacy and levels of bioactive compounds extracted using selected solvents from bark, the leaves and the root; to determine interaction effects between the treatments and the crude extracts. The experiment involved the isolation of bioactive crude extracts from the roots, bark and leaves of *Croton jatrophioides* plant using three solvents, namely hexane (non-polar), ethyl acetate (moderately polar) and methanol (highly polar). The isolated crude extracts from each solvent were concentrated using rotary evaporator, then purified using micro-filters. These extracts were then used for testing their antifungal activity in Potatoes Dextrose Agar (PDA) petri-plates containing *Fusarium oxysporum* inoculum. The results indicated that the quantity of the solvent required for exhaustive extraction of bioactive compounds from the ground material was at 180 mL. The minimum inhibition concentration that elucidated response against *F. oxysporum* was found to be 50.0 mg/mL. Non-polar solvents such as hexane and ethyl acetate were found to have the highest abilities in extracting bioactive compounds from *C. jatrophioides* materials since most of these compounds that had antifungal activity were non-polar. Hexane derived extracts had the highest significant efficacy in control of *Fusarium oxysporum*, comparable to Rindomil, a positive control. Dimethyl sulfoxide (DMSO) had the least effect on *Fusarium oxysporum*. In the absence of hexane, ethyl acetate was the second most suited solvent for extraction of bioactive compounds from *C. jatrophioides*. Methanol had the least abilities in extraction of bioactive compounds from *C. jatrophioides*. There were interaction effects between the parts of the plants from which bioactive compounds were derived and the type of solvents used.

1. Introduction

Tomato is one of the most important vegetables in the world that is consumed in almost every house-hold [1]. Its demand and therefore production has been on the increase due to the ever-increasing population. However, tomato production faces a myriad of challenges among which *Fusarium oxysporum f. sp. lycopersici* disease is the most debilitating and economically serious disease of tomatoes both in the field and greenhouse [2, 3]. *Fusarium oxysporum f. sp. lycopersici* is a soil borne fungus which can persist for many years in the soil without a host. Its inoculum is transmitted through the air or carried in plant residues. It attacks any parts of the plant including both the rooting system and the stems of susceptible plants, especially most solanaceae family causing blockage of xylem, the vascular water transmitting vessels, resulting in wilting of the entire plant [4]. Most infections originate from the population associated with infected tomato debris [5]. *Fusarium* wilt (*Fusarium oxysporum*) also affects a wide variety of other plant species of any age, including, tobacco, legumes, cucurbits, sweet potatoes, banana and even other herbaceous plants [6].

Due to this Kilifi county and coastal region as a whole has been a net importer of tomatoes from other upcountry regions of Kenya. Hence the study would give a way forward in terms of containing the disease and

enhancing tomato production and consumption in the region thereby moving towards attaining sustainable development goals numbers one, two and three of poverty eradication, ensuring no hunger and maintaining good health and well-being of the community [7].

Tomato is the second most important vegetable in the world after Irish potatoes and is grown in almost all the countries [1]. Therefore, this tasty and highly nutritious vegetable is a must-have vegetable in almost all the kitchens in Kenya. Therefore, any effort towards effective control of the pathogen is thus a highly welcome move since *Fusarium* wilt is among the main soil borne systemic disease that causes significant economic losses in tomatoes [3, 8, 9]. Over the years, continuous application of systemic fungicides commonly used against *Fusarium oxysporum f. sp. lycopersici* had a negative impact on the environment, and biodiversity of most agro-ecosystems [2, 6]. Also, use of biological, chemical and cultural control and other treatment measures have either been unsuccessful, less effective or still at trial stages. In the recent past, extracts from medicinal plants that produce secondary metabolites have proved to be effective against pathogenic fungi, bacteria, parasites and viruses in plants and also in animals. In the recent past, extracts from medicinal plants that produce secondary metabolites have proved to be effective against pathogenic fungi, bacteria, parasites and viruses in plants and also in animals. With the advent of new novel science for extraction and use of plant bioactive compounds against fungal diseases, this would go a long way in saving the environment and fragile ecosystems against pollution from synthetic pesticides [4]. Therefore, use of natural products extracted from potential medicinal plants to control plant diseases would save the natural environment from the negative impacts of use of synthetic pesticides,

*Corresponding Author: s.muti@pu.ac.ke (Simon Muti)

thereby help in protecting its flora and fauna, and attaining millennium development goal number seven (7) of living in a sustainable environment [4, 7, 10]. *Croton jatrophioides* is one such plant whose bioactive compounds have been postulated to have inhibitory effects against Fusarium wilt disease of tomatoes [11–13]. However, little research has been done on this plant regarding its effectiveness in control of Fusarium wilt disease of tomato. It is in this regard that this study was conceived to evaluate the efficacy of solvent extracted bioactive compounds from *Croton jatrophioides* plant using selected polar and non-polar solvents against fusarium wilt disease of tomato, namely *Fusarium oxysporum* f. *Sp lycopersici* fungus. Thus, the new knowledge gained on the use of extracts from *Croton jatrophioides* as an antifungal against *Fusarium oxysporum* f. *sp lycopersici* would go a long way in improving tomato production and therefore food security especially in Kilifi County, leading to a healthy and wealthy nation which would enhance eradication of hunger and poverty and lead to attainment of millennium development goal number one [7]. With the current trend to near zero-market tolerance for pesticide residues in fresh vegetables and fruits, the majority of the population is going for safe foods obtainable in organic farming. This has provided an additional impetus and motivation for search on non-chemical means to control pests and diseases through use of natural phyto-metabolites [10]. Therefore, exploring alternative means to control the pathogen by use of an extract from a medicinal plant *Croton jatrophioides* is expected to draw a lot of interest among the end-users. Also, the findings would provide scientific data that would give credible support towards their conservation and cultivation for their phyto-chemical value in control of plant diseases [12].

Kilifi county in Coastal region of Kenya, is blessed as ecological home of both Neem tree and *Croton jatrophioides*, natural resources that can be exploited to solve local pests and disease problems, with minimal costs and minimal effects on the environment. Langat [13] and Nihei [14] in their studies showed that extracts from *Croton jatrophioides* (Msinduzi) tree had indications of insecticidal and fungicidal activities attributed to presence of limonoids that are biologically active. However, a major threat to *Croton* plants is risk of extinction due to rapid loss of their natural habitats from uncontrolled human activities [15]. This calls for urgent documentation of their phyto-chemical properties and values. Therefore, this study was also designed to document characteristics and physicochemical properties from the leaves, bark and roots of *C. jatrophioides* with a view to contributing to the knowledge of this important medicinal plant for its pharmacognostic identification, preservation and standardization.

2. Experimental Methods

2.1 Characteristics of the Study Site

The research was conducted at Pwani University, Chemistry and Biological Science Laboratories from April to July 2016 and 2017. Pwani University is located 60 km from Mombasa and from Malindi and lies at an altitude of about 30 m above sea level, and at latitude 3.6° S and longitude 39.8° E [16, 17]. The region experiences temperatures in the ranges between 21 °C and 32 °C and receives bimodal type of rainfall, where long rains occur during the months of March to July, and short rains in September to December, with annual total rainfall of about 1100 mm [17]. The cool seasons occur during rainy periods between mid-April to end of June, due to presence of overcast clouds associated with occurrence of ITCZ in the region [18]. Relative humidity tends to be relatively high for most parts of the year due to nearness to the sea, except during the peak dry periods of August to March when the sun is too hot. The soils in the region are predominantly sandy loam with pockets of clayey soil [19].

2.2 *Croton jatrophioides* Plant Samples Sources and Preparation

Leaf, bark and root samples of *Croton jatrophioides* plant were sourced from Bamba area, 50 km away from Kilifi town in Kilifi County. The bark, root and leaf samples were then air dried under shade for two weeks at room temperature, then cut into small pieces and ground into powder using an electric blender (for leaves) and hammer mill (for barks and roots) at Pwani University micro-biological laboratory as described by Ndunda *et al.* [20] and Gichui *et al.* [21]. It is from these air dried powdered samples of the bark, leaves and roots from where the bioactive compounds were extracted. In this study, solvent extraction method was used with the solvents hexane, ethyl acetate and methanol.

2.3 Methodology for Isolation of *Fusarium oxysporum* f. *sp. lycopersici* inoculum

The operation was undertaken in the clean bench. Diseased tomato plants infested with *Fusarium oxysporum* f. *sp. lycopersici* pathogen were

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obtained from infested tomato field at Pwani University farm. The diseased plants were washed in sterilized distilled water (SDW) and cut into small portions. The cut portions were immersed in 70% ethanol for 10 seconds. The pieces were then removed and washed successively in 3 different containers each containing SDW. The cut pieces were then transferred into petri plates containing PDA media and incubated for 5 days. Using a clean and sterilized stock borer, a plug was incised from the developed mycelium, plugged into a PDA slant and some on petri plates (containing PDA media) and then incubated at 25 °C for 4 days. The developed mycelium was transferred and stored in the fridge set at 20 °C for next use.

2.4 Methodology for Acquisition of *Croton jatrophioides* Plant Materials

Acquisition and preparation of plant materials (roots, stem bark and leaves) was done as described by Mbwapo *et al.* [15]. Plant materials weighing 15 kg of roots (root barks); 15 kg of stem barks and 22.5 kg of leaves were air-dried as follows: roots (root barks) and stem barks were air dried from 6th /06/14 to 21st /06/14 (i.e., 15 days to dry). Leaves were dried from 13th /06/14 to 21st /06/14 (i.e., for 8 days to dry). Dry weight of the plant samples was: Roots (root barks) 6.5 kg; stem barks (barks) 7 kg and leaves 6 kg. The materials were ground into powder form using a hammer mill. The weight of the ground samples was: Root powder samples 4.7 kg; bark (stem barks) powder samples 6.2 kg and leaf powder samples 4.25 kg. The moisture content of all the ground powder materials were separately determined and packed into 1 kg zip lock bags, ready for extraction of bioactive compounds levels.

2.5 Methodology for Extraction of Bioactive Compounds from *Croton jatrophioides*

30 g of each of the plant sample, Root (R), Bark (B) and Leaf (L) were weighed separately. Each of the three (3) samples were separately soaked in three (3) different solvents, namely hexane (H), ethyl acetate (E) and methanol (M) to aid in the solvation and extraction of the relevant crude bioactive extracts (Fig. 1). The quantity of the solvent required for soaking the plant material to effectively effect the extraction process was applied on trial and error basis at different levels, as follows: Soaking the 30 g in 90 mL, 120 mL, 150 mL, 180 mL, and 210 mL solvents for 24 hours and 48 hours (Table 1). This gave a total of 45 solvent extracted samples (coded as: Hexane-Root extract (HR), Ethyl acetate Root extract (ER), Methanol Root extract (MR); Hexane-Bark extract (HB); Ethyl Acetate-Bark extract (EB), Methanol-Bark extract (MB); Hexane-Leaf extract (LH); Ethyl Acetate-Leaf extract (LE) and Methanol Leaf extract (ML). The contents were soaked in reagent bottles which were then put on hot plates. Magnetic stirrers were immersed in each bottle to effect the stirring and thus accelerate the extraction process. After 48 hours, the solution was decanted, and re-soaking done using similar solvents. Entire extraction process thus took 96 hours (Table 1). After exhaustive extraction, the samples were separately concentrated in the Rotary evaporator at the Pwani University laboratory. Filtration of crude bioactive extracts was also done. After concentration, 25mg were separately dissolved in 10 mL of mixtures of DMSO and sterilized distilled water (SDW) in a ratio of 10:90, then filtered with what-man filter paper. Using vertpunctures/ vertpure filters mounted onto a syringe the contents were further micro-filtered into sterile vials, labelled and stored at -20 °C to await next use on determination of minimum inhibition concentration and determination of efficacy of the crude extracts against *Fusarium oxysporum*.

Table 1 Composition of exhaustive extraction of bioactive compound

S. No.	Type of solvent	Quantity of solvent used to exhaustive extraction (mL)	Quantity of solvent used to exhaustive extraction (mL)	Material dissolved	Extracts produced
1	Hexane	90	120 150 180	Bark; Root; leaves	Bark-Hexane (HB) Root Hexane (HR) Leaf-Hexane (HL)
2	Ethyl acetate	90	120 150 180	Bark; Root; Leaves;	Bark-Ethyl acetate (EB) Root Ethyl acetate (ER) Leaf- Ethyl acetate (EL)
3	Methanol	90	120 150 180	Bark; Root; Leaves	Bark-Ethyl acetate (MB) Root Ethyl acetate (MR) Leaf-Ethyl acetate (ML)

2.6 Methodology for Determination of Minimum Inhibition Concentration of Crude Extracts

Using the micro-filtered crude extracts in the sterile vials, different levels of concentrations of extracts were constituted through serial dilution and each screened with the aim of determining the concentration

levels that elucidated peak reaction against *F. oxysporum* f. sp. *lycopersici*. The dilutions ranged from 6.25 mg/mL to 56.25 mg/mL, where optimum dilution was observed at 50 mg/mL for all solvents. These serial dilutions of the extracts for each solvent were tested *in-vitro* and screened for their activity against cultured fungal pathogen in petri-plates. Each concentration tested comprised of 8 levels of dilutions for each number of plant parts (root, bark and leaves) and then replicated 3 thrice to give a total of 72 petri plates. Allocation of the treatments among and within the petri-plates was completely randomized, with three replications in each set of experiment.

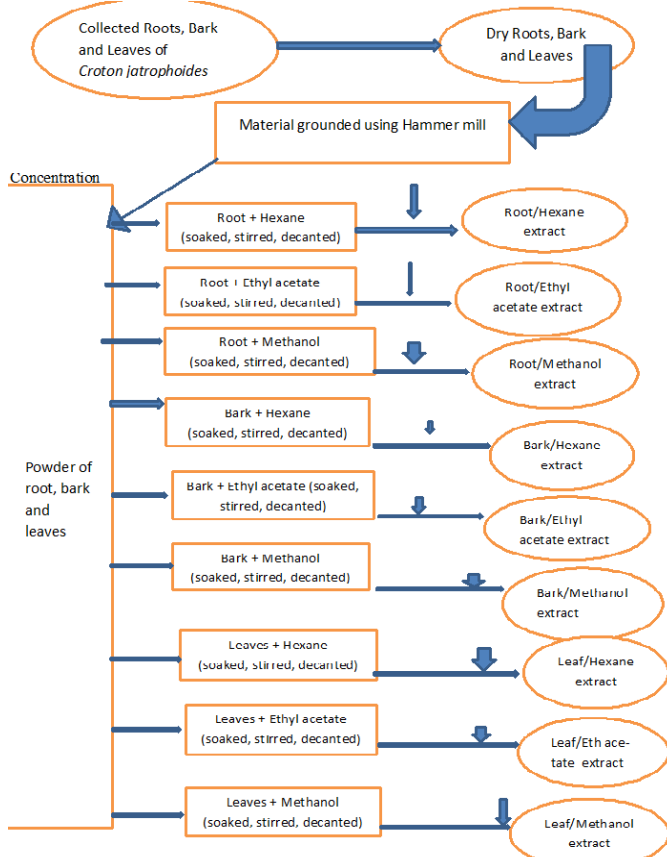


Fig. 1 Summary of protocol for extraction of crude extracts from *C. jatrophaoides*

2.7 In-Vitro Testing of the Efficacy and Levels of Bioactive Compounds Extracted from *Croton jatrophaoides*

The 4-day sub-cultured inoculum of *F. oxysporum* f. sp. *lycopersici* pathogen was centrally mounted in the petri-plates. All the crude bioactive extracts were constituted at a dilution of 50 mg/mL, since this level of dilution proved to be the most effective as the minimum inhibition concentration. The extracts were separately mounted on 5 sterilized discs in Petri-plates which were placed at equidistant positions to each other at the edges of the petri plates (Table 2). Each petri plate contained the following extract:

Petri-plate No. 1: Root extracts

This tested root extracts and contained: Root/Methanol (RM); Root/Ethyl acetate (RE); Root/Hexane (RH); Metalaxyl/Ridomil (F), a positive control; and DMSO (D), a negative control.

Petri-plate No. 2: Bark extracts

This tested bark extracts and contained: Bark/Methanol (BM); Bark/Ethyl acetate (BE); Bark/Hexane (BH); Metalaxyl/Ridomil (F), a positive control and DMSO (D), a negative control.

Petri-plate No. 3: Leaf extracts

This tested leaf extracts and contained: Leaf/Methanol (LM); Leaf/Ethyl acetate (LE); Leaf/Hexane (LH); Metalaxyl/Ridomil (F), a positive control and DMSO (D), a negative control.

Allocation of treatments within the petri-plates was completely randomized, with three replications for each set of the three (3) petri-plates to give a total of 9 petri plates. In every petri-plate, the 5 extracts surrounded the centred *Fusarium oxysporum* f. sp. *lycopersici* pathogen (Table 2).

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Table 2 Summary of treatments of bioactive extracts from the bark, root and leaves with positive and negative standard controls of Ridomil and DMSO/SDW respectively for screening against fusarium oxysporum

Source of Sample	Crude extract test sample (at dilution rate of 50 mg/mL)			Positive control	Negative control
Root sample	Hexane Root extract (H _{R1})	Ethyl acetate Root extract (E _{R3})	Methanol Root extract (M _{R3})	Metalaxyl/Ridomil (F) at 2.5 mg/mL	DMSO at 10% and SDW at 90%
Bark sample	Hexane Bark extract (H _{B1})	Ethyl acetate Bark extract (E _{B3})	Methanol Bark extract (M _B)	Positive control	Negative control
Leaf sample	Hexane Leaf extract (H _{L1})	Ethyl acetate Leaf extract (E _{L3})	Methanol Leaf extract (M _L)	Metalaxyl/Ridomil (F) at 2.5mg/ml	DMSO at 10% and SDW at 90%

All the collected data was subjected to analysis of variance and the means of the test values obtained compared against the control values for significance

3. Results and Discussion

3.1 Moisture Content of Ground Sample Materials from Bark, Roots and Leaves of *C. jatrophaoides*

The results of moisture content of *C. jatrophaoides* ground materials of the bark, roots and leaves indicate that the bark had highest levels of moisture content of 4.22% followed by the leaves samples with 3.81% while the roots had the least moisture content of 2.67% (Table 3). Both the bark and the leaves had significantly higher moisture content than the roots. Thus, the bark dried materials of *C. jatrophaoides* for extraction of bioactive compounds had 36.7% and 9% more moisture content than the root and leaves respectively. This could have been attributed to the fact that the bark has high bulk density and more mass of tissues of xylem and phloem compared to the roots and the leaves.

Table 3 Moisture content of ground plant samples of bark, root and leaves of *C. jatrophaoides*

S. No.	Plant material	Moisture content (%)	Average moisture content (mc) of <i>C. jatrophoides</i> (%)
1	Root sample –	Sample 1 2.74%	2.67%
		Sample 2 2.44%	
		Sample 3 2.82%	
2	Bark/stem bark -	Sample 1 4.08%	4.22%
		Sample 2 4.09%	
		Sample 3 4.48%	
3	Leaf sample -	Sample 1 3.75%	3.81%
		Sample 2 3.89%	
		Sample 3 3.77%	

However, the dried leaf materials of *C. jatrophaoides* had 29.9% more moisture content than the roots. The moisture content of the dried ground samples was in the order bark > leaves > roots (Table 3). Thus, the results of crude extracts indicated that the bark and the roots had highest levels of bioactive compounds when extracted using Hexane, while the leaves had the least levels of bioactive compounds.

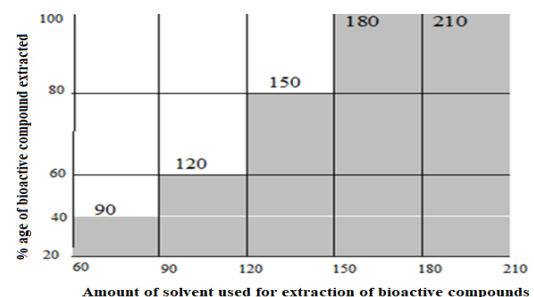


Fig. 2 Amounts of solvent used for exhaustive extraction of bioactive compounds from ground samples of *C. jatrophaoides*

3.2 Amounts of Solvents Used for Exhaustive Extraction of Bioactive Compounds from *C. jatrophaoides*

Results of extraction of bioactive compounds from *C. jatrophaoides* using the various solvents, namely Hexane, ethyl acetate and methanol indicated that exhaustive extraction of bioactive compounds was observed after using 180 mL of the solvent that took 96 hours, as confirmed by TLC (Thin layer chromatography) (Fig. 2). Any further addition of the solvents did

not yield any significant amounts of bioactive compounds. This suggest that 180 mL was the most economical and efficient amounts of solvent for complete extraction of bioactive compounds from *C. jatrophoides* samples.

3.3 Properties of Crude Extracts Obtained from *C. jatrophoides* Materials

The results indicate that hexane, a non-polar solvent, derived crude extracts were observed to be oily and insoluble in water. However, ethyl acetate which was moderately polar had its derived crude extracts being waxy and fairly soluble in water (Table 4). Methanol which was highly polar had its derived extracts being highly soluble in water.

Table 4 Properties of crude extracts obtained from *C. jatrophoides* material samples using selected solvents

Batch	Solvent	Nature of solvent	Properties
1 - 3	Hexane: Root samples (RH) Bark samples (BH) Leaf samples (LH)	Non-polar	Oily and least soluble in water
4 - 6	Ethyl acetate: Root samples (RE) Bark samples (BE) Leaf samples (LE)	Moderately polar	Waxy and fairly soluble in water.
7 - 9	Methanol: Root samples (RM) Bark samples (BM) Leaf samples (LM)	Very/highly polar	Soluble in water

3.4 Minimum Inhibition Concentration Given by Serial Dilution

The results on various serial dilutions against activity on *Fusarium oxysporum* showed that from concentrations of 6.25 mg/mL up to 43.75 mg/mL. The activity against *Fusarium oxysporum* was observed to be on gradual increase, attaining a sharp peak at 50 mg/mL (Fig. 3). However, dilutions beyond 50.0 mg/mL, resulted in drastic decline in activity against *F. oxysporum*. This indicates that serial dilutions of 50.0 mg/mL of the crude extracts elucidated the highest response against *Fusarium oxysporum* growth. Concentration lower than and above 50 mg/mL gave negative results (Fig. 3). This would appear to be the most effective and economical diluent of the extract to elucidate action against *Fusarium oxysporum*. As such, this reflects the minimum inhibition concentration for the specific crude extracts

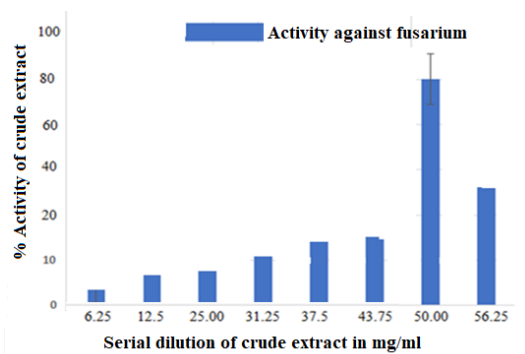


Fig. 3 Serial dilution with highest activity against fusarium oxysporum, giving minimum inhibition concentration

3.5 Comparison of Activity of Bioactive Compounds Extracted from the Bark, Leaves and Root of *C. jatrophoides*

The results of both ANOVA and Fisher's pairwise comparison indicate that the levels of bioactive compounds in the bark, leaves and roots from where the phytochemicals were extracted were not significantly different at $P \leq 0.05$ (Tables 5 and 6, and Fig. 4). The P value was 0.279, which was much greater than the set value of 0.05 significance level.

The results in Table 5 and Fig. 4 indicate that the mean values of concentration of bioactive compounds in the bark, roots and leaves were comparable at 5% level of significance. This is despite the fact that the bark had 18.4% more bioactive compounds than the leaves, and 7.2% more bioactive compounds than the roots.

Table 5 Analysis of variance for treatments and crude extracts from *C. jatrophoides*

Source	DF	Dj SS	Adj MS	F-Value	P-Value
Tx	2	0.8236	0.4118	1.50	0.279
Crude extract	4	6.8360	1.7090	6.24	0.014
Error	8	2.1895	0.2737		
Total	14	9.8492			

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Table 6 Fisher's pairwise comparison of levels of concentration of bio-active compounds in the bark, root and leaves of *C. jatrophoides*

Treatment (sample source)	N-Number of Samples	Concentrations of bio-active compounds
Bark	5	9.11a
Root	5	8.45a
Leaf	5	7.43a
Mean	5	8.33
P-Value (5%)		0.279
Verdict		NS

^aNot significantly different at 5% level of significance

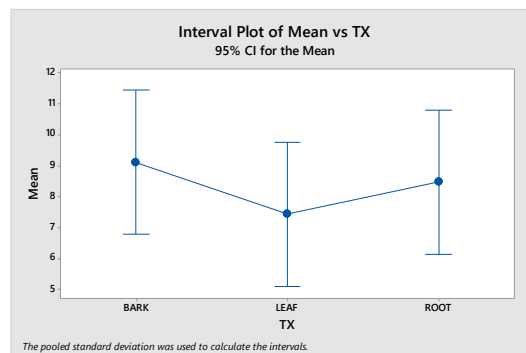


Fig. 4 Concentration of bio-active compounds extracted from the bark, leaves and roots of *Croton jatrophoides*. TX= treatment

3.6 Efficacy of Solvent Extracted Bioactive Compounds of *C. Jatrophoides* on *Fusarium oxysporum*

The analysis of variance (ANOVA) and Fisher's pairwise comparison results on the activity of bio-active compounds in crude extracts derived using the different solvents, indicate that the P-Values for the crude extracts were 0.014, much less than the set value of 0.05 level of significance (Tables 5 and 6). This indicates that there were significant differences in activity against *F. oxysporum f. sp. lycopersici* among the crude extracts derived using the different solvents namely, hexane, ethyl acetate and methanol.

Table 7 Fisher's pairwise comparison on efficacy of bioactive compounds in crude extracts derived using Hexane, ethyl acetate and methanol as compared to activity of Ridomil and DMSO against *Fusarium oxysporum f. sp. Lycopersici*

Crude extract	Polarity	Mean activity
Hexane (H)	Non-polar	11.4a
Ethyl acetate (E)	Semi-polar	7.9bc
Methanol (M)	Polar	7.3bc
Ridomil (R)	Non-polar	9.2ab
DMSO (M)	Semi-polar	5.9c
Mean		8.3

Means that do not share a letter are significantly different at 5% level of significance Letters a-c, next to a value indicate level of significant differences between the values

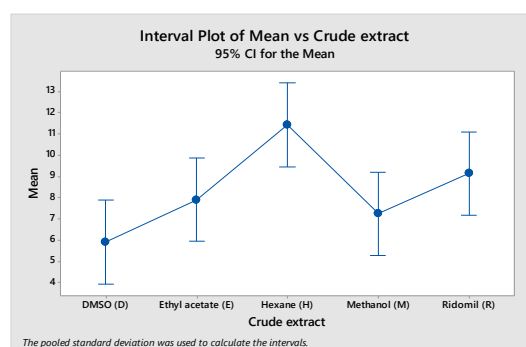


Fig. 5 Main effects (efficacy) of different bio-active crude extracts and controls on *Fusarium oxysporum f. sp. lycopersici*

Hexane derived crude extracts were significantly 30.7% and 36% more effective ($P \leq 0.05$) against *Fusarium oxysporum f. sp. lycopersici* compared to crude extracts derived using Ethyl acetate and Methanol, respectively, and the activity of DMSO, a negative control (Tables 7 and Fig. 5). However, hexane derived crude extracts had comparable activity to Ridomil, the positive control. Ridomil, a commercial fungicide had second highest suppressive effects after Hexane extracts against *fusarium oxysporum*, but had more superior effects when compared to ethyl acetate, methanol and DMSO. DMSO had the least suppressive effects on *fusarium oxysporum*

compared to all other treatments. Ethyl acetate and Methanol derived crude extracts had comparable effects against *Fusarium oxysporum* f. sp. *lycopersici* while DMSO had significantly the least effects against *Fusarium oxysporum* f. sp. *lycopersici* (Table 7 and Fig. 5). It is notable that Ridomil was significantly more effective ($P \leq 0.05$) compared to DMSO, both of which were controls. However, Ridomil had comparable effects to ethyl acetate and methanol derived crude extracts (Table 7 and Fig. 5). Part of the differences in activity against *Fusarium oxysporum* f. sp. *lycopersici* could be attributed to the types of compounds extracted from *C. jatrophioides* and the synergistic activity from combination of the solvent and the bioactive compounds polarities.

3.7 Interaction Effects between Treatments and Crude Extracts

The study indicated that the highest interaction effects of hexane extracts against *Fusarium oxysporum* was observed on extracts from the bark, followed by extracts from the root and least from the leaves (Fig. 6). That is the highest levels of bioactive compounds were extracted using Hexane from the bark followed by extracts from the root, and least from the leaves. Indeed, Hexane as a solvent was able to extract 42.9 % more bioactive compounds from the bark than ethyl acetate (Fig. 6). Hexane also had the highest extraction abilities of bioactive compound against *Fusarium oxysporum* f. sp. *lycopersici* from the root compared to ethyl acetate. Therefore, from the three solvents, Hexane had significantly the highest abilities of extracting highest levels of bioactive compounds (14 mg/mL) from the bark and the root (11 mg/mL) of *Croton jatrophioides*.

The results also indicate that ethyl acetate solvent had the second highest level of interaction effects compared to methanol treatments. Ethyl acetate extracted the highest amounts of bioactive compounds from the root than from the bark. Thus, ethyl acetate extracted 40% more bioactive compounds from the root than the 20% from the bark. The highest interaction effects of Ethyl acetate extracts against *Fusarium oxysporum* was observed on extracts from the root, followed by extracts from the bark and least from the leaves (Fig. 6). This suggests that the highest levels of bioactive compounds extracted using ethyl acetate were from the root followed by extracts from the bark, and least from the leaves. This implies that Ethyl acetate solvent had the second highest abilities of extracting bioactive compounds from the root (10 mg/mL) and from the bark (8 mg/mL). A comparison of extraction abilities of bioactive compounds that could act against *Fusarium oxysporum* f. sp. *lycopersici* from the bark of *Croton jatrophioides* shows that hexane and ethyl acetate were the most suited since they exhibited highest extraction potential. Although methanol had the least extraction abilities of bioactive compounds from *C. jatrophioides* from the bark and the root, when compared to hexane and ethyl acetate, it had superior extraction abilities of bioactive compounds from the leaves compared to ethyl acetate (Fig. 6).

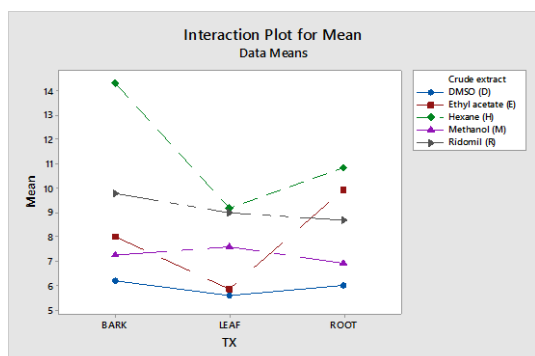


Fig. 6 Interaction effects between the sources of crude extracts, namely the bark, leaves and roots and concentrations/activity of bioactive compounds in crude extracts derived using solvents hexane, ethyl acetate and methanol and activity of Ridomil and DMSO

The highest interaction effects of hexane extracts against *Fusarium oxysporum* was observed on extracts from the bark, followed by extracts from the root and least from the leaves (Fig. 6). This suggests that the highest levels of bioactive compounds were extracted using hexane from the bark followed by extracts from the root, and least from the leaves.

3.8 Discussions

3.8.1 Extraction of Bioactive Compounds from *Croton jatrophioides* for Use against *F. oxysporum* f. sp. *lycopersici*

3.8.1.1 Moisture Content of Ground Materials from Bark, Roots and Leaves

From the study it is evident that of the powdered dried samples, the bark had the highest levels of moisture content followed by the leaf samples while the roots had the least moisture content. Thus, the bark

dried samples of *C. jatrophioides* for extraction of bioactive compounds had 36.7% and 9% more moisture than the root and leaves, respectively. However, the dried leaf materials of *C. jatrophioides* for extraction of bioactive compounds had 29.9% more moisture content than the roots. The moisture content of the dried ground samples was in the order: Bark > Leaves > roots. This could have been attributed to the fact that the bark and roots had high bulk density and more mass of tissues of xylem and phloem and depositions of calcium oxalate crystals compared to the leaves which did not have depositions of calcium oxalate crystals and lignified scleroids. This study is in agreement with similar observations by Adeniran *et al.* [16] who in his studies on *Spondias mombin* plant stem and roots, reported presence of compacted layers of periderm, lined with cork cortex layers that were characterized by numerous prismatic calcium oxalate crystals, starch grains, xylem fibre and thickened scleroids appearing as concentric rings and lignified, which were absent in the leaves.

The dried tissue level of moisture content, a physico-chemical parameter is regarded as an indicator of extractive value, and bears a resemblance of weights of chemical constituents from the crude extract [16]. Kunle *et al.* [17] observed that in general, a high extractive value suggests a better extraction of phytochemicals from the plant material and that extractive values aid in selecting the best solvent that will further assist in having optimum yield of bioactive compounds. In similar studies, Adeniran *et al.* [16] observed that the amount of moisture content in a crude dried sample becomes significant during storage. According to Kunle *et al.* [17], the lower the moisture content in a natural herbal drug material, the less likely is the microbial contamination, and this reduces and prevents spoilage of herbal medicines during storage and extraction. Specifically, the British Pharmacopoeia [18] recommends moisture content of not more than 14% for crude dried samples for storage and the possibility of microbial contamination and degradation will be minimized during storage. Adeniran *et al.* [16] reported that the physicochemical parameters, such as extractive values of moisture content of a powdered sample could be used as pharmacognostic standards which is useful in the preparation and compilation of monograph for the identification of the leaf, bark and root of a study plant such as *Croton jatrophioides*, thus contributing to the knowledge of its collection and preservation. Thus, the values of the moisture content of the dried samples of *Croton jatrophioides* obtained in this study can be considered for pharmacognostic standards for purposes of preparing and compiling a monograph for the identification of the leaf, bark and root of *Croton jatrophioides*, an important contribution to the knowledge and perhaps classification of *C. jatrophioides*.

3.8.1.2 Extraction of Bioactive Compounds from *C. jatrophioides*

From the study, it was evident that the amounts of solvents required for complete and exhaustive extraction of bioactive compounds from *C. jatrophioides* using the various solvents, namely hexane, ethyl acetate and methanol was 180 mL as confirmed by thin layer chromatography (TLC). Any further addition of the solvents did not yield any significant amounts of bioactive compounds. This suggests that 180 mL was the most economical and efficient amounts of solvent for complete extraction of bioactive compounds from *C. jatrophioides* samples. Soquetta *et al.* [19] reported that the yield and the amount of the extract obtained from a powdered dried plant sample depend on several other factors such as the type of extract, temperature, extraction time and method, and there was no single method or solvent that was ideal for all situations. Soquetta *et al.* [19] observed that the efficiency of conventional extraction methods depends on the choice of solvent and the polarity of the compound and the solvent. The polarities of compounds vary and it is difficult to develop a single method for the efficient extraction of all compounds. Thus, the amounts of solvents used for exhaustive extraction of bioactive compounds from *C. jatrophioides* were depended on the prevailing conditions at time of extraction, among them, temperature, pressure, solvent types and concentrations among other factors. It is also important to take cognizance of observations by Muhamad *et al.* [22] who observed that different solvent systems could give or extract different chemical compounds; and that polar solvents such as water, ethanol, and methanol are common solvents for extraction of phenolic compounds, while nonpolar solvents such as hexane, chloroform and petroleum ether are used for oil and fats extraction.

3.8.1.3 Properties of Crude Extracts Obtained from *C. jatrophioides* Materials

The study revealed that the crude extracts derived using hexane solvent were oily and insoluble in water, while ethyl acetate derived crude extracts were waxy and fairly soluble in water. However, methanol extracts were highly soluble in water. In this study Hexane which is liquid at room temperature was used as solvent. It is hydrophobic in nature and

a non-polar solvent. It therefore dissolved and extracted non-polar bioactive compounds. On the other hand, Ethyl acetate is known to have medium polarity for both polar and non-polar compounds and therefore due to its biphasic actions, it is able to extract both polar and non-polar compounds, hence its crude extracts appeared to be both waxy and fairly soluble in water. These findings are in agreement with those observed by Jain *et al.* [23], who reported that the types of bioactive compounds dependent on the type of solvents used and their polarity.

The methanol extracts were observed to be highly soluble in water. Methanol has been described as being highly polar, and therefore dissolves polar compounds which are normally soluble in water, which is in agreement with observations of this study. These findings are consistent with those observed by Altemimi *et al.* [24] and Kathare *et al.* [25] who reported that polar solvents dissolve polar compounds and non-polar solvents dissolve non-polar compounds; and that polar solvents such as water, ethanol, and methanol are common solvents for extraction of phenolic compounds, while nonpolar solvents such as hexane, chloroform and petroleum ether are used for extraction of phenolic compounds, oils and fats. Soquetta *et al.* [19] observed that the efficiency of conventional extraction methods depends on the choice of solvent system, the polarity of the bioactive compound and the solvent, solubility, hydrophilic or hydrophobic properties of the bioactive compound and the prevailing conditions of temperature and pressure during the process of extraction. This therefore, explains why the crude extract from hexane solvent appeared oily and least soluble in water, while those extracts derived using ethyl acetate (which is both partially polar and partially non polar) were waxy and fairly soluble in water, while those extracts derived using methanol were highly soluble in water.

3.8.2 Determination of Minimum Inhibition Concentration of Crude Extracts

3.8.2.1 Results of Serial Dilution for Determination

The study shows that serial dilutions of 50 mg/mL elucidated the highest activity against *Fusarium oxysporum*. Any dilutions below or above 50 mg/mL resulted in drastic decline in activity against *Fusarium oxysporum* and gave negative results. This suggests that serial dilutions of 50.0 mg/mL of the crude extracts elucidated the highest response against *Fusarium oxysporum* growth. This dilution would appear to be the most effective and economical diluent of the extract to elucidate action against *Fusarium oxysporum* and reflects the minimum inhibition concentration for the specific crude extracts.

3.8.3 Comparison of Bioactive Compounds of *C. jatrophioides* Extracted from the Bark, Leaves and Root

The study showed that the mean levels of concentration of bioactive compounds (secondary metabolites) in the bark, leaves and roots were comparable. This could have been attributed to the fact that the sites where these bio-active secondary metabolites are synthesized are located in different parts of the plant, then re-distributed to various parts of the plant depending on the stimuli generated as a result of pest or disease activity or vagaries of the season. This therefore, suggests that once the bioactive compounds are synthesized in the production sites, mechanisms exist in plants that ensure that they are immediately re-distributed to all parts of the plant, including the roots, bark and leaves, either through the xylem or phloem tissues, to assure uniform distribution in the plant. These observations are in agreement with observations made by Isah [26] and Pavarini *et al.* [27] who observed that biosynthesis of secondary metabolites and compound storage occurs in specialized plant structures which are important to consider when searching for important shifting levels of bioactive compounds in various parts of the plant. These bioactive compounds in most cases serve to protect and increase the immunity of the plants against injurious pests and diseases or harsh weather conditions and may act like shock 'proteins'. Attack on any part of the plant by a pest or diseases initiated mobilization of these secondary metabolites for defenses against the pathogen [28]. Dziadek *et al.* [29] observed that the concentration of the different bioactive compounds in *C. alba* tree were different among the different parts of the plants. The leaves exhibited higher levels of α -terpineol (27.4%), eucalyptol (23.3%), and phellandrene (16.3%) which exhibited antifungal and antibacterial activities, and also insecticidal effect [49]. The bark had lower levels of these bioactive compounds. Jin [30] in their studies on cannabis plants found that cannabinoid content decreased in order from inflorescences to leaves, stem barks and roots. Cannabis inflorescence and leaf material were found to contain sufficient cannabinoids, mono- and sesquiterpenoids, and flavonoids while the stem, bark and roots were found to be important sources of triterpenoids and sterols. The findings of this study appear to contradict the findings by the above stated authors. However, the occurrence, concentration, variability and distribution of the various secondary metabolites in plants are reported to be influenced or

dictated by seasonality, occurrence of ultradian and circadian rhythms in the plants, seasonal variation in both biotic and abiotic stress signals and such factors as light, stress, drought, the age and stages of growth of the plants, insect attack, mechanical damage, fungal infection, among others [26-28]. However, the fact that the mean values of concentration of bioactive compounds (secondary metabolites) in the bark, roots and leaves of *Croton jatrophioides* were observed to be comparable could also have been influenced by the prevailing external environmental, abiotic or biotic stimuli that elicited fair distribution of the bioactive secondary metabolites within the whole plant at that particular time of the growing season and harvesting of the plant samples. Perhaps further studies would establish whether these levels vary depending on season or stimuli.

3.8.4 Efficacy of Solvent Extracted Bioactive Compounds of *C. jatrophioides* on *Fusarium oxysporum*

This study showed that there were significant differences in activity against *F. oxysporum* f. sp. *lycopersici* among the crude extracts derived using the different solvents namely, Hexane, ethyl acetate and methanol and when compared to the activities of the control treatments, namely, Ridomil and DMSO. Part of the differences in activity against *Fusarium oxysporum* f. sp. *lycopersici* could be attributed to the types of compounds extracted from *C. jatrophioides* coupled with the variability in the polar nature of the solvents. Hexane derived crude extracts were found to be significantly more effective ($P \leq 0.05$) against *Fusarium oxysporum* f. sp. *lycopersici* (F.o.I) compared to crude extracts derived using ethyl acetate and methanol and the activity of DMSO. This could be attributed to the fact that the different solvents had different polarities and therefore different abilities to dissolve different bioactive compounds. Hexane was mainly non-polar solvent and therefore dissolved and extracted corresponding non-polar bioactive compounds, that may have contained bioactive compounds such as terpenes, alkaloids and polyphenols which have been reported to have more fungicidal effects, than those bioactive compounds derived using semi aqueous solvents such as ethyl acetate and methanol [11, 21]. Lattanzio *et al.* [31] and Bennett [32] reported that some groups of alkaloids, terpenes and polyphenols were toxic against fungi, through phyto-alexins, free radicals and deposition of lignin and tannins which inhibited fungal growth enzymes responsible for metabolic activities and acted as barriers to further growth and development. In similar extraction studies Gichui [21], Akhtar [33] and Naqvi [34] observed that crude plant extracts had tremendous potential in management of fungal plant pathogens.

In their studies, n-hexane and chloroform sub-fractions used in extraction of crude plant extracts showed the highest inhibitory effect against fungal pathogens. According to Vance *et al.* [35], the formation of lignin and tannins as a defense mechanism against fungal pathogens has long been recognized. This explains the observed significance in effectiveness of hexane derived extracts in inhibiting further growth of the fungal pathogen in the petri-dishes during experimentation. Kalia and Sharma [36] reported that resistance in pea to powdery mildew (*Erysiphe polygoni*) was strongly correlated with the concentrations of total and ortho-dihydroxyphenols in the leaves. The resistant cultivars expressed much higher levels of both phenolics and oxidative enzymes (peroxidases and polyphenoloxidases) and this created a very toxic environment in and around the infecting fungus in the tissue, brought about by very reactive phenylpropanoid free radicals and the actual process of lignification.

The study also indicated that while Hexane derived crude extracts had the highest suppressive effects on *fusarium oxysporum*, it had comparable activity to Ridomil, which was a positive control. Ridomil, a commercial fungicide had second highest suppressive effects after Hexane extracts against *fusarium oxysporum*, but had more superior effects when compared to ethyl acetate, methanol and DMSO. DMSO had the least suppressive effects on *fusarium oxysporum* compared to all other treatments. Sukul and Spittler [37] reported that Ridomil is a systemic broad spectrum fungicide used in control of many fungal diseases in plants. The chemical composition of the active ingredient is Metalaxyl, specifically mefenoxam (of the formula $C_{15}H_{21}NO_4$) which has both curative and systemic properties. Its mode of action is that being systemic, it is rapidly taken up by the green plant parts, transported upwards in the sap stream and is distributed throughout the plant, thus providing control of fungi from within the plant [37]. Its effectiveness results from inhibition of uridine incorporation into RNA and specific inhibition of RNA polymerase-1. The 2,6-dimethylphenyl group belongs to the phenolic group of secondary compounds found in plants which has anti-fungal properties and is believed to be a constituent in the *Croton jatrophioides* extracts [13,20]. The methyl N-(2,6-dimethylphenyl)-N-(methoxyacetyl) alaninate in Ridomil is an aromatic amide, a carboxamide, an ether and a methyl ester which is non-polar compound with formal charge of zero [37]. This perhaps explains the observations that Hexane derived extracts

had comparable effects to Ridomil. Ridomil, being non-polar, it exhibited similar properties to Hexane derived extracts, hence giving the observed comparable results. Also, Ridomil had comparable effects to Ethyl acetate and Methanol derived crude extracts. It is notable that Ridomil was significantly more effective ($P \leq 0.05$) compared to DMSO, both of which were controls. However, Ridomil had comparable effects to Ethyl acetate and Methanol derived crude extracts. In this study Ridomil, a fungicide, was used as a positive control while DMSO, was used as a negative control.

The study showed that ethyl acetate and methanol derived crude extracts had comparable effects against *Fusarium oxysporum* f. sp. *lycopersici* while DMSO had significantly the least effects against *Fusarium oxysporum* f. sp. *lycopersici*. The comparable effects of ethyl acetate and Methanol derived crude extracts could be explained by the fact that both solvents are polar and are miscible with water. Therefore, they were able to dissolvate similar bioactive compounds perhaps in comparable proportions, hence causing similar comparable levels of inhibition of the *Fusarium oxysporum* in the petri-plates. H. However, Ruan *et al.* [38] and Koffi *et al.* [39] had observed that ethanolic extracts of Ivorian plants extracted higher concentrations or amounts of phenolics compared to methanol, acetone and water. This could have been attributed to the fact that ethyl acetate is reportedly more non-polar and therefore dissolves most non-polar compound than methanol, which is more polar.

The study has revealed that DMSO had significantly the least effects against *Fusarium oxysporum* compared to Hexane, Ethyl acetate and Methanol derived crude extracts yet it was used as a negative control against *Fusarium oxysporum*. The reason for this is that DMSO does not have the phenolic ring structure which is responsible for inhibition of fungal growth enzymes [37]. Most bioactive compounds that have activity against *fusarium oxysporum* had the phenolic ring structure as exhibited by groups of alkaloids, terpenes and polyphenols that had been proven to be toxic against fungi, through phyto-alexins, free radicals and deposition of lignin and tannins which inhibited fungal growth enzymes responsible for metabolic activities and acted as barriers to further growth and development [35].

Presence of these phenolic compounds normally create a very toxic environment in and around the infecting fungus, which is brought about by very reactive phenylpropanoid free radicals and the actual process of lignification making its growth impaired. Thus, DMSO, which is an organosulfur compound with the formula (C_2H_6OS or $CH_3-SO-CH_3$) in its chemical structure and composition does not have phenolic derivatives that are responsible and effective against *Fusarium*. This explains its least effects against *fusarium oxysporum*.

3.8.5 Interaction Effects between Treatments and Crude Extracts

The study revealed that the highest interaction effects of hexane derived crude extracts against *fusarium oxysporum* was observed on extracts from the bark, followed by extracts from the root and least from the leaves. Thus, the highest levels of bioactive compounds were extracted using hexane from the bark followed by extracts from the root, and least from the leaves. Therefore, from the three solvents, hexane had significantly the highest abilities of extracting highest levels of bioactive compounds (14 mg/mL) from the bark and the root (11 mg/mL) of *Croton jatrophioides*. This observation suggests that, Hexane being highly non-polar solvent, much of the bioactive compounds extracted from the bark and root of *C. jatrophioides* were non-polar in nature and that hexane had best penetrating abilities to extract the bioactive compounds from the matrix of the plant tissues compared to ethyl acetate and methanol [24].

Thus, hexane being mainly non-polar solvent, dissolvated and extracted from the bark and roots corresponding non-polar bioactive compounds from the matrix of ground plant samples of *C. jatrophioides*, such as terpenes, alkaloids and polyphenols which have been reported to have more fungicidal effects, than those bioactive compounds derived using semi aqueous solvents such as ethyl acetate and methanol [11, 21]. It is also notable that in general, stems, barks and roots of most perennial plants tend to have more lignin and tannin depositions and also contain more groups of alkaloids, terpenes, polyphenols, phyto-alexins and free radicals than leaves. These complex compound are more effective in inhibiting fungal growth and development which explains the high efficacy of Hexane derived bioactive compounds against *fusarium oxysporum*.

The study also showed that ethyl acetate solvent had the second highest level of interaction effects compared to methanol treatments. The highest interaction effects of Ethyl acetate extracts against *fusarium oxysporum* was observed on extracts from the root, followed by extracts from the bark and least from the leaves. This suggests that the highest levels of bioactive compounds extracted using ethyl acetate were from the roots followed by extracts from the bark, and least from the leaves. This implies that ethyl acetate solvent had the second highest abilities of extracting bioactive compounds from the root (10 mg/mL) and from the bark (8 mg/mL) after

hexane. It also had higher abilities to extract bioactive compounds from the roots than the bark of *C. jatrophioides*.

Soquetta *et al.* [19], Altemimi *et al.* [24] and Truong *et al.* [40] reported that different solvents had different polarities and therefore different abilities to dissolve and extract different bioactive compounds from the compact matrix of plant tissues and fibre materials. Ethyl acetate having both a polar and non-polar properties is able to dissolve and extract both polar and non-polar bioactive compounds [41, 42].

A comparison of extraction abilities of bioactive compounds that could act against *Fusarium oxysporum* f. sp. *lycopersici* from the bark of *Croton jatrophioides* shows that hexane and ethyl acetate were the most suited since they exhibited the highest extraction potential. Hexane as a solvent was able to extract 42.9% more bioactive compounds from the bark than ethyl acetate. Hexane also had the highest extraction abilities of bioactive compound against *Fusarium oxysporum* f. sp. *lycopersici* from the root compared to ethyl acetate. This suggests that Hexane was the solvent most suited for extracting bioactive compounds from the bark, roots and the leaves. Ethyl acetate extracted the highest amounts of bioactive compounds from the root than from the bark. Thus, ethyl acetate extracted 40% more bioactive compounds from the root than the 20% from the bark, suggesting it was more prudent to use ethyl acetate in extracting bioactive compounds from the roots rather than from the bark and leaves.

In the overall, Hexane was the best choice of solvent for extraction of bioactive compounds from *C. jatrophioides* from the bark, and the root. However, in absence of Hexane solvent, Ethyl acetate was second best solvent of choice for extraction of bioactive compounds from *C. jatrophioides* on roots and bark. Methanol had the least abilities to extract bioactive compounds from the different parts of the *C. jatrophioides*, namely the bark (7.2 mg/mL), the root (7.0 mg/mL) and the leaves (7.8 mg/mL). Thus, Methanol exhibited highest extraction abilities on leaves. Given that methanol is mainly a polar solvent, it suggests that much of the bioactive compounds extracted by methanol from the leaves were polar in nature. Although methanol had the least extraction abilities of bioactive compounds from *C. jatrophioides* from the bark and the root, when compared to Hexane and Ethyl acetate, it had superior extraction abilities of bioactive compounds from the leaves compared to ethyl acetate. The fact that methanol is a highly polar solvent and that it had superior extraction abilities of bioactive compounds from the leaves than ethyl acetate suggests that most of the bioactive compounds in leaves were polar and soluble in aqueous solutions. This implies that if the leaves are the sites of synthesis and therefore sources of some of these bioactive metabolites, they undergo further processing and transformation as they reach the bark and roots to form the insoluble non-polar complex compounds. Thus, the findings of this study are consistent with observations made in similar studies on extraction of bioactive compounds from other plants by Pavarini *et al.* [27] and Jin [30] who reported occurrence of different levels of bio-active compounds in various parts of the stem, leaves and the bark.

4. Conclusion

The study has shown that it was possible to extract bioactive compounds from *C. jatrophioides* using hexane, ethyl acetate and methanol solvents. Non-polar solvents proved to be more effective in extracting the non-polar bioactive compounds as they exhibited significant antifungal activity, and therefore were more effective in suppressing the growth of *fusarium oxysporum* pathogen. The study has also shown that the bioactive crude extracts obtained using the different solvents are able to suppress *fusarium oxysporum* pathogen that causes the serious fusarium wilt disease of tomatoes. Thus, farmers can now have alternatives to use of pesticides that have had negative effects on the environment and induced development of resistant strains. The study has also been able to show that a minimum inhibition concentration of crude extracts that elucidated response against *F. oxysporum* occurs at a dilution of 50 mg/mL of the extract. The study revealed that the different solvents had different abilities in extracting bioactive compounds from the various materials of *C. jatrophioides*. Hexane solvent exhibited the highest extracting abilities of bioactive compounds followed by ethyl acetate while methanol had the lowest abilities. Thus, hexane was the most suited solvent for extraction of bioactive compounds from the bark and roots of *C. jatrophioides* and in its absence it would be prudent to use ethyl acetate as a solvent.

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