Biopesticidal potentials of plants extracts against *Cochliobolus lunatus* R.R. Nelson & F.A. Haasis. Anamorph: *Curvularia lunatus* (Wakker) Boedgin

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ABSTRACT

Ethanolic extracts of leaves of *Chromolaena odorata, Emilia sonchifolia* and *Tridax procumbens* were evaluated for their bioactivity potentials on *Cochliobolus lunatus* under *in-vitro* condtions. To determine their bioactivity, food poisoning technique using Potato Dextrose Agar medium at concentrations of 0 to 80 mg/ml was used. Tested plant extracts significantly ($P<0.05$) suppressed the mycelia growth of *C. lunatus* with minimum inhibitory concentration of extracts as: *T. procumbent* (64mg/mL), *E. sonchifolia* (72mg/mL) and *C. odonata* (80mg/mL). Among the plants tested, the lowest and highest extract concentrations from leaves of *T. procumbens* were superior to the other extracts in its inhibitory activity. The extracts were also tested for the presence of various phytochemicals reveals the presence of alkaloids, anthraquinones, flavonoids and steroids. Totally, 8 compounds among which are Caryophyllene oxide (22.16%), Ethyl iso-allocholate (20.36%), Estra-1, 3, 5(10)-trien-17, beta-ol (15.03%) and Naphthalene, decahydro-4a-methyl-1- (11.18%) were identified in *C. odorata*; 9 compounds among which are Phytol (27.41%), Squalene (18.68%), n-Hexadecanoic acid (17.30%) and 9,12,15-Octadecatrienoic acid (Z,Z,Z)- (15.55%) were identified in *E. sonchifolia* and 13 compound which include Phytol (22.87%), n-Hexadecanoic acid (15.25%), Cyclohexene, 1-methyl -4- (1-methylethyl)- (14.34%) and 1,5,9,-Decatriene, 2,3,5,8-tetramethyl (10.37%) identified from *T. procumbens* through Gas Chromatography-Mass Spectrometry analysis of extracts. Phytol was the most abundant constituent in both *E. sonchifolia* (27.41%) and *T. procumbens* (22.87%) with Canyophyllene oxide (22.16%) in *C. odorata*. The portrayed potentials of these extracts indicated that they could be excellent candidates to be harnessed in the biosafety formulation of biopesticides for the control of plant diseases incited by *Cochliobolus lunatus*.

Keywords: Leaf extracts, biofungicides, *Cochliobolus lunatus*

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INTRODUCTION

Plant diseases play an important role in determining the amount and cost of food, and major part of crop loss as a result of disease is due to fungal pathogens (Mehrotra and Aggarwal, 2004). *Cochliobolus lunatus* Nelson & Haasis (Pleosporaceae), ascospores are airborne, septate and four celled brown to black in colour growing rapidly in potato dextrose agar medium. It is an opportunistic pathogen infecting immune-compromised patient such as advanced age patients and cancer patients (Berman, 2012; Nelson, 1964). *C. lunatus* is a natural enemy of various agricultural crops with six genetically varied pathogenic types (Gao *et al.,* 2017). It has the ability to adapt to host tissues, counter attack the defense mechanism and causing devastating disease such as leafspots on sweet potato (Ilondu, 2013a), maize (Akinbode, 2010), rice (Tann and Soytong, 2017), seed-borne pathogen of *Dalbergia sissoo* (Gupta *et al.,* 2017). Having been reported as a human pathogen, it is often referred to as across kingdom pathogen (Dharmici *et al.,* 2015;

Louis *et al.,* 2017).Plants serve as rich source of biochemicals and are continually being investigated for their bioactive potentials against plant pathogens hence extracts of medicinal plants play a vital role in the control of different phytopathogenic fungi (More *et al.,* 2017, Adeyemo *et al.,* 2018, Ilondu and Bosah, 2017). In this study, three medicinal plants of no-food values, commonly available and ecofriendly species in the Family of Asteraceae including *Chromolaena odorata* (L) R.M.King & H.Rob, *Emilia sonchifolia* (L) D.C. ex Wight and *Tridax procumbens* (L) were selected. The fungitoxic activities of these weed plants *C. odorata* (Vital and Rivera 2009, Okigbo *et al.,* 2010, Ijato 2016, Adeyemo *et al.,* 2018); *E sonchifolia* (Toga *et al.,* 2009, Okey and Asuqwo, 2016) and *T. procumbens* (Sandeep and Srivastava 2010, Jindal and Kumar 2013, Priyadarshini and Priya 2013; Sarkar *et al.,* 2016) were documented. Although, previous attempts have been made to control *C. lunatus* with other plant extracts (Akinbode 2010, Ilondu 2013b; Ilondu *et al.,* 2014; Bhajbhuje 2015, Ojha and Goyal 2017), information is lacking on the use of *C. odorata, E. sonchifolia* and *T. procumbens* extracts in the control of *C. lunatus*. In continuous attempt to find solution to the devastating effect of *C. lunatus* on crops, the following objectives were evaluated; (i) *in-vitro* assay of different concentrations of *C. odorata, E. sonchifolia* and *T. procumbens* leaf extracts against *C. lunatus* and (ii) phytochemical screening and GC-MS analysis of the extracts for the presence of antifungal compounds. It is hoped that this study will provide a new source of biofungicides and lead compounds to be harnessed in the management of diseases caused by *C. lunatus*.

MATERIALS AND METHODS

Source of organism for the study

C. lunatus (IMI394871) was obtained from pathology section of the Department of Botany Laboratory, Delta State University, Abraka. It was previously isolated from sweet potato leaf spot disease, identified and maintained in McCartney bottles on PDA slants at 4^0C (Ilondu, 2013a). The culture was revived on fresh PDA medium thrice before use.

Plant Sample Collection and Extraction

Healthy leaves of *C. odorata, E. sonchifolia* and *T. procumbens* were harvested from the premises around the Faculty of Science, Delta State University Site III, Abraka. Samples were washed separately in sterile distilled water, air dried and pulverized with electric blender. 100 grams of each sample was steeped in 300ml of ethanol and extracted by Soxhlet method (Oyewale and Audu, 2007). The yield of the extracts was computed and recorded (Ilondu *et al*., 2014).

Antifungal assay

The negative control was setup using PDA plates containing 1ml of distilled water without plant extracts while the positive control was setup using PDA plates containing 8 – 80mg/ml of plant extracts. The plant extract were screened for their antifungal potential on the mycelial growth of test fungus by poisoned food technique (Swami and Alane, 2013). Different concentrations (8 – 80 mg/ml) were prepared and 1 ml of each concentration was incorporated into 20 ml of cool molten Potatoes Dextrose Agar (PDA) medium in sterile 9 cm diameter petri dishes (Ilondu *et al.,* 2014). Small disc (0.4 cm diameter) from the edge of actively growing 5-day old culture in PDA was aseptically transfer to center of PDA extract plates in triplicates as well as the control plates. At the end of 7 days incubation period at room temperature of 28 ± 2^0C, the diameter of the fungal growth in the treated plate was compared with the control and used as a measure of fungitoxicity. The inhibition percentage was computed using the method adopted from Okigbo *et al.* (2010). The minimum inhibitory concentration (MIC) where there is no physical growth of the fungus in extract treated plates was recorded for each plant extract.

Phytochemical examination

Phytochemical tests for the presence or absence of the secondary metabolites for all the extracts were carried out following the standard techniques and methods adapted from

Evans and Trease (2002). Characterisation, identification and relative amount of the components of the leaf extracts were determined. The analysis was carried out in the Department of Chemistry, Usman Danfodio Univeristy, Sokoto, Gas Chromatography – Mass Spectrometry (GC-MS) and GC Co injection of the extracts with authentic standards following the method of Asawalam *et al.* (2008). GC-MS analysis were performed on a capillary GC-MS Agilent 122–5532 equipped with a split capillary injector system DB-5 ms, 0.25 mm*30 mm* 0.25 µm. maximum temperature of 100^0C, nominal film thickness 0.25 µm, constant flow mode, nominal initial pressure of 3.06 psi. The carrier gas was Helium, at a flow rate of 0.5 ml/min. The MS was operated in the Electron Impacted (EI) mode and the generated chromatogram recorded.

Statistical Analysis

All data were analysed using simple descriptive statistics, and results presented as mean and standard error (M±SE). Mean were separated by Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS

The yield of the leaf extracts recorded in the plant samples was 2.45%, 2.80% and 4.80% in *C. odorata*, *E. sonchifolia* and *T. procumbens* respectively. Phytochemical screening showed the presence of alkaloid, anthraquinones, flavonoids, glycosides, phenols, saponins, sterol, tannins and terpenes were in all extracts.

Figure 1. GC-MS Chromatogram for *Chromolaena odorata* extract

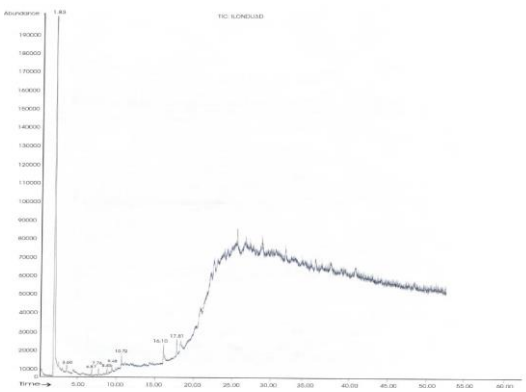


Table 1. Phytochemicals tested in the plant extracts used for the study

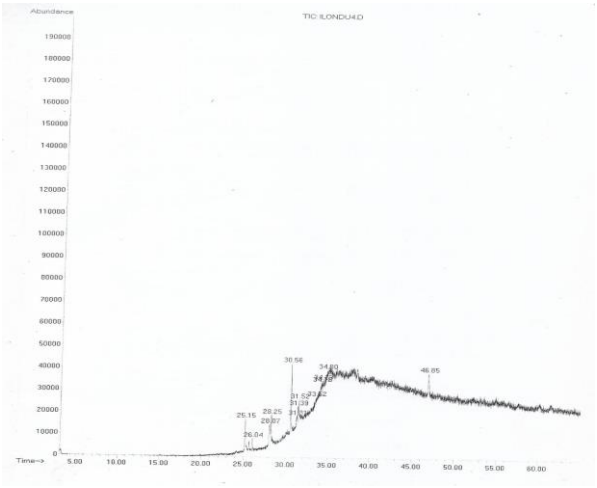
Phytochemicals tested	Plant extracts		
	Chromolaena odorata	*Emilia sonchifolia*	*Tridax procumbens*
Alkaloids	+	+	+
Glycosides	+	+	-
Tannins	+	+	+
Flavonoids	+	+	+
Anthraquinones	+	+	-
Sterols	+	-	+
Terpenes	+	+	+
Phenols	+	+	+
Saponins	+	+	-

(+) = Positive, (-) = Negative

However, anthraquinones, glycosides and saponins were absent in *T. procumbens* while sterol is absent *in E. sonchifolia* (Table 1). From the GC/MS analysis of the extracts, a total of 8-13 compounds were detected. Of all these, 9 compounds were identified in *E. sonchifolia*, 8 compunds in *C. odorata*, and 13 compounds from *T. procumbens* extracts with varied percentage abundance. Among the identified compounds, phytol (27.41%), squalene (18.68%), n-Hexadecanoic acid (17.30.26%) and 9,12,15-Octadecatrienoic acid (Z,Z,Z)- (15.55%) were among the constituents in *E. sonchifolia*.

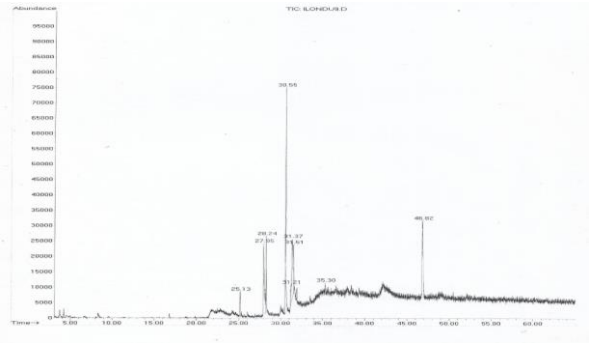
T. procumbens has phytol (22.87%), n-Hexadecanoic acid (15.25%) and Cyclohexane 1-methyl-4-(1-methylethyl)- (14.34%), 1,5,9,-Decatriene, 2,3,5,8-tetramethyl (10.37%) and Hexadecanoic acid ethyl ester (8.64%) while Caryophyllene oxide (22.16%), Ethyl iso-allocholate (20.31%), Estra-1,3,5(10)-trien-17, beta-ol (15.03%), Naphthalene, decahydro-4a-methyl-1- (11.18%) and 4H-Pyran-4-one, 2, 3-dihydro-3, 5-dihyroxy-6-methyl- (10.25) in *C. odorata* (Table 2). The GC peaks for each extract are presented in Figure 1-3. The inhibitory action of all plant extracts on the mycelial growth of *C. lunatus* was observed in various concentrations compared to the control.

Figure 2. GC-MS Chromatogram for *Emilia sonchifolia* extract



At the lowest extract concentration of 8mg/ml, high mycelial growth reduction of the test fungus was recorded for all plants. There was no significant difference (P<0.05) between the The effects of all extract concentration of *T. procumbens* on the mycelia growth were superior over that of *C. odorata* and *E. sonchifolia*. Similarly, the effect of *C. odorata* at concentrations of 16-mycelial growth inhibition at 32mg/ml and 40mg/mL in *C. odorata* and that of 48mg/mL and 56 mg/mL in *E. sonchifolia.* 56mg/mL was greater than that of *E. sonchifolia* at the same concentrations. The mycelia growth inhibition and the minimum inhibitory concentration where there is no visible mycelial growth in the plates treated with extracts of *T. procumbens* occurred at 64mg/ml, *E. sonchifolia* at 72mg/ml and *C. odorata* at 80mg/ml (Table 3).

Figure 3. GC-MS Chromatogram for *Tridax procumbens* extract



The percentage inhibition of the extract concentrations on the test fungus also followed the same trend. Higher concentrations gave higher percentage inhibition (Figure 4). The maximum percentage inhibition (100%) was recorded in *T. procumbens* at 64mg/ml while *E. sonchifolia* and *C. odorata* were at 72mg/ml and 80mg/ml respectively.

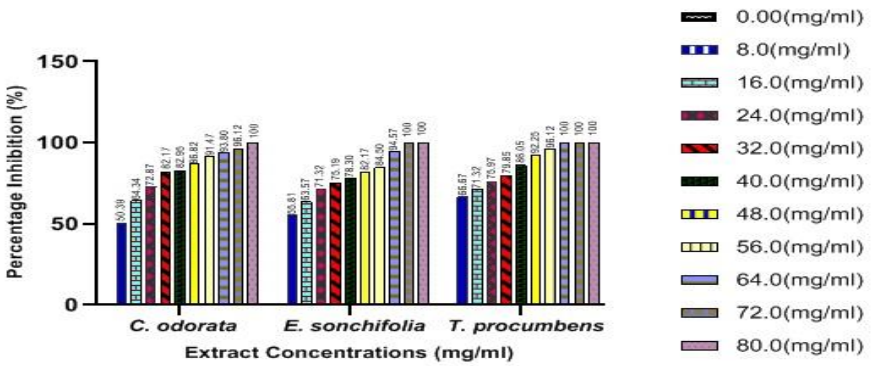


Figure 4. Mean percentage growth inhibition of *C. lunatus* to concentrations of plant extracts after 7 days on agar plates

Table 2. Major identified constituents of the three plant extracts used for the study

Plant Extract	Serial No.	Peak area (%)	Retention time (min)	Name of component
Chromolaena odorata	1	10.25	3.60	lH-Pyran-4-one, 2, 3-dihydro-3, 5-dihyroxy-6-methyl-
	2	7.08	6.87	Cedrene
	3	5.70	7.76	Cis-(-)-2, 4a, 5, 6, 9a-Hexahydro-3, 5, 5, 9-tetramethyl (1H) benzocycloheptene
	4	8.32	8.85	12, 15-Octadecatrienoic acid, methyl ester
	5	11.18	9.48	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-(4aR-trans)-
	6	22.16	10.72	Caryophyllene oxide
	7	15.03	16.10	Estra-1, 3, 5(10)-trien-17, beta-ol
	8	20.36	17.81	Ethyl iso-allocholate
Emilia sonchifolia	1	3.14	25.13	lR*, 5R*, 9S*) –5, 9-Dimethylspiro [3, 5] nonan-1-one
	2	17.30	27.95	n-Hexadecanoic acid
	3	8.49	28.24	Hexadecanoic acid ethyl ester
	4	27.41	30.55	Phytol
	5	1.95	31.21	9, 12, 15 – Octadecatrienoic acid, (Z, Z, Z)-
	6	15.55	31.37	9,12,15-Octadecatrienoic acid (Z,Z,Z)-
	7	5.97	31.51	1, 3 – Cyclooctadiene, (Z, Z)-
	8	1.51	35.30	Heptadecanoic acid heptadecyl ester
	9	18.68	46.82	Squalene
Tridax procumbens	1	14.34	25.15	Cyclohexene, 1-methyl -4- (1-methylethyl)-
	2	3.84	26.04	11,13-Dimethyl-12-tetradecen-i-ol acetate
	3	15.25	28.07	n-Hexadecanoic acid
	4	8.64	28.25	Hexadecanoic acid ethyl ester
	5	22.87	30.56	Phytol
	6	2.37	31.31	4,4,8-Trimethyl-non-5-enal
	7	5.61	31.39	9, 12-Octadecadienoic acid (Z, Z)-
	8	5.59	31.52	2(IH)-Naphthalenone, Octahydro-49-methyl – 7 (1-methyl)-, (4a.alpha., 7. Beta., 8a.beta.)-
	9	4.12	33.52	E-8-Methyl-9-tetradecen-i-ol acetate
	10	4.10	34.12	Cyclopropaneoctanal, 2-octyl-
	11	2.09	34.24	1-Bromo-11iodoundecane
	12	0.81	34.80	, 1'-Bicyclohexyl] –4-carboxylic acid, 4-pentyl-, 4-pentyl phenyl ester
	13	10.37	46.85	1,5,9,-Decatriene, 2,3,5,8-tetramethyl

Table 3. The potency of the plant extracts concentrations (mg/ml) against the mycelia diameter (cm) of *C. lunatus*

Extract concentration (mg/ml)	Plant Extracts		
	C. odorata	***E. sonchifolia***	***T. procumbens***
0	4.30±0.00^{a}	4.30±0.00^{a}	4.30±0.00^{a}
8	2.13±0.023^{b}	1.90±0.231^{b}	1.43±0.006^{b}
16	1.53±0.006^{c}	1.57±0.159^{c}	1.23±0.032^{c}
24	1.17±0.040^{d}	1.23±0.032^{d}	1.03±0.032^{d}
32	0.77±0.009^{e}	1.07±0.038^{e}	0.87±0.052^{e}
40	0.73±0.032^{e}	0.93±0.032^{f}	0.60±0.058^{f}
48	0.57±0.052^{f}	0.77±0.009^{g}	0.33±0.032^{g}
56	0.37±0.049^{g}	0.67±0.052^{g}	0.17±0.040^{h}
64	0.27±0.055^{h}	0.23±0.032^{h}	0.00±0.00^{i}
72	0.17±0.046^{h}	0.00±0.00^{i}	0.00±0.00^{i}
80	0.00±0.00^{1}	0.00±0.00^{i}	0.00±0.00^{i}

Values with the same superscript(s) in the same column are not significantly different at P>0.05 by DMRT.

DISCUSSION

This study showed that leaf extract of *C. odorata, E. sonchifolia* and *T. procumbens* contained various phytochemicals among which are alkaloids, antraquinones, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenes. Some of these phytochemical metabolites have been reported by different researchers including Sandeep and Srivastava (2010), Okey and Asuqwo (2016), Sarkar *et al.* (2016) and Adeyemo *et al.* (2018) to possess antifungal activities. Similarly, Sarkar *et al.* (2016) documented the great impact of alkaloids, flavonoids and tannins on antimicrobial potentiality. These phytochemicals may have been the cause of antifungal activities of the extracts. They may act directly on the pathogen or disrupt important developmental and metabolic processes (Ilondu and Bosah, 2017). Tanins are toxic to bacteria, filamentous fungi and yeast (Okolie *et al.,* 2009). Vital and Rivera (2009) reported that antimicrobial activity of *C. odorata* extracts was by inhibition of cell wall synthesis due to presence of flavonoids and tannins while *Uncaria perrottetil* targeted cell wall and cell membrane due to its content of alkaloids and tannins. Okey and Asuquo (2016) implicated the antifungal activities of plants used in their study to presence of flavonoids, tannins and saponins. The effectiveness of alkaloid in the inhibition of *Alternaria alternate* has been reported (Raghavendra *et al.,* 2009). Dania *et al.* (2015) attributed the growth inhibition of *Collectotrichum gloeosporioides* and *Alternaria* sp. to the abundance of alkaloids, flavonoids and phenols in the ethanol extracts used.

All the plant extracts showed a significant reduction of the mycelia growth of *C. lunatus*. Much work has been done on the use of plant extracts against plant pathogenic fungi (Bhajbhuje, 2015). The plant extracts under investigation showed a dose dependent effect. The higher concentrations favoured higher reduction of mycelia growth. Similar reports have been documented by Ilondu *et al.* (2014), Ijato (2016) and Mir *et al.* (2017). Akinode (2010) opined that plants are known to contain chemicals which when present in adequate concentration show toxic effect on plant pathogens. Hence, Adeyemo *et al.* (2018) reiterated that some plant have higher antifungal potentials and higher power of diffusion. Adeyemo *et al.* (2018) also observed that higher concentration of antimicrobial substances showed appreciation in growth inhibition.

The antifungal effect observed in the study is also species dependent with respect to the recorded MIC of each plant extract. The MIC value of *T. procumbens* was of vibrant significance in this study as *C. lunatus* showed greater sensitivity to the extract. Species dependent effect of other Asteraceous extracts on *C. lunatus* has been reported by Ilondu *et al.* (2014) which included *Ageratum conyzoides* (88mg/ml), *Spiranthes filicaulis* (72mg/mL) and *Tithonia diversifolia* (56mg/mL).

The antifungal activities of *T. procumbens* on other fungi such as *Rhizoctonia solani, Helminthosporium oryzae* (Sandeep and Srivastava, 2010), *Candida* species (Kamble and Moon, 2015), fruit rot causing fungi of tomatoes such as *Aspergillus niger, Rhizopus stolonifer* among others (Ijato *et al.,* 2011) has been reported. Other reports on the bioactivities of *T. procumbens* include that of Priyadarshini and Priya (2013), Sarkar *et al.* (2016) and Mir *et al.* (2017). Similarly inhibitory effect of *C. odonata* extracts has been reported on some bacterial and fungal species (Vital and Rivera, 2009), *Aspergillus* species and *Botryodipodia theobromae* (Ijato, 2016), *Phythopthora megakarya* (Adeyemo *et al.,* 2018).

The antimicrobial activities of *E. sonchifolia* (Yoga *et al.,* 2009) as well as antifungal effect of its leave extracts on *Aspergillus niger* and *Rhizopus oryzae* (Okey and Asuqwo, 2016) has been documented. All these reports confirmed the antifungal activity of the plant extracts under investigation against *C. lunatus*. Different extracts of these plants in the family of Asteraceae showed different efficacy against the tested fungus. The *in-vitro* bioassay with the extracts showed that *T. procumbens*

proved better than those of *C. odorata* and *E. sonchifolia* in inhibiting the mycelia growth of *C. lunatus*. The fact that all the plant extract tested did not contain the same constituents implies different antifungal activities. These disparity may be caused by the nature and level of the antifungal agents present in the extracts, their mode of action on this pathogen, or as the result of high solubility of the metabolites in one extract than the other in the organic solvent used (Dania *et al.,* 2015). Moreso, these compounds vary in composition from plant to plant due to environmental or genetic factors (Musa *et al.,* 2015).

The phytoconstituents of the extracts responsible for antifungal effects on *C. lunatus* were explored through GC-MS analysis. Understanding the chemical constituents of plants is necessary for isolating the compounds that can be applied to the etiological agent of a disease. GC-MS studies have been used in plant analysis because this technique is very simple, sensitive and effective in separating mixtures of compounds (Varsha *et al.,* 2016). Each extract showed a mixture of constituents such as Phytol, Ethyl iso-alocholate, Caryophyllene oxide, Squalene, n-Hexadecanoic acid, 9,12,15-Octadecatrienoic acid (Z,Z,)- among others which may have conferred inhibitory effect on the test fungus. Antifungal and antimicrobial activities of some of the identified compounds have been reported. Various reports have proven the antifungal and antimicrobial activities of phytol (Inoue *et al.,* 2005; Pejina *et al.,* 2014). Meanwhile, Bharathy *et al.* (2012) indicated that phytol is a diterpene with antimicrobial properties against many bacteria strains.

Phytol acts by damaging the cell membrane of fungal cell (Ilondu, 2013). The antibacterial activity of phytol against *Staphylococcus aureus* have been documented (Inoue *et al.,* 2005). Compounds such as phytol, ethyl iso-allocholate, squalene and n-hexadecanoic acid have shown antimicrobial property (Varsha *et al.,* 2016). Kumar *et al*. (2010) has reported the antimicrobial activities of plant constituents with compound nature of palmitic acid (including hexadecanoic ethyl ester, n-hexadecanoic acid) and unsaturated fatty acid (including octadecatrienoic acid). Methyl ester of hexadecanoic acid isolated from leaves of *Annona muricata* showed antifungal activity against *Alternaria solani, Aspergillus fumigates* and *Penicillium chrysogenum* (Abubakar and Deepalakshmi, 2013). n-hexadecanoic acid has been reported as a highly bioactive compound and its methyl ester showed extreme antifungal potentials (Abubackar and Deepalakshmi, 2013; Karim *et al.,* 2017). 9,12-octadecadienoic acid, squalene and ethyl iso-allocholate have been reported for their antimicrobial properties (Francis and Jose, 2016; Malathi *et al.,* 2016; Varsha *et al.,* 2016). Musa *et al.* (2015) and Haider *et al.* (2016) have reported that Hexadecanoic acid ethyl ester is a natural lipid soluble form of palmitic acid with antimicrobial activity as well as that of 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z). The antifungal activity could possibly be accentuated by synergistic effect of different chemical components present in the extracts (Ilondu *et al.,* 2014; Musa *et al.,* 2015). Therefore, these extracts especially *T. procumbens* could be harnessed as source of cheap and eco-friendly biofungicides and lead compounds for the management of plant diseases incited by *C. lunatus*.

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