FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 4, December, 2020, pp 213 –222
DOI: https://doi.org/10.33003/fjs-2020-0404-474

ANTIFUNGAL ACTIVITY OF CRUDE EXTRACTS OF *Euphorbia tirucalli*; ISOLATION AND CHARACTERIZATION OF ONE OF ITS BIOACTIVE PRINCIPLES TIRUCALLOL

***[1]Ekhuemelo, D. O., [2]Anyam, J. V. and [3]Ekhuemelo C.**

[1]Department of Forest Production and Products, Federal University of Agriculture Makurdi, Nigeria.
[2]Department of Chemistry, Federal University of Agriculture Makurdi, Nigeria.
[3]Department of Crop and Environmental Protection, Federal University of Agriculture Makurdi, Nigeria.

***Correspondent email:** *davidekhuemelo@gmail.com*;+234-703 133 2803

ABSTRACT

Antifungal properties of extracts from *Euphorbia tirucalli* against some wood decay fungi were studied**.** *E. tirucalli* fresh sample was harvested, cleansed and chipped into chips of 3 x 2 cm length and breadth. Chipped sample (846.3 g) was macerated in 1000 mL of ethyl acetate, n hexane and methanol solvents, respectively. Column chromatography experiment on *E. tirucalli* was carried out and antifungal screening was done according to standard method for 7 days to observe zones of inhibition of fungi growth. Broth dilution method was adopted to determine the Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *E. tirucalli* extracts and fraction. Results show that *Fibroporia vaillantii, Fomitopsis pinicola, Gloeophyllum sepiarium, Rhizopus sp.* and *Serpula lacrymans* were sensitive to ethyl acetate, n' hexane *E.tirucalli* extracts and at concentrationmethanolof200mg/mL with zone of inhibition of 18-26 mm and methanol extract had the highest value of 26 mm. The fraction had zone of inhibition of between 18 and 20 mm which are not significantly different ($p<0.05$) from 29-35 mm obtained from antibiotics. The MIC of 10 mg/mL and MFC of 20 mg/mL were recorded for extracts against the test fungi while MIC of 50 µg/mL and MFC 200 µg/mL were noted for fraction (Et15), respectively. Fraction (Et15) obtained from *E. tirucalli* was characterised as tirucallol compound. In conclusion, *E. tirucalli* extracts and fraction have proved to be effective in the control of wood decay fungi and may serve as control of diseases caused by the test fungi.

Keywords: Antifungal, *E. tirucalli*, Extract, Fraction, Wood, Fungi, Tirucallol

INTRODUCTION

Euphorbia tirucalli L**.** is of the family Euphorbiaceae. It is commonly called pencil plant, milk bush and petroleum plant among others. *E. tirucalli* is the most prevalent of all the *Euphorbia* species. It is a large armless shrub or a small tree that grows up to 5 meters in height. Mwine and Damme (2011**)** reported that *E. tirucalli is* of the genus *Euphorbia* and is one of the 8,000 species of the family Euphorbiaceae. This plant bears erected branches, possesses rough bark, greenish stem and produces poisonous milky sap when cut. The branch is slender, smooth, cylindrical, polished, whorled and formed into phylloclade (Baniakina and Eyme, 1997).

E. tirucalli is reported to be one of the very important trees of family Euphorbias ever known globally because of its many uses (Mwine and Damme, 2011**)**. *E. tirucalli* is normally planted to serve as boundary demarcation and as a live fence surrounding house compounds, farms and gardens because of its ability to survive severe aridity and resistance to herbivore pressure (Mwine and Damme, 2011**).** Kumar A. (1999) reported that *E. tirucalli* plant has been used in the treatment of many ailments such as asthma, leprosy, and enlargement of spleen, whooping cough, jaundice, tumours, gonorrhoea and bladder stones. The latex of *E. tirucalli* is used for the treatment of tumours, ear ache, tooth ache, rheumatism, abdominal pains, intestinal worms and skin diseases while the root is used to reduce coli pains (Swapna and Prasad, 2011).

E. tirucalli exudates possess pesticidal properties and it is toxicity to aphids (Mwine and Van Damme, 2010), nematodes (Siddiqui *et al*., 2003). It contains anti-bacteria properties against *S. mutans* and *S. sobrinus* (Yi *et al*., 2017). *E. tirucalli* is also containing antibacterial properties against some micro-organisms. Yi *et al.* (2017) reported that methanol and ethanol stem extracts of *E. tirucalli* possessed antibacterial activity against *S. mutans* and *S. sobrinus.* Although, many studies have been done on the medicinal properties of *E. tirucalli*, there is dearth of information on its fungicidal potentials.

Wood decay fungi carry out vital role in forest ecosystems. They recycle dead wood and other biomass in the forest ecosystem. However, they damage the strength and durability wood in service leading to huge economic loses. Although, synthetic chemicals have been conventionally used in the treatment of wood before application, they constitute hazardous impact to human and the environment hence the search for alternative bioactive pesticides which are eco-

friendly, less hazardous, cost effective and accessible. Hence, this study was designed to evaluate the antifungal properties of crude extracts and fraction from *E. tirucalli* against selected wood decay fungi.

MATERIALS AND METHODS

Sample Collection, Preparation and Extraction of *E. tirucalli*

A fresh sample of *E. tirucalli* was harvested from household in Makurdi town where it was used as live fence. Sample was cleaned to remove sand and unwanted particles. It was chopped into small chips measuring 3 cm x 2 cm in dimension. The fresh chopped sample was weighed to obtain initial weight of 846.3 g and macerated in a cleaned glass bottle. N-hexane, ethyl acetate, and methanol solvents (1000 mL each) were sequentially used for maceration for the period of 24 hours respectively. After the extraction process, the mixture was filtered, evaporated and air dried to obtain n hexane, ethyl acetate, and methanol extracts respectively. Plate 1 A shows sample of *E. tirucalli* plant at live fence in Makurdi.

Plate 1: *E. tirucalli* plant in live fence in Makurdi, Benue State, Nigeria

Column Chromatography

Column chromatography on *E. tirucalli* extract was carried out according to standard methods described by Hostettmann and Marston (1995) to obtain fractions. Vail with white crystals (Et15) was sent for Nuclear Magnetic Resonance (NMR) spectrometry for characterisation. NMR spectra obtained were further analysed using MestreNova 12 software.

Antifungal Screening of Test Fungi

The antifungal activities of Et15 fraction and crude extracts were assessed on selected wood fungi namely: *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicola, Gloeophyllum sepiarium Phaeolus schweinitzii, Rhizopus* spp., *Serpula lacrymans* and *Sclerotium rolfsii*. Fulcin Ketoconazole and Fluconazole antibiotics were used as control. Disk diffusion method was employed for the screening of the extracts and fraction as explained by Chand *et al.* (1994) for 7 days to observe zones of inhibition of fungi growth.

Determination of Minimum Inhibition Concentration (MIC) andMinimum Fungicidal Concentration (MFC)

Broth dilution method described by Chand *et al.* (1994) was adopted for this experiment. Petri dishes with lowest concentration of extracts and fraction without colony growth were noted for MIC and MFC respectively.

Data Analysis

One-way Analysis of Variance (ANOVA) was applied to determine significant effects of treatments on test fungi. Follow up test was carried out using Duncan Multiple Range Test DMRT) using Statistical Package for the Social Sciences (SPSS version 20).

RESULTS

Effect of Antifungal Actives of *E. tirucalli* Crude Extracts and Antibiotic against Test Fungi

Table 1 presents sensitivity and zone of inhibition of *E. tirucalli crude* extracts and antibiotics against test fungi. *F. vaillantii, F. pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans* were sensitive to ethyl,*E.* n' *tirucalli extracts* at concentration of 200 mg/mL at zone of inhibition ranging from 18-26 mm. Although the zones of inhibition were not significantly extract recorded the highest val hexane had the least value of 18 mm. *Aspergillus fumigatus,*

Coniophora puteana, Phaeolus schweinitzii, and *Serpula lacrymans* test fungi species were all resistant to the three extracts. Fulcin was sensitive to seven out of the nine fungi tested at zone of inhibition of 29-35 mm while the meth hexane and ethyl acetate extracts inhibited the growth of five test fungi

Table 1: Sensitivity and zone of inhibition of *E. tirucalli* crude extracts and antibiotic against test fungi

S/No.	Test Fungi	Crude Extract (200 mg/mL)			Antibiotic (200 mg/mL)		
		E. tirucalli EAE	*E. tirucalli* ME	*E. tirucalli* NHE	Fulcin	Ketoconazole	Fluconazole
		AFA (ZOI)	AFA (ZOI)	AFA (ZOI)	AFA (ZOI)	AFA (ZOI)	AFA (ZOI)
1	*A. fumigatus*	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S (32.00±1.00^{bc})	R (0.00±0.00^{a})	R (0.00±0.00^{a})
2	*C. puteana*	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S (30.00±3.00 ^{bc})	R (0.00±0.00^{a})	S (27.00±1.00 ^{b})
3	*F. vaillantii*	S (23.00±1.00^{b})	S (24.00±1.00 ^{bc})	S (20.00±1.00^{b})	R (0.00±0.00^{a})	S (28.00±1.00 ^{b})	R (0.00±0.00^{a})
4	*F. pinicola*	S (21.00±2.00^{b})	S (24.00±1.00 ^{bc})	S (18.00±2.00 ^{b})	S (31.00±3.00^{bc})	S (29.00±1.00 ^{bc})	R (0.00±0.00^{a})
5	*G. sepiarium*	S (23.00±2.00^{b})	S (26.00±2.00 ^{d})	S (19.00±1.00 ^{b})	S (35.00±1.00d)	R (0.00±0.00^{a})	R (0.00±0.00^{a})
6	*P. schweinitzii,*	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S (30.00±2.00 ^{c})	R (0.00±0.00^{a})
7	*Rhizopus sp.*	S (22.00±7.00 ^{b})	S (23.00±3.00^{b})	S (18.00±4.00 ^{b})	S (29.00±3.00 ^{b})	R (0.00±0.00^{a})	R (0.00±0.00^{a})
8	*S. rolfsii*	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S (31.00±1.00^{bc})	R (0.00±0.00^{a})	S (28.00±1.00 ^{c})
9	*S. lacrymans*	S (21.00±2.00^{b})	S (24.00±1.00 ^{bc})	S (20.00±1.00^{b})	S (34.00±4.00 ^{cd})	S (29.00±1.00 ^{bc})	R (0.00±0.00^{a})

Key: *S = Sensitive, R = Resistance, AFA = Anti-fungicidal Activities, ZOI = Zone of Inhibition, EAE = Ethyl acetate extract, ME = Methanol extract, NHE = N-Hexane extract*

Mean values in the same column with same alphabet are not significantly different from each other (P< 0.05

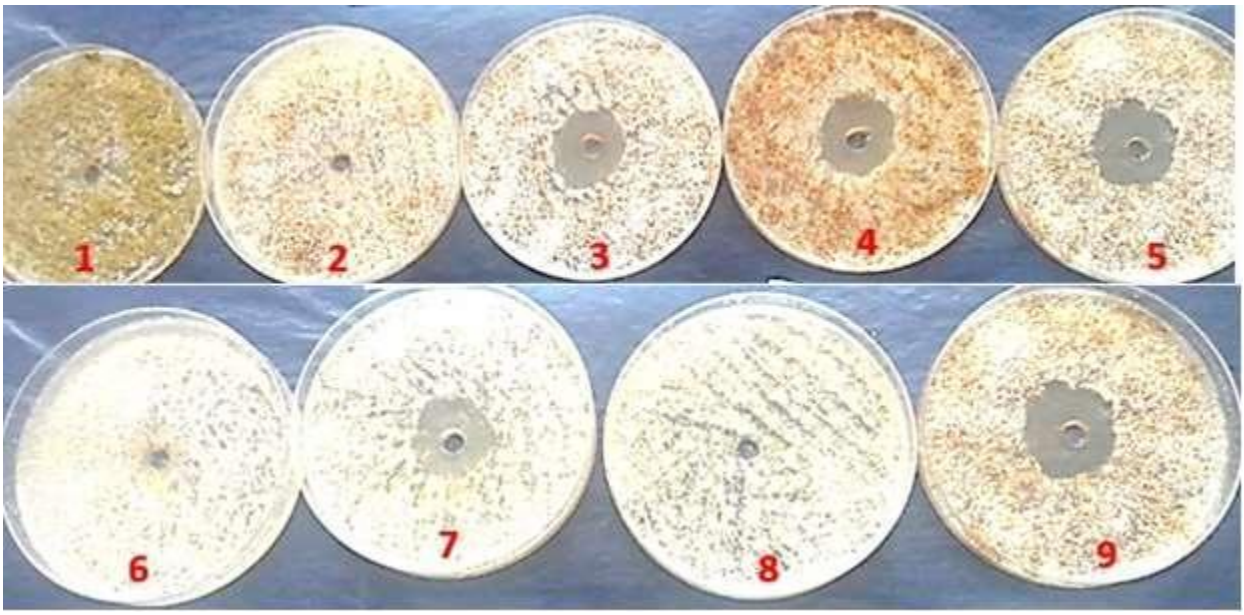
Effect of Minimum Inhibition Concentration and Minimum Fungicidal Concentration of *E. tirucalli Crude* Extracts on Test Fungi

Ethyl acetate and methanol *E. tirucalli extracts* inhibited the growth of *F. vaillantii,* F. *pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans* at MIC of 10 mg/mL and at MFC of 20 mg/mL the test fungi were all killed (Table 2). Plate 2 shows the effect of antifungal activities of ethyl acetate *E. tirucalli* extract on test fungi. Zone of inhibition was observed as the empty core centre in Petri dishes containing *F. vaillantii,* F. *pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans*.

Table 2: Effect of Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC) of *E. tirucalli* crude extracts on test fungi

S/No.	Test Fungi	Effect of Minimum inhibition Concentration (mg/mL)			Effect of Minimum Fungicidal Concentration (mg/mL)		
		E. tirucalli EAE	*E. tirucalli* ME	*E. tirucalli* NHE	*E. tirucalli* EAE	*E. tirucalli* ME	*E. tirucalli* NHE
1	*A. fumigatus*	.	-	-	-	-	-
2	*C. puteana*	.	-	-	-	-	-
3	*F. vaillantii*	10	10	10	20	20	40
4	*F. pinicola*	10	10	20	40	20	40
5	*G. sepiarium*	10	10	20	20	20	40
6	*P. schweinitzii*	.	-	-	-	-	-
7	*Rhizopus sp.*	10	10	20	40	20	40
8	*S. rolfsii*	.	-	-	-	-	-
9	*S. lacrymans*	10	10	10	40	20	40

Key: *EAE = Ethyl acetate extract, ME = Methanol extract, NHE = N-Hexane extract,*



Key: 1 - *Aspergillus fumigatus,* **2** - *Coniophora puteana,* **3** - *Fibroporia vaillantii,* **4** - *Fomitopsis pinicola,* **5** - *Gloeophyllum sepiarium,* **6** - *Phaeolus schweinitzii,* **7** - *Rhizopus sp.,* **8** - *Sclerotium rolfsii,* **9**- *Serpula lacrymans*

Plate 2: Antifungal activities of Ethyl acetate *E. tirucalli* extract

Characterization of Et15 as Tirucallol ($C_{30}H_{50}O$)

Fraction Et15 was obtained as white needle-shaped crystals. Thin Layer chromatographic analysis (TLC) (Hexane/ethyl acetate 9:1) showed a pink spot on spraying/heating with methanol-sulfuric acid 20 % at R_f =0.28. Its proton nuclear magnetic resonance spectrum (^{1}H NMR) (400 MHz, Chloroform-*d*) revealed the following distinct signal regions: ^{1}H NMR :- olefinic:*J*= δ 5.09 7.2, 5.1 Hz, 1H), oxymethine: 3.23 (dd, *J* = 11.6, 4.6 Hz, 1H), methylene/methylic: 1.68 (s, 3H), 1.60 (s, 3H), 1.25 (s, 3H), 1.00 (s, 3H), 0.95 (s, 3H), 0.87 (s, 3H), 0.85 (d, 4H), 0.79 (s, 3H), 0.75 (s, 3H). The carbon 13 NMR (^{13}C NMR) revealed the following 30 signals: ^{13}C NMR (100 MHz, Chloroform-*d*): olefinic: δ 134.13, 133.65, 130. methyls (–CH3), methylenes (–CH2), methines (CH) quaternaries (C) and ring methylene carbon signals: 51.07, 50.15, 49.74, 44.22, 39.06, 37.38, 36.01, 35.53, 35.38, 31.02, 29.88, 29.85, 28.29, 28.18, 28.05, 27.80, 25.89, 24.87, 24.60, 21.66, 20.27, 19.07, 19.06, 17.82, 15.74, 15.67. The structure was arrived at using extensive 2D NMR studies of its COSY, HSQC and HMBC (Table 3, Supplementary information S1-S5). Figure 1 shows tirucallol structure/numbering obtained from Et15 fraction. Figures 4, 5, 6, 7 and 8 show Proton NMR Spectrum, H-H COSY NMR Spectrum, Carbon 13 NMR Spectrum, HMBC Spectrum and HSQC Spectrum of Et 16.

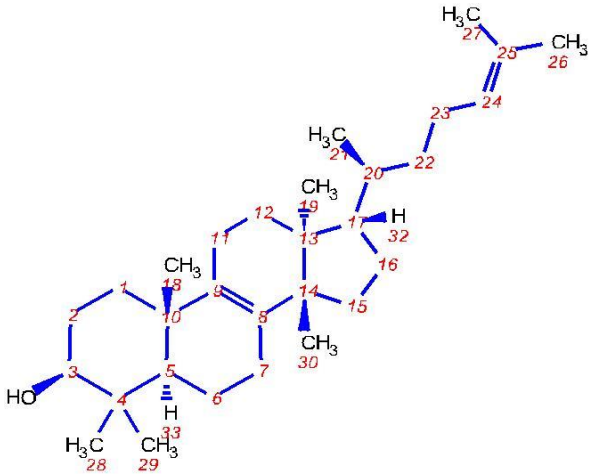


Figure 1: Structure of Et15 (Tirucallol)

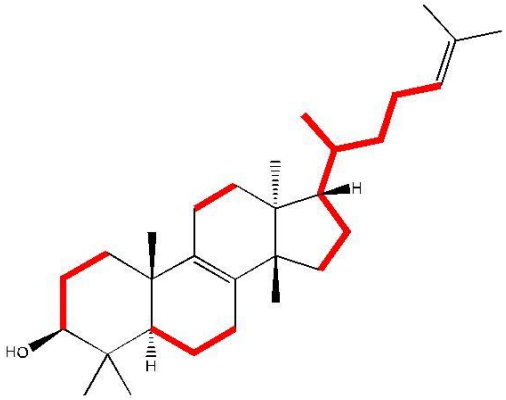


Figure 2: Prominent COSY correlations for Et16

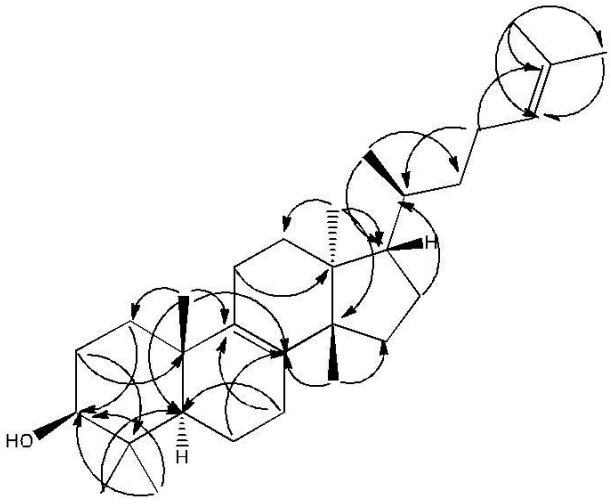


Figure 3: Prominent HMBC correlations for Et16

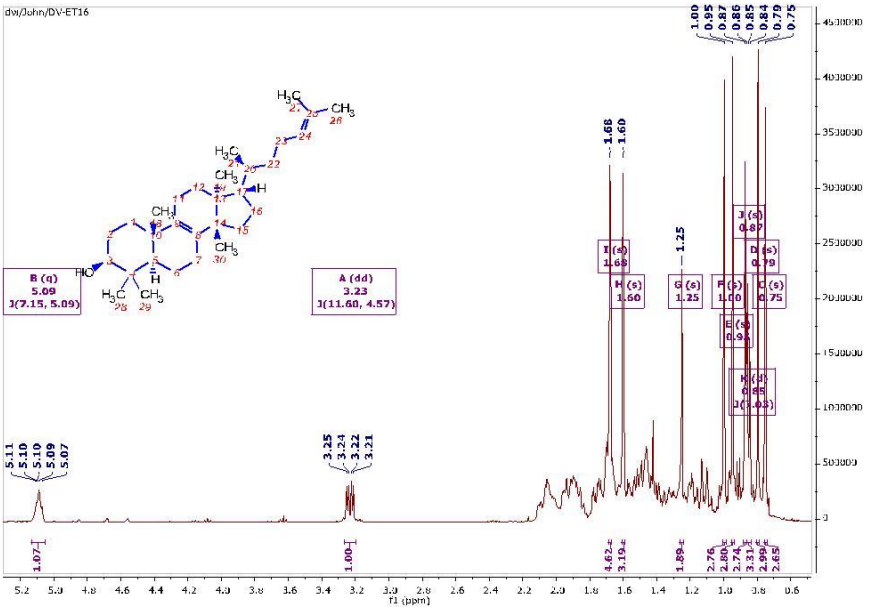


Figure 4: Proton NMR Spectrum of Et 16

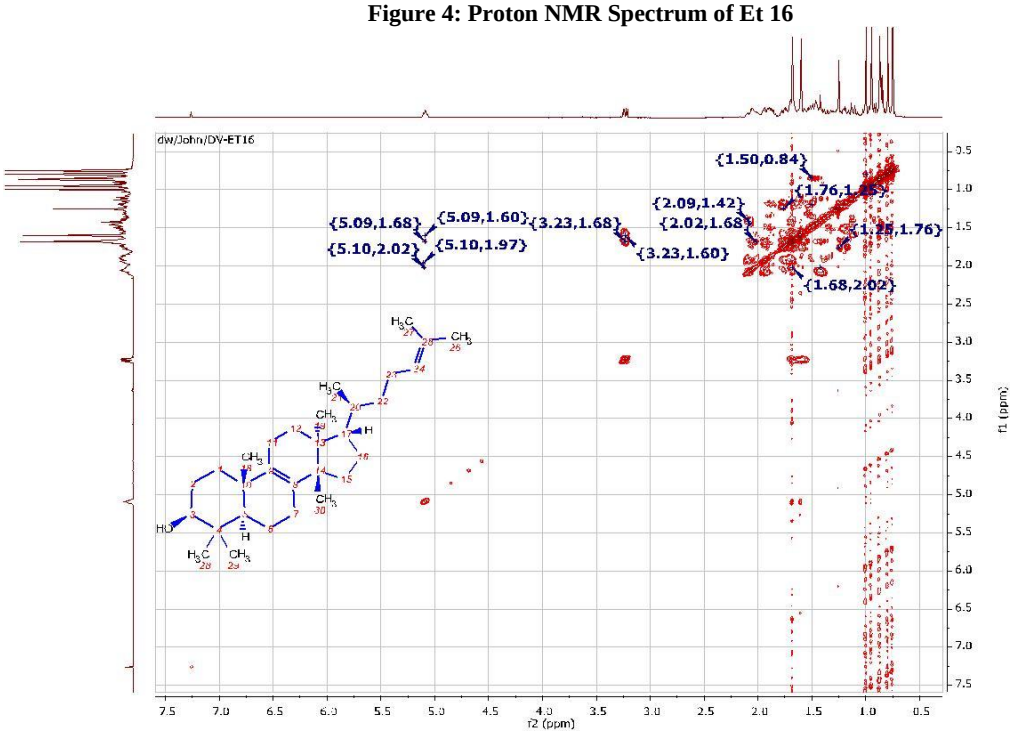


Figure 5: H-H COSY NMR Spectrum of Et 16

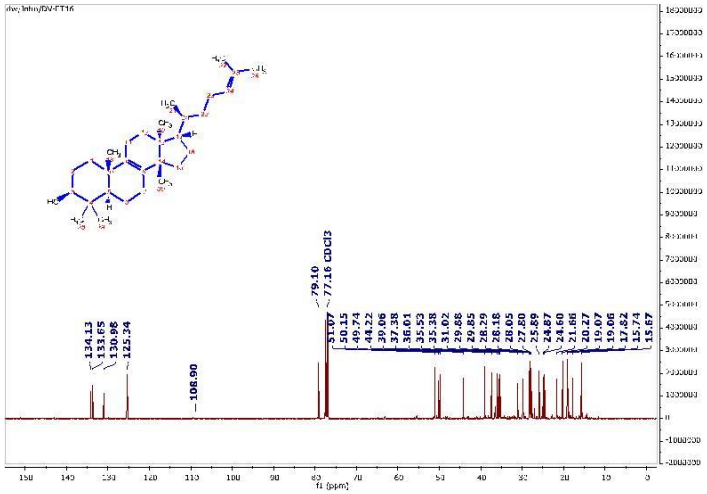


Figure 6: Carbon 13 NMR Spectrum of Et 16

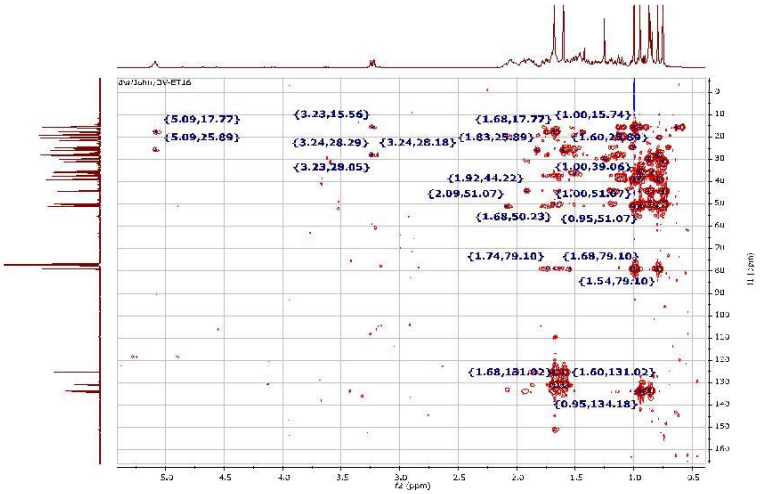


Figure 7: **HMBC Spectrum of Et 16**

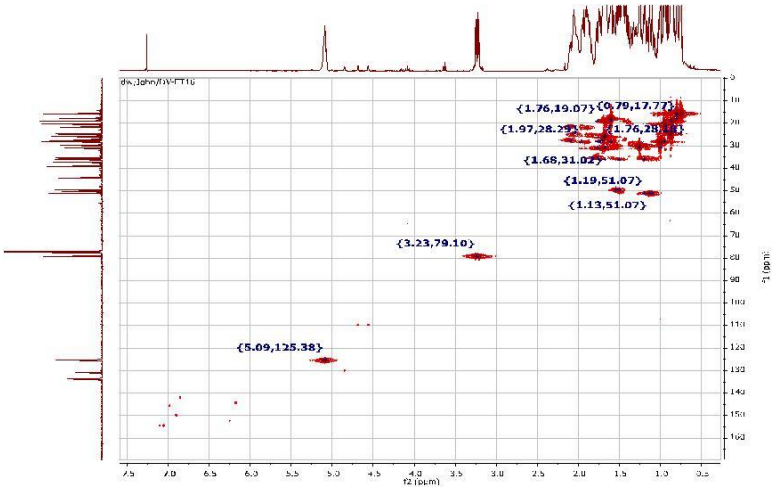


Figure 8: HSQC Spectrum of Et 16

Table 3: Characterization of Et15 as Tirucallol

Atom number	^{13}C	^{1}H	HMBC	HH-COSY
	δ [ppm]	δ [ppm],*J*[Hz]	($^{3}J/^{2}J$ in Hz)	
1	35.38	1.25, 1.76	3	2, 1 (geminal)
2	28.05	1.68, 1.60	3	3, 2 (geminal)
3	79.1	3.23 (dd, *J* = 11.6, 4.6 Hz)	2, 28, 29	2 a &b
4	39.06			
5	51.07	1.1		6
6	19.06	1.76, 1.60, 1.42		5
7	27.8	2.09	5,	6
8	133.65			
9	134.13			
10	37.38			
11	21.66	1.97	13	
12	29.88	1.25 (s)		
13	44.22			
14	49.74			
15	31.02	1.68, 1.25	17	16
16	28.18	1.74		15
17	50.15	1.5		
18	20.27	0.95 (s)	5, 10, 1, 8, 9	
19	15.67	0.79 (s)	18, 16, 14, 12, 17	
20	35.53	1.76, 1.19		
21	19.07	0.87, 0.84	17, 22	
22	36.01	1.5	20	21a&b, 23
23	24.87	2.02, 1.68		24, 23 (geminal) , 22
24	125.34	5.09 (q, J = 7.2, 5.1 Hz)	24, 25	23 a & b
25	130.98			
26	25.89	1.68 (s)	24, 25, 27	
27	17.82	1.6 (s)	24, 25, 26	
28	15.74	0.75 (s)	3, 4, 5	
29	28.29	1 (s)	3, 4, 28, 5	
30	24.6	0.87 (s)	8, 9	

Effect of Antifungal activities of *E. tirucalli* Fraction (Et15), Antibiotics Minimum inhibition concentration and Minimum Fungicidal Concentration of *E. tirucalli* Fraction (Et15)

Table 4 shows the effect of antifungal activities and *E. tirucalli* fraction (Et15) and antibiotics MIC and MFC against test fungi. *Euphorbia tirucalli* fraction (Et15) was sensitive to *A. fumigatus, F. vaillantii, G. sepiarium, Rhizopus sp. S. rolfsii* at zone of inhibition that ranged between 18 and 20 mm. The fraction was more active on *A. fumigates* and *Rhizopus sp.* at zone of inhibition of 20 mm. Fulcin was the most active the three antibiotic used against *A. fumigatus, C. puteana, F. pinicola, P. schweinitzii*, and *S. lacrymans* between 28 and 31 mm zone of inhibition.

The effect of MIC and MFC of *E. tirucalli* fraction (Et15) on test fungi is shown in Table 5. At MIC of 50 µg/mL, the growth of *A. fumigatus* and *Rhizopus sp.* fungi were inhibited while it was 100 µg/mL for *F. vaillantii* and *G. sepiarium.* However, *A. fumigatus* and *Rhizopus sp., F. vaillantii* and *G. sepiarium* were all killed at MFC of 200 µg/mL.

Table 4: Antifungal activities of *E. tirucalli* fraction (Et15), antibiotics, Minimum Inhibition Concentration (MIC) and Minimum Fungicidal Concentration (MFC) on test fungi

Key: *S = Sensitive, R = Resistance, Et = E. tirucalli; R = Resistance; MIC = Minimum inhibition concentration; MFC*

S/No.	Test Fungi	*E. tirucalli* Fraction (Et15)	Antibiotics			MIC of Et15 Fraction	MFC of Et15 Fraction
			Fulcin	Ketoconazole	Fluconazole		
		Anti-fungicidal Activities/ Zone of Inhibition					
1	*A. fumigatus*	S (20)	S (29)	S 25)	R (0)	-	200
2	*C. puteana*	R (0)	S (31)	R (0)	R (0)	-	-
3	*F. vaillantii*	S (18)	R (0)	R (0)	R (0)	100	200
4	*F. pinicola*	R (0)	S (28)	R (0)	R (0)	-	
5	*G. sepiarium*	S (19)	R (0)	S (28)	S (29)	-	200
6	*P. schweinitzii*	R (0)	S (25)	R (0)	R (0)	-	-
7	*Rhizopus sp.*	S (20)	S (29)	S (27)	R (0)	50	200
8	*S. rolfsii*	R (0)	S (31)	S (30)	R (0)	-	-
9	*S. lacrymans*	R (0)	R (0)	S (25)	R (0)	-	-

=Minimum Fungicidal Concentration of E. tirucalli; ZOI = Zone of Inhibition; When *ZOI < 10 mm is inactive; 10 -13 mm is partially active; 14 -19 mm is active, and >19 mm is very active.*

DISCUSSION

The study showed that ethyl, n hexane and methanol *E. tirucalli* extracts at concentration of 200 mg/mL were effective in the control of *F. vaillantii, F. pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans* at zone of inhibition ranging from 18-26 mm while it was between 18 and 20 mm for Et15 fraction**.** These values are within the values obtained by Kirbag *et al.* (2013), who reported zone of inhibition of 10-21 mm for *Candida albicans, Candida tropicalis and Candida vulgaris* fungi from methanol extracts of *Euphorbia aleppica, Euphorbia szovitsii, Euphorbia falcate, Euphorbia denticulate, Euphorbia macroclada, Euphorbia cheiradenia, Euphorbia virgata and Euphorbia petiolata*. However, the values in this study are lower than what was recorded by Waheed *et al*. (2020) who reported zone of inhibition of 24 and 32 mm on *Rhizopus nigricans*, 16 and 34 mm on Acremonium and 28 and 34 mm against *A. niger* from aqueous *Euphorbia helioscopia* extract at a concentration of 500 mg mL-1 and 1,000 mg/mL respectively. Guevara, (2005) noted that zone of inhibition of antibiotics values less than 10 mm could be said to be inactive, 10-13 mm as partially active, 14-19 mm as being active and values greater than 19 mm are regarded as being very active. It therefore implies that *E. tirucalli* extracts and fraction as reported from this study were very potent in the control of five wood test fungi.

The MIC of 10 mg/mL and MFC of 20 mg/mL were recorded as the concentrations that were most effective against *F. vaillantii, F. pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans* test fungi. From, literature search, no much work has been done on the use of *E. tirucalli* extracts and fractions in the control of wood decay fungi.*E. tirucalli* fraction (Et15) was characterized as Tirucallol. Fernandez-Arche, (2010) reported 0.3 % tirucallol from *Euphorbia lactea* latex to exert a topical anti-inflammatory influence in vivo, through a mechanism of feat in relation to the neutrophil migration. Fernandez-Arche, (2010) also reported that topical function of tirucallol isolated from *Euphorbia lactea* latex appreciably reduced Myeloperoxidase levels in ear homogenates.

CONCLUSION

It was noted that both *E. tirucalli* extracts and fraction had fungicidal activities against of *F. vaillantii,* F. *pinicola, G. sepiarium, Rhizopus sp.* and *S. lacrymans*. Tirucallol compound was characterized from *E. tirucalli.* Zones of inhibition and MIC/MFC recorded for *E. tirucalli* extracts and fraction on test fungi implies that, the species was very potent and could therefore be used in the control of diseases caused by the wood decay fungi and their activities on wood products.

REFERENCES

Baniakina, J. and Eyme, J. 1997. Studies on the morphological and anatomical structures in the Family Euphorbiaceae. *Revue de medicines et Pharmacopee africaine,* 12: 27-48.

Chand, S., Lusunzi, I., Veal, D. A., Williams, L. R. and Karuso, P. 1994. Rapid screening of the antimicrobial activity of extracts and natural products. *The Journal of Antibiotics*, 47 (11), 1295-1304.

Fernandez-Arche, A., Saenz, M. T., Arroyo, M., de la Puerta, R. and Garcia, M. D. 2010. Topical anti-inflammatory effect of tirucallol, a triterpene isolated from *Euphorbia lactea* latex. *Phytomedicine,* 17 (2): 146–148. doi:10.1016/j. phymed. 2009.05.009.

Guevara, B. Q. 2005. A Guidebook to Plant Screening: Phytochemical and Biological, Revised Edition, UST Publishing House, Manila. Pp 156.

Hostettmann, K. and Marston, A. 1995. *Chemistry and Pharmacology of Natural Products*, 548: 326-327. Cambridge, UK: Cambridge University Press.

Kirbag, S., Erecevit, P., Zengin, F. and Guvenc, A. 2013. Antimicrobial activities of some Euphorbia species.

African Journal of Traditional, Complementary and Alternative Medicines, 10 (5): 305-309.

Kumar A. (1999). Some potential plants for medicine from India, Ayurvedic medicines, University of Rajasthan, Rajasthan. Pp. 1-12.

Loizou, S., Paraschos, S., Mitakou, S., Chrousos, G. P., Lekakis, I., and Moutsatsou, P. (2009). *Chios Mastic Gum Extract and Isolated Phytosterol Tirucallol Exhibit Anti-Inflammatory Activity in Human Aortic Endothelial Cells. Experimental Biology and Medicine*, 234 (5): 553–561. Doi: 10.3181/0811-rm-338.

Mwine J. and Van Damme P. (2010). Evaluation of selected pesticidal plant extracts against major cabbage insect pests in the field. Tropentag 2010: "World Food System – A Contribution from Europe", ETH Zurich, Switzerland, 14-16 September 2010.

Mwine, J. and Damme, P. V. 2011. *Euphorbia tirucalli* L. (Euphorbiaceae) –The miracle tree: Current status of available knowledge. *Scientific Research and Essays*, 6 (23): 4905-4914

Siddiqui, M., Alam, M., and Trivedi, P. 2003. Management of plant parasitic nematodes with latex bearing plants, Nematode management in plants. Scientific Publishers, India, Pp. 173-174.

Swapna, N. L. and Prasad, M. 2011. Efficacy of *Euphorbia tirucalli* (L.) towards microbicidal activity against human pathogens. *International Journal of Pharmaceutical and Bio Sciences*, 2 (1): 229-235.

Waheed, K., Muhammad, S. K., Shomaila, A., Muhammad, Z., Izhar, U. and Ullah, S. 2020. Antimicrobial activity and phytochemical screening of *Euphorbia helioscopia*, *Planta daninha*, 38: 1-11.

Yi, Q., Wan, Z. Z., Nurulhidayah, C., Mohamad, E. Y., Azlina, A. and Suharni, M. 2017. The Antibacterial Properties of *Euphorbia tirucalli* Stem Extracts against Dental Caries-Related Bacteria. *Medicine and Health*, 12 (1): 34-41.