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Antifungal Activity of Compounds obtained from Sawdust and Stem Bark of Sasswood Tree (*Erythrophleum suaveolens*) on Wood Rot Fungi

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ABSTRACT: Extracts and compounds from the sawdust and stem bark of *Erythrophleum suaveolens* were studied for antifungal activity. The plant materials were extracted using hexane, ethyl acetate and methanol to obtain the crude extracts. Column chromatography over silica gel was used to purify the extracts and obtain pure compounds. Betulin and 21-acetoxylupenone were obtained from the stem bark and cycloeucalenol from the sawdust. The compounds were very active on most of the fungi with zones of inhibition between 18 to 23 mm. Minimum Inhibition Concentration (MIC) was highest at 50 µg/mL against *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicoca, Gloeophyllum sepiarium, Phaeolus schweinitzii* and *Rhizopus* spp. whereas antifungal activity was least at MFC of 200 µg/mL. The study demonstrated that *E. suaveolens* stem bark and sawdust isolates possess antifungal activity and could be used in the control of the soft-rot, brown-rot, wet rot and white-rot of wood and diseases caused by wood fungi.

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Wood fungi help to remove nitrogen, phosphorus, and potassium in the early stages of wood decay. Woody tissues are decomposed by soft-rot, brown-rot, and white-rot fungi (Deacon, 2005). White-rot fungi degrade lignocelluloses and they are capable of degrading cellulose, hemicellulose and lignin (Schmidt, 2006) while brown rot fungi degrade hemicellulose, cellulose and modify or cleave lignin but are not able to metabolize it. Soft rot fungi degrade only cellulose and hemicelluloses (Prasher and Lalita, 2013). Soft rot usually occurs in areas with excessive moisture. Wood rot fungi can decompose the main components of wood because they produce hydrolytic and oxidative enzymes (Lalita and Prasher, 2014). Fungi activities decrease the strength and properties of wood over time (Harmon *et al.,* 1994; Hattenschwiler *et al.,* 2005).

Wood and wood products are majorly treated with synthetic chemicals which are costly and harmful to users and the environment (Venmalar and Nagaveni, 2005). These challenges have led to an increasing interest in the development of biological alternatives for the control of wood degrading microbes. Plants with pesticidal properties have proven to be viable alternative (Syofuna *et al.,* 2012). Evaluation of plants possessing antimicrobial activities has been variously carried out to ascertain their potency (Odelade and Oladeji, 2016; Nascimento *et al.,* 2000).

Erythrophleum suaveolens or Sasswood tree belongs to the family leguminosae-caesalpinioideae. It is a perennial tree of about 30 m in height and has been used as a biopesticide (Dongmo *et al*., 2001). The stem bark extract of *E. suaveolens* has been reported to possess significant anti-fungal activity against *C. albicans* and *C. krusei* as well as having antibacterial activity (Ngounou *et al.* (2005). However, the fungicidal activity of the sawdust of *E. suaveolens* has not been evaluated. As a result, the fungicidal activities of isolated compounds from *E. suaveolens* stem bark and sawdust on selected wood fungi is hereby reported.

MATERIALS AND METHODS

Extraction of stem bark and sawdust: Extraction of dried samples of *E. suaveolens* was by successively macerating 1000 g and 600 g of stem bark and sawdust

into 1000 mL (w/v) of n-hexane, ethyl acetate and methanol respectively, for a period of 24 hours. The resulting extract were filtered and dried as described by Ekhuemelo *et al.* (2019).

Column chromatography: A slurry of silica gel (50 g) in hexane: ethyl acetate 95:5 was poured into a glass column and *E. suaveolens* extracts was loaded onto the column bed and eluted as previously described. This was carried out for each of the extracts to obtain several column fractions. Thin layer chromatography was used to examine and combine similar fractions. Nuclear magnetic resonance spectroscopic analysis of the combined fractions identified compounds ES-24, ESS-25 and ESS-31 (Ekhuemelo *et al.,* 2019).

Antimicrobial and fungi screening: Antifungal screening was carried out at the Nigerian Institute for Leather Science and Technology (NILEST), Zaria. The antifungal activities of fractions ES-24, ESS-25 and ESS-31 were evaluated against selected wood fungi: *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicoca, Gloeophyllum sepiarium Phaeolus schweinitzii, Rhizopus* spp*., Serpula lacrymans* and *Sclerotium rolfsii*. The antibiotics, Fulcin Ketoconazole and Fluconazole were used as control. A disk diffusion method was used for the screening of the fractions. An initial concentration of the fractions was prepared by dissolving 0.002 g in 10 mL of dimethyl sulfoxide (DMSO) to obtain a concentration of 200 µg/mL. Media were prepared in accordance to manufacturer's manual and purified at 121^{o}C for a period of 15 minutes. The media was emptied into germ-free Petri dishes and left to cool and harden. Sabouraud Dextrose agar was seeded with a 0.1 mL standard inoculum of the fungi spread uniformly on the surface of the medium with aid of a disinfected swab. A cork borer measuring 6 mm in diameter was used to cut a well at the middle of each injected medium. In the well of the inoculated medium, 0.1 mL of the stock solution of compound (200 µg/mL) was introduced at 30 ^{o}C for 1-7 days and the media plates were observed for zones of inhibition of fungi growth.

Technique for Minimum Inhibition Concentration (MIC): A broth dilution method was used to determine MIC of compounds. Sabouraud dextrose broth was prepared while 10 mL of the stock solution was dispensed into test tubes; the broths were sterilized at 121 ^{o}C for 15 minutes and allowed to cool. Mcfarland turbidity scale number 0.5 was made to produce turbid solution while normal saline was prepared as 10 mL was emptied into sterilized test tubes. The fungi was inoculated and nurtured at 30 ^{o}C for 1 – 7 days. Dilution of the fungi was carried out in the standard saline until the turbidity synchronised with the scale of Mcfarland by visual assessment. At this point, the fungi had a concentration of about $1.5x10^{8}$ cfu/ml.

To attain concentration levels of 200 µg/mL, 100 µg/ mL, 50 µg/ mL, 25 µg/ mL and 12.5 µg/ mL, two-fold serial dilution of fraction was carried out in the sterilized broth. Initial concentration was obtained by dissolving 0.002 g of the fraction in 10 mL of the sterile broth and 0.1 mL of fungi in the normal saline was then introduced into the different concentrations. The fungi were introduced into the Sabouraud Dextrose broth. Incubation was at 30 ^{o}C for 1-7 days to observe fungi growth. The least concentration of the fraction in the sterilized broth that indicates no growth was determined as the MIC.

Minimum Fungicidal Concentration (MFC): Sabouraud Dextrose agar were prepared and sterilized at 121^{o}C for a period of 15 minutes, emptied into sterilized Petri dishes and left to cool and congeal. The contents of the MIC in the serial dilution were then sub-cultured on to the prepared medium. The fungi were incubated at 30 ^{o}C for 1-7 days, after which the plates of the media were observed for colony growth. MFC were observed as the plates with lowest concentration of the fraction without colony growth.

Data Analysis: Analysis of Variance (ANOVA) was used to determine significant effects of treatments on ZOI. Follow up tests were carried out using Duncan Multiple Range Test (DMRT) where significant differences exist.

RESULTS AND DISCUSSION

The yield of extracts is given in Table 1 and most of the extracts appeared as dark brown, purple or yellow.

Table 1: Yield of extracts

Solvent	Extracts	Weight of plant material (g)	Weight of dry extract (g))	Percentage yield of extracts (%)
Ethyl acetate	*E. suaveolens* stem bark	1000	3.6	0.36
	E. suaveolens sawdust	600	6.4	1.06
Methanol	*E. suaveolens* stem bark	1000	51.9	5.19
	E. suaveolens sawdust	600	20.5	3.42
n-Hexane	*E. suaveolens* stem bark	1000	1.6	0.16
	E. suaveolens sawdust	600	0.7	0.12

Characterization of ES-24, ES-28, ESS-25 and ESS-37: The compounds in the fractions were identified as 21-acetoxylupenone (ES-24, Fig. 1), Betulin (ES-28, Fig. 2), Cycloeucalenol (ESS-25, Fig 3) and a mixture of Sitosterol, Stigmasterol and Cycloeucalenol (ESS-37) as previously reported (Ekhuemelo *et al* 2019).

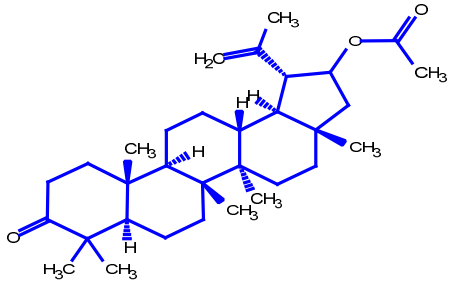

Fig 1: Structure of ES-24 (21-acetoxylupenone)

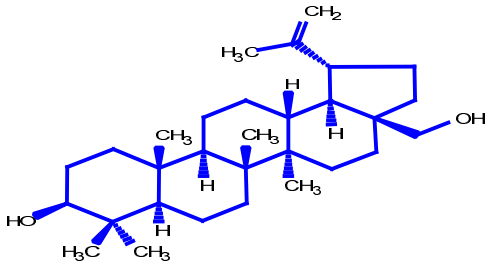

Fig 2: Structure of ES-28 (Betulin)

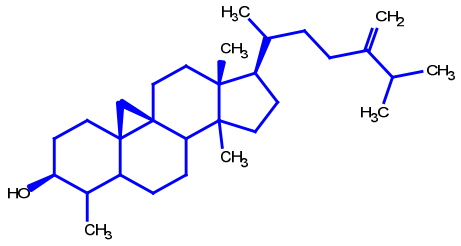

Fig 3: Structure of ESS-25 (Cycloeucalenol)

Sensitivity and ZOI of antibiotics and E. suaveolens isolated compounds (ES-24, ESS-25 and ESS-31) on fungi: Fulcin was the most active antibiotic on fungi at concentration of 100 µg/mL followed by Ketoconazole and Fluonazole (Table 2). Their ZOI ranged from 25 – 31 mm. *Erythrophleum suaveolens* fractions were active against seven of the fungi at ZOI of between 18 – 23 mm. *Fibroporia vaillantii* microbe was resistant to all antibiotics but sensitive to the three *E. suaveolens* isolated compounds at ZOI between 21 – 22 mm. Whereas *Serpula lacrymans* and *Sclerotium rolfsii* microbes were resistant to all *E. suaveolens* compounds, *Serpula lacrymans* growth was inhibited by Fulcin at 30 mm while, *Sclerotium rolfsii* growth by Ketoconazole at 25 mm. The ZOI of antibiotics and isolated compounds were not significantly different ($p<0.05$).

The compounds isolated from the sawdust and stem bark extracts of *E. suaveolens* showed high antifungal activity against all the test fungi with ZOI between 18 and 23 mm. Omachi (2015) reported a similar ZOI of 20 - 21 mm for fungi tested with extracts of *Morinda lucida* leaf and 25 - 30 mm for *Uapaca togoensis* stem bark extracts. However, Ezeonu *et al*. (2018) reported a higher ZOI against *Aspergillus niger* (39.5 mm), *Rhizopus stolonifer* (91.5 mm) and *Aspergillus oryzae* (84.0 mm) from *Azadirachta indica* ethanolic stem bark extract. Ezeonu *et al*. (2018) also reported zone of inhibition of aqueous stem bark extract from *Azadirachta indica* against *Aspergillus oryzae* (96.0 mm), *Rhizopus stolonifer* (94.0 mm) and *Aspergillus niger* (87.0 mm).

Effect MIC of E. suaveolens isolated compounds (ES-24, ESS-25 and ESS-31) on fungi: MIC results of *E. suaveolens* compounds (ES-24, ESS-25 and ESS-31) on fungi (Table 3) indicates that at 50 µg/mL, the growth of *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicoca* and *Rhizopus* spp. fungi were completely inhibited *by* ES-24 while, *Coniophora puteana, Fibroporia vaillantii, Gloeophyllum sepiarium* and *Phaeolus schweinitzii* growth were inhibited by ESS-25. Similarly, *Aspergillus fumigatus, Fibroporia vaillantii, Fomitopsis pinicoca, Phaeolus schweinitzii,* and *Rhizopus* spp., were completely inhibited by ESS-31. However, *Serpula lacrymans* and *Sclerotium rolfsii* were not affected by any of the *E. suaveolens* compounds. Minimum inhibition concentration for *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii, Fomitopsis pinicoca, Gloeophyllum sepiarium, Phaeolus schweinitzii* and *Rhizopus* spp was 50 µg/mL. This result is similar with the finding of Johann *et al.* (2011) who reported hexane extract of *P. paniculata* and EtOAc fraction of *P. sabulosa* to have antifungal activity, with MIC values of 60 and 30 µg/mL, respectively, against *C. tropicalis, C. gattii and S. schenckii.*

The MIC values obtained in this study are at variance with the report by Mendes de Toledo *et al*. (2015) for *Candida* species with MIC between 15.3 to 31.3 µg/mL for crude extracts, 3.9 to 15.6 µg/mL for an ethyl acetate fraction, and 7.8 to 31.3 µg/mL for sub-fractions of the *Curatella Americana* extract. The isolated compounds were identified as 4-*O*-methylcatechin, epicatechin-3-*O*-gallate, and 4-*O*-methyl-catechin-3-*O*-gallate showed antifungal activity higher than the crude extracts and fractions with MIC from 31.3 to 125.0 µg/mL which is within the range obtained in this study.

Effect of MFC of E. suaveolens isolated compounds (ES-24, ESS-25 and ESS-31) on fungi: Table 4 shows

the MFC of *E. suaveolens* fractions on fungi. Compound ES-24 completely inhibited *Aspergillus fumigatus, Coniophora puteana, Fibroporia vaillantii,* and *Fomitopsis pinicoca* fungi at 200 *µg/mL and Rhizopus* sp. at 100 µg/mL.

Table 2: Sensitivity and Zone of inhibition of *E. suaveolens* compounds against wood fungi

Fungi	Fulcin (100 µg/mL)	Ketoconazole (100 µg/mL)	Fluconazole (100 µg/mL)	ES-24	ESS-25	ESS-31
	AFA (ZOI) Mean ± SD	AFA (ZOI) Mean ± SD	AFA (ZOI) Mean ± SD	AFA (ZOI) Mean ± SD	AFA (ZOI) Mean ± SD	AFA (ZOI) Mean ± SD
Aspergillus fumigatus	S (29.00±1.00^{bc})	S (25.00±1.00^{b})	R (0.00±0.00^{a})	S(20.33±1.53^{b})	R(0.00±0.00^{a})	S(20.00±2.00^{b})
Coniophora puteana	S (31.00±1.00^{c})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S(20.00±5.00^{b})	S(23.00±1.00^{b})	R(0.00±0.00^{a})
Fibroporia vaillantii	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S(21.00±4.00^{b})	S(22.00±2.00^{b})	S(21.00±2.00^{b})
Fomitopsis pinicoca	S (28.00±4.00^{bc})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	S(23.00±1.0^{b})	R(0.00±0.00^{a})	S(20.00±2.00^{b})
Gloeophyllum sepiarium	R (0.00±0.00^{a})	S (28.00±6.00^{b})	S (29.00±5.00^{b})	R(0.00±0.00^{a})	S(21.00±5.00^{b})	R(0.00±0.00^{a})
Phaeolus schweinitzii,	S (25.00±5.00^{bc})	R (0.00±0.00^{a})	R (0.00±0.00^{a})	R(0.00±0.00^{a})	S(20.00±5.00^{b})	S(20.00±3.00^{b})
Rhizopus sp.	S (29.00±2.00^{bc})	S (27.00±2.00^{b})	R (0.00±0.00^{a})	S(23.00±3.00^{b})	R(0.00±0.00^{a})	S(18.00±2.00)
Serpula lacrymans	S (30.33±3.05^{c})	S (30.00±2.00^{b})	R (0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})
Sclerotium rolfsii	R (0.00±0.00^{a})	S (25.00±5.00^{b})	R (0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})	R(0.00±0.00^{a})

Key: *AFA = Antifungal Activities; ES = E. suaveolens stem bark, ESS = E. suaveolens sawdust, ZOI = Zone of Inhibition. ZOI = Zone of Inhibition. ZOI < 10 mm is inactive; 10 -13 mm is partially active; 14 -19 mm is active, and >19 mm is very active.*

Table 3: Minimum Inhibition Concentration (MIC) of *E. suaveolens* fractions (ES-24, ESS-25 and ESS-31) against wood fungi

Fungi	ES-24					ESS-25					ESS-31				
	Concentration *(µg/mL)*					Concentration (µg/mL)					Concentration (µg/mL)				
	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
Aspergillus fumigatus	-	-	δ	+	#	R	R	R	R	R	-	-	δ	+	#
Coniophora puteana	-	-	δ	+	#	-	-	δ	+	#	R	R	R	R	R
Fibroporia vaillantii	-	-	δ	+	#	-	-	δ	+	#	-	-	δ	+	#
Fomitopsis pinicoca	-	-	δ	+	#	R	R	R	R	R	-	-	δ	+	
Gloeophyllum sepiarium	R	R	R	R	R	-	-	δ	+	#	R	R	R	R	R
Phaeolus schweinitzii,	R	R	R	R	R	-	-	δ	+	#	-	-	δ	+	#
Rhizopus spp.	-	-	δ	+	#	R	R	R	R	R	-	δ	+	#	##
Serpula lacrymans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sclerotium rolfsii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key: *ES = Erythrophleum suaveolens stem back; ESS = Erythrophleum suaveolens sawdust; R = Resistance; - = No turbidity (no growth);* ***δ*** *= Minimum inhibition concentration (MIC); += Turbid (Light growth); # = Moderate turbidity; ### = High turbidity*

Table 4: Minimum Fungicidal Concentration *(*MFC) of *E. suaveolens* fractions (ES-24, ESS-25 and ESS-31) against wood fungi

Fungi	ES-24					ESS-25					ESS-31				
	Concentration *(*µg/mL)					Concentration (µg/mL)					Concentration (µg/mL)				
	200	100	50	25	12.5	200	100	50	25	12.5	200	100	50	25	12.5
Aspergillus fumigatus	δ	+	#	###	##	R	R	R	R	R	δ	+	#	###	##
Coniophora puteana	δ	+	#	###	##	-	δ	+	#	###	R	R	R	R	R
Fibroporia vaillantii	δ	+	#	###	##	δ	+	#	###	##	δ	+	#	###	##
Fomitopsis pinicoca	δ	+	#	###	##	R	R	R	R	R	δ	+	#	###	##
Gloeophyllum sepiarium	R	R	R	R	R	δ	+	#	###	##	R	R	R	R	R
Phaeolus schweinitzii,	R	R	R	R	R	δ	+	#	###	##	δ	+	#	###	##
Rhizopus sp.	-	δ	+	#	###	R	R	R	R	R	δ	+	#	###	##
Serpula lacrymans	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Sclerotium rolfsii	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Key*: ES = Erythrophleum suaveolens stem back; ESS = Erythrophleum suaveolens sawdust; R = Resistance; - = No turbidity (no growth); δ = Minimum Fungicidal concentration (MFC); + = Turbid (Light growth); # = Moderate turbidity; ### = High turbidity; ##= Very High turbidity*

It was also observed that compound ESS-25 completely inhibited by *Coniophora puteana* at 100 µg/mL, whereas *Fibroporia vaillantii, Gloeophyllum sepiarium* and *Phaeolus schweinitzii,* were inhibited at MFC of 200 µg/mL. At *200* µg/mL MFC also, *Aspergillus fumigatus, Fibroporia vaillantii, Fomitopsis pinicoca, Phaeolus schweinitzii* and *Rhizopus* sp. were completely inhibited by ESS-31. while ES-24 and ESS-25 inhibited the growth of *Rhizopus sp.,* and *Coniophora puteana* at 100 *µg/mL.* Whereas *Aspergillus fumigatus, Fibroporia vaillantii, Fomitopsis pinicoca, Gloeophyllum sepiarium, Phaeolus schweinitzii* pathogens were inhibited at MFC of 200 µg/mL. Joshi *et al*. (2003) reported a lower MFC of fungicidal activity for the chloroform extract of the stem bark of *Alianthus excelsa* on *A.*

niger (500 mg/mL), *Aspergillus fumigatus* (160 mg/mL), *P. frequentance* (80 mg/mL), *P. notatum and B. cinerea* to be 160 mg/mL, respectively.

The efficacy of *Erythrophleum suaveolens* stem bark isolates (ES-24 and ES-28) and *E. suaveolens* sawdust isolate (ESS-25) from this study implies they can control wood-trimmers' disease caused by *Aspergillus fumigatus* (Land *et al.,* 1987); brown rot decay caused by *Aspergillus fumigatus* and *Coniophora puteana* (Mahmood *et al.,* 2016*;* Korner *et al.,* 1992) The isolated compounds can also be effective in treating wet rot disease in buildings caused by *Fibroporia vaillantii* (Property Care Association, 2016); stem decay in wood caused by *Fomitopsis pinicoca* and *Gloeophyllum sepiarium* (Haight *et al.,* 2016). Brown cubical rot of the heartwood of butt and roots caused by *Phaeolus schweinitzii* (Sinclair *et al.,* 1987) and head rot disease or decay fruits and vegetables caused by *Rhizopus* sp. (Kirk *et al.,* 2008) could be controlled by *E. suaveolens* isolates. The antifungal activity of *E. suaveolens* sawdust and stem bark extracts can be attributed to the presence of 21-acetoxylupenone, betulin and cycloeucalenol isolated and identified in this study.

*Conclusion***:** This study has demonstrated that *E. suaveolens* stem bark and sawdust isolates possess antifungal activity and could be used in the control of the soft-rot, brown-rot, wet rot and white-rot disease of wood caused by *Aspergillus fumigatus*, *Aspergillus fumigatus,* fungi *Coniophora puteana, Fibroporia vaillantii, Gloeophyllum sepiarium, Phaeolus schweinitzii* and *Rhizopus* sp. Therefore, this study has scientifically validated the use of *E. suaveolens* stem bark and sawdust as effective antifungal agents.

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