Malaysian Journal of Microbiology, Vol 8(1) 2012, pp. 34-41

Bioactivity guided isolation of the antifungal components in sawdust extracts of *Piptadeniatrum africanum,* and *Terminalia ivorensis*.

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Received 10 May 2011; received in revised form 27 November 2011; accepted 25 December 2011

ABSTRACT

Aim: The antifungal activities of extracts from fresh sawdust of *Piptadeniatrum africanum,*and *Terminalia ivorensis* was determined using the agar well diffusion method. Aqueous, methanol, chloroform,and n-hexane extracts obtained from the sawdust samples were tested against fungi isolated from some decayed wood samples.
Methodology and Results: Fungal isolates included: *Aspergillus. niger*, *Aspergillus. flavus*, *Aspergillus. fumigates*, *Aspergillus. wentii*, *Aspergillus. tamari*, and *Penicillium. chrysogenum* (please write the microorganisms' name in full when first time introducing them). Results of inhibitory activities showed that the chloroform extracts showed the highest inhibitory abilities with zones of inhibition ranging from 14 mm-24 mm for *P. africanum,* and 11 mm-15 mm for *T. ivorensis*. However, the aqueous extracts exhibited the least antifungal activity with zones of inhibition ranging between 10 mm-13 mm and 8 mm-11 mm respectively.
Conclusion, significance and impact of study: The major secondary plant metabolites identified are alkaloids, anthraquinones, anthraglycosides, arbutin, glycosides, flavonoids, phenolics, saponins, coumarins and valepotriates. The antifungal components in *P. africanum* were identified to be 3, 7, 8, 3'–Tetramethoxy-6–C-methyl-5, 4'–dihydroxyflavone and 3–methoxy–6–C–methyl–3',4',5,7,8–pentahydroxyflavone while *Terminalia ivorensis* contained 5,7,8-Trihydroxy-2′5′-methoxy-3′,4′-methylenedioxyisoflavanone as the bioactive component.

Keywords: antifungal, extracts, bioactive components, fungal isolates, wood, sawdust.

INTRODUCTION

Wood is a natural and renewable resource used extensively in the home, office, for furniture and construction of fences, utility poles, etc. Wood is one of the oldest and least costly of available construction materials. This renewable natural resource is used for a variety of purposes because of its unique combination of properties which include high strength–to-weight ratio, resiliency, and toughness (Bultman and Southwell, 1976). Throughout recorded history, the unique characteristics and relative abundance of wood have made it man's most valuable and useful natural resources. Today, literally thousands of available products from solid wood, wood pulp and chemicals are derived from wood (Hoadley, 1990).

Blanchete and Shaw (1978) explained that wood decay is deterioration of wood by primarily enzymatic activities of microorganisms and that fungi are the major agents causing wood decay. There are other kinds of deterioration though, caused by insects and marine animals, but this is not decay, nor is it quantitatively as important as decay. microbes act directly on individual components (such as cellulose and lignin) of the wood cell wall by the production of extracellular enzymes, such as cellulase and pectinase which destroy the wood components (Hoadley 1990 and Adeleye and Lashebikan, 2003).

In the natural forest, the tree grows, reaches full maturity, falls and is broken down by organisms. Humus and essential materials are returned to the forest soil, more trees grow and the cycle continues. Organisms have evolved depending heavily on wood as their main source of nutrition.

In wood decay, three classes of fungi are of prime importance:
(1) Basidiomycetes – Spores are borne on outside of a special organ called basidium. The basidiomycetes include the fungi giving rise to brown and white rots. They are capable of metabolising the lignin and carbohydrate components of wood (white rot) or primarily the carbohydrate component (brown rot) (Bultman and Southwell, 1976). The Basidiomycetes generally occur in moist and fairly well-aerated conditions.
(2) Ascomycetes – Spores borne inside a special structure – the Ascus. The Ascomycetes give rise to soft rot by the degrading the secondary layers of wood cellwalls and they can tolerate lower levels of aeration than Basidiomycetes.
(3) Fungi imperfecti also contain species capable of causing soft rot in wood. These fungi resemble Ascomycetes in many ways but differ from them by not

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ISSN (print): 1823-8262, ISSN (online): 2231-7538

undergoing a perfect spore bearing phase (Blanchette and Shaw, 1978).

Soft rot fungi, primarily ascomycetes and fungi imperfecti degrade secondary layers of wood cell walls. Under favourable conditions of temperature, air and moisture content of the wood, brown and white rot fungi penetrate deeply into the wood, spreading from cell to cell and enzymatically degrading the cell wall components and storage products from which they obtain the nutrients and energy necessary for their survival. (Barry *et al.,* 2002). The soft rot fungi attack the outer layers of wood cells and cause a gradual softening of wood from the surface inward as successive layers of cells are colonized and decayed (Bultman and Southwell, 1976).

It has been established that woods contain extractives known to confer protection against wood-decaying microorganisms (Dinwoodie, 1981 and Barry *et al.,* 2002). Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Iwu *et al.,* 1999). Most are secondary metabolites in many cases. These substances serve as plant defense mechanisms against microorganisms, insects and herbivores (Cowan, 1999 and Khan *et al.,* 2002).

Many constituents of wood are toxic or inhibitory to fungi (Cobb *et al.* 1968). Of these, tannins and phenols have received most attention.In view of the continued problems and daily constraints on plant disease control methods and applications particularly those arising through the use of chemicals such as pesticides, insecticides and fungicides plant pathologist are increasing their efforts in developing areas for the achievement of successful biological control systems. In turn this has led to its recognition as a possible practical method for soil borne and aerial pathogens control (Madigan *et al,* 2000 and Fatubarin, 2006).

MATERIALS AND METHODS

Isolation of microorganisms

The wood samples, were surface sterilized by flooding with 0.1% v/v solution of mercuric chloride (Adeleye and Omotosho, 2003). Isolation was by the method described by Levy and Dickinson (1981). A small cut made below the surface and a piece of underlying wood tissues (about 2 g) was removed aseptically and placed in a sterile pestle and mortar. The wood sample was crushed and suspended in 9 mL of sterile distilled water. Serial dilution was carried. Exactly 1ml of the 10^{-6} diluted solution of each wood sample was inoculated on potato dextrose agar plates enriched with 0.1% sawdust. The plates were incubated at 30 ^{0}C for 48 h and later purified by sub-culturing onto fresh potato dextrose agar at ambient temperature for 48 h.

Identification of Fungal Isolates

Isolated fungi were identified based on the observation of cultural and morphological characteristics, colour of colony and sporulation. Examination was carried out using needle-mount preparation whereby fragments of the sporing surface of the culture was taken. This was teased out in drop of alcohol on a cleaned glass slide using needle. The fragment was stained by adding a drop of lactophenol. A cover slip was applied carefully avoiding air bubbles and the preparation was examined under light microscope (Barnett and Hunter, 1972).Potato dextrose agar (PDA) and Czapek Dox agar (CDA) were used for cultivation of cultures (Haung and Ling, 1973 and Difco, 1984).

Sawdust sample extraction

Exactly 300 g each of the sawdust sample from the healthy woods of *Piptadeniatrum africanum,* and *Terminalia ivorensis* was soaked in 750 mL of methanol. The solution was left on the laboratory bench for 72 h after which it was filtered first with muslin cloth, then with No. 1 Whatman filter paper. The filtrate was dried using the rotary evaporator and the extract was obtained. Similar procedure was used to obtain chloroform and n-hexane extracts (Ogundare, 2005 and Wang and Weller, 2006).

Antifungal activities of the crude extract

Each of the crude extracts were screened for antifungal activities against the fungal isolates implicated in wood decay using the agar-well diffusion method described by Olutiola and Akintunde (1991). Spores of the fungal isolates were introduced into sterile water; one mL of the stock was introduced into sterile Petri dish on to which sterile potato dextrose agar was poured. Using a 4 mm sterile cork borer, holes were made inside the agar, and into these holes solution of the crude extracts were introduced. The plates were incubated at room temperature (28^{o}C ± 2 °C) for 3-7 days and they were examined for fungal growth. The antifungal activity of the extracts, as indicated by clear zones of growth inhibition around the wells, was examined and recorded in millimeters. Control experiment was carried out alongside introducing sterile water into the holes borne instead of the crude extract solution.

Phytochemical Screening

Basic phytochemical analysis was carried out to determine the bioactive ingredient present in the extracts. The conventional methods of Harbone (1984) and Wagner and Bladt (2001) were adopted.

Fractionation of the extracts

Exactly 3 g of the crude extracts that exhibited antifungal activity was each absorbed on silica gel of 60-120 mesh (BDH) and chromatographed on a column of silica gel 60 slurry packed in petroleum ether. The column was gradient eluted first with petroleum ether and then with ethyl acetate: methanol 40:1 and finally with 100% methanol.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

The fractions collected were analyzed by thin layer chromatography (TLC) on a precoated plates Merck silica gel 60 F254, 0.2 mm thickness using ethyl acetate : methanol (40:1), butanol : water (1:1), chloroform : pyridine (1:1), methanol: ammonium hydroxide(200:3); acetate : pyridine : water (5:1:4); chloroform : hexane : ethanol : acetic acid (5:4:1:1) as the mobile phases. Fractions showing the same TLC characteristic were bulked together and numbered. This was also confirmed by measuring their absorbance with the aid of spectrophotometer and later concentrated *in vacuo.* Visualization of the spots on plates was by observing under ultra violet light and by spraying separately with vanillin-sulphuric acid reagent followed by heating at 100 ^{0}C for 5 min.

Each of the numbered fractions was tested against each fungi implicated in wood decay. Fractions that gave antifungal activity were spotted on the thin layer chromatograph (TLC). The fractions were analysed by thin layer chromatography with the stationary phase on silica gel (60 Fe_2SO_4, 0.2 mm thickness) and the mobile phase as n-hexane: chloroform: ethanol: acetic acid (5:2:1:1 v/v). All the fractions that gave antifungal activities were subjected to IR, MS and NMR to obtain the structure of the active compounds. All the pure fractions from each of the extracts above were subjected to IR, MS and NMR to get the active compounds.

All the IR spectra were recorded with a Perkin Elmer (Precisely) Spectrum 100 series FT-IR spectrometer, while UV spectra were obtained from a Unicam UV 4-100 UV/Vis spectrophotometer. The MS were obtained using JEOL JMS-AX505HA double-focusing probe at 70 eV, while NMR spectra (both 1D and 2D) were obtained on a Bruker AMX- 400 (at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR) spectrometer in C_5D_5N.

All data obtained were subjected to statistical analysis of variance (ANOVA) to determine the significance of the sources of variation while Duncan Multiple Range Test was used to determine the significant difference between the means at 5% level of significance ($p \leq 0.05$) (Duncan, 1995).

RESULTS AND DISCUSSION

The various fungi isolated from decayed wood samples included *Aspergillus flavus, Aspergillus niger, Aspergillus fumigates, Aspergillus wentii, Aspergillus tamari,* and *Penicillium chrysogenum* (Table 1). These fungi identified in decayed wood samples in this study, have been implicated in the deterioration of wood and wood products (Dickinson and Pugh, (1974); Levy and Dickinson, (1981) ; Adeleye and Omotosho, (2003). *A. Flavus, A. tamarrii* and *A. fumigatus* among other *Aspergillus* species have been implicated in various types of wood decomposition (Adeleye and Omotosho, 2003).

In general, the extracts from each of the solvents (methanol, chloroform, and n-hexane) exhibited zones of inhibition against the decay fungi (Table 2). Extracts from -hexane gave the highest antagonistic activities followed by chloroform, and methanol in that order.

Table 1: Cultural characterization and microscopic observation of the fungal isolates.

S/N	Observation On Potato Dextrose Agar	Microscopic observation	Isolates
1.	Colonies spreading rapidly. Yellowish at first then yellowish green with age.	Vesicles small, phialides entire and conidia globose and in chains. Mycelium branched and septate.	*Aspergillus flavus*
2.	Colonies spread rapidly with mycelium white at first and bear black heads with age.	Vesicles present. Metullae and phialides were born on the vesicle. Non-septate and no collumella.	*Aspergillus niger*
3.	Colonies spead rapidly with mycelium dirty white first then blue with age	Vesicles present. Interwoven mass of hyphae which were septate.	*Aspergillus fumigatus*
4.	Colonies spread rapidly. Yellowish at first then turned greenish with age	Vesicles present. The mycelium branched and septate.	*Aspergillus wentii*
5.	Colonies spread rapidly with mycelium whitish at first and became dark grey with age	Vesicle present. Metulae and phialide were born on the vesicle. Heads were globose when seen in petridish	*Aspergillus tamari*
6.	Colonies bluish-green and velvety	Brush arrangements of conidia and were borne on metullae. The branching of the spore-bearing head was monoverticillate	*Penicillium chrysogenum*

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 2: Inhibitory effect of crude sawdust extracts (50 mg/mL of solvent) on fungi associated with wood decay.

Sample	Solvent	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus fumigatus*	*Aspergillus wentii*	*Aspergillus tamarii*	*Penicillium chrysogenum*
Piptandeniastrum africanum							
	methanol	12.50c ±0.29	15.17c ±0.17	12.83c ±0.17	14.67c ±0.33	11.50c ±0.29	13.50c ±0.76
	chloroform	14.00b ±0.00	20.83b ±0.60	16.33b ±0.33	24.67b ±0.33	15.67b ±0.33	22.00b ±1.53
	n-hexane	18.17a ±0.17	25.67a ±0.33	28.00a ±0.58	27.50a ±0.29	17.33a ±0.33	26.67a ±0.67
Sterculia rhinopetala							
	methanol	12.00c ±0.00	12.33c ±0.17	10.00c ±0.58	13.00c ±0.00	10.00b ±0.00	14.67c ±0.33
	chloroform	12.83b ±0.17	18.33b ±0.17	14.33b ±0.67	20.00b ±0.00	15.33a ±0.33	16.67b ±0.33
	n-hexane	13.83a ±0.17	20.17a ±0.17	21.33a ±0.67	21.67a ±0.33	15.00a ±0.00	19.00a ±0.58

Values are mean of triplicate measurements.
Values along column with same superscripts are not significantly different ($p \geq 0.05$).
± = Standard errors of mean.

Table 3: Phytochemical screening of sawdust extracts of wood samples.

		Metabolites								
Wood samples	Solvents	Tann	Alkd	Antq	Glc	Flvd	Phnl	Spni	Cumr	Vlpt
Sterculia rhinopetala	M	+	+	+	+	+	-	+	+	-
	C	+	+	+	+	+	+	+	+	-
	H	+	+	+	+	+	+	+	+	-
Piptandeniastrum africanum	M	+	+	+	+	+	+	+	+	+
	C	+	+	+	+	+	+	+	+	+
	H	+	+	+	+	+	+	+	+	+

Key (+) = Positive to test, Alkd – Alkaloids, Vlpt – Valepotriate, Antq – Anthraquinones, Glc – Glycosides, Tann – Tannins
(-) = Negative to test, Phnl – Phenolics, Flvd – Flavonoids, Spni – Saponins, Cumr – Coumarins, H – n-Hexane, M – Methanol, C - Chloroform

Piptandeniastrum africanum's n-hexane extract gave highest zone of inhibition (28.00 mm) against *Aspergillus wentii.* Earlier workers (Shain, 1976; Cobb *et. al.,* 1968; Ahmad *et. al.,* 1998; Wang and Weller, 2006; Abhilash and Singh, 2008; Ahmad *et al.* 2009) hadhad reported n-hexane to be a good solvent for wood extractives.

Phytochemical screening of the crude extracts revealed the presence of constituents such as alkaloids, anthraquinones, glycosides, flavonoids, valepotriates, phenolics, saponins, and coumarins (Table 3). These extractable organic compounds naturally deposited in the heartwood during its transformation from sapwood generally are more important contributors to the natural resistance of wood to biodegradation than are inorganic constituents or physical factors such as wood density and degree of cellulose crystallinity (Bultman and Southwell, 1976; Ghoshal *et al.,* 1996; Iwu *et al.,* 1999). Terpenoids, essential oils and some flavonoid compounds were reported to exhibit antifungal activities on some brown and white rot fungi (Cobb *et al*., 1968; Carll and Highley, 1999). Some of these compounds, such as saponin, were found to be fungistatic (Ahmad *et al.*, 1998; Zehavi *et al*, 2008) while others, such as terpenoids, were found to be fungicidal (Fabry *et al.,* 1996; Molina-Torres *et al.,* 2004; Tripathi *et al.,* 2008).

Phytochemical compounds reported in this research work have also been reported by Abiy *et al.* (2005) who detected flavonoids from *Erythrina burttii* as active against some microorganisms. Flavonoids and tannins have also been reported as responsible for the antimicrobial activities of forty-five medicinal plants (Ahmad and Berg, 2001). Moreover, it is reported that flavonoids are components synthesized by plants in response to microbial infection (Dixon *et al,.* 1983) thus justifying their antagonistic activities in the test extracts.

Development of the fractions on thin layer chromatography (TLC) plates showed the presence of flavonoids, coumarins, anthraquinones, alkaloids, saponins and valepotriates. Anthraquinones were detected as a consistent bioactive component in all the wood species. Saponin was found in all the wood species. However, valeopotriates was detected only in *P. africanum* and found to be one of the active components

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

in same. This compound may be responsible for its unusual resistance to wood decay by fungi.

The structure of the bioactive components in *Sterculia rhinopetala* (Aye) extract was elucidated and it gave a yellow waxy solid (C1). The structure was established by UV, IR, MS and a series of 1D and 2D NMR analyses and suggested as 5,7,8-Trihydroxy-2'5'–methoxy–3',4'–'methylenedioxyisoflavanone (Figure 1). **C1** is a Yellow waxy solid, melting point 185 – 187^{0}C, with UV λ_{max} nm (log ε): 214(4.10), 218 (4.12), 288 (4.00), 333 (sh) (3.25). IR ν_{max} cm^{-1} pronounced peaks at: 3421, 2914, 1634, 1472, 1386, 1358, 1257, 1224, 1161, 1100, 1068, 925; the ^{1}H NMR, (Figure 2), ^{13}C NMR (Figure 3) and heteronuclear multiple bond correlation (HMBC) is as shown in Tables 4 and 5. Electron Impact Mass Spectrum (EIMS) molecular ion peak $(M)^{+\cdot}$ *m/z* 378.0625 (calculated for $C_{18}H_{18}O_9$, 378.0623), 5,7,8-Trihydroxy-2′,5′-dimethoxy-3′,4′-methylenedioxyisoflavanone (Figure 3).

Table 4: ^{1}H NMR (400 MHz) data of C1 and ^{13}C NMR (100 MHz) data of C1 in C_5D_5N

Position	^{1}H	^{13}C
2	4.69, *t*, *J* = 11.0 Hz	71.1
	4.50, *dd*, *J* = 10.5, 5.6 Hz	-
3	4.43, *dd*, *J* = 11.0, 5.6 Hz	48.5
4	–	197.6
5	–	166.0
6	6.52, *d*, *J* = 2.0 Hz	97.7
7	–	168.6
8	–	96.5
9	–	164.7
10	–	103.3
1′	–	121.4
2′	–	142.5
3′	–	137.8
4′	–	149.8
5′	–	145.6
6′	6.80, *d*, *J* = 8.3 Hz	125.1
5-OH	12.89, *br. s*	–
7-OH	12.98, *br. s*	–
8-OH	13.02, *br. s*	–
2′-OMe	3.96, *s*	59.6
5′-OMe	3.87, s	60.2
–OCH_2O–	5.90, *s*	102.0

For *Piptandeniastrum africanum* extract, the bioactive component also gave a yellow amorphous powder melting point (m.p.) 234 – 236 °C **(C2)** and a yellow fluffy powder m.p. 217 – 219 °C **(C3).** The C2 properties were observed as V λ_{max} nm (log ε): 255 (4.64), 357 (4.62); +NaOAc: 271, 365; +NaOH: 276, 343, and 410. $^+AlCl_3$: 279, 440. IR (v cm^{-1}) pronounced peaks: 3401, 2932, 1653, 1612, and 1556 EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 388.0103. (calculated molecular formula $C_{20}H_{20}O_8$).

Table 5: HMBC data (400 MHz, C_5D_5N) of C1
J = Coupling constants i.e. difference in chemical shifts in ppm

Protons	C1 (H → C)	
	J_2	J_3
H-2	C-3	C-9, C-4, C-1′
H-3	C-2, C-4, C-1′	C-2′, C-6′
H-6	C-5, C-7	C-10, C-8
H-6′	C-5′	C-3, C-2′, C-4′
HO-5	C-5	C-6, C-10
HO-8	C-8	C-7, C-9
MeO-2′	–	C-2′
MeO-5′	–	C-5′
–OCH_2O–	–	C-3′, C-4′

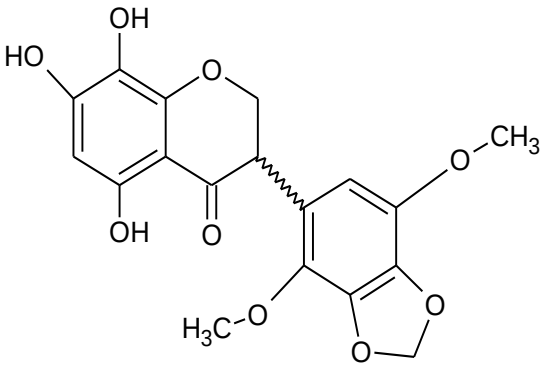

Figure 1 : (C1) 5,7,8-Trihydroxy-2′5′-methoxy-3′,4′-methylenedioxyisoflavanone

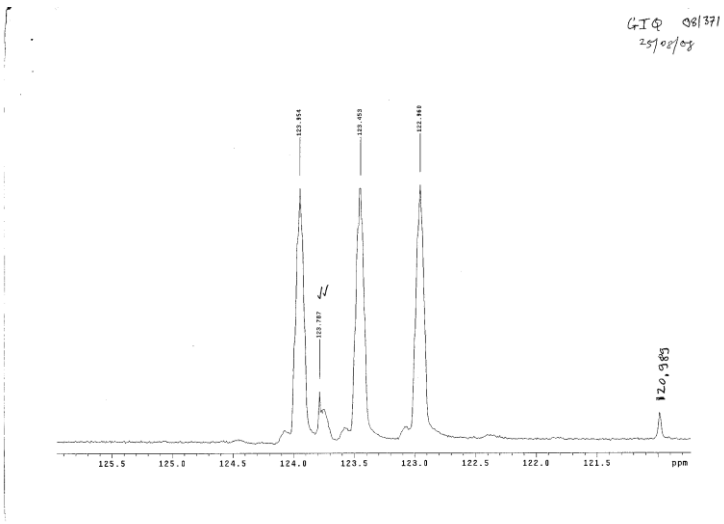

Figure 2: ^{1}H NMR for C1

ISSN (print): 1823-8262, ISSN (online): 2231-7538

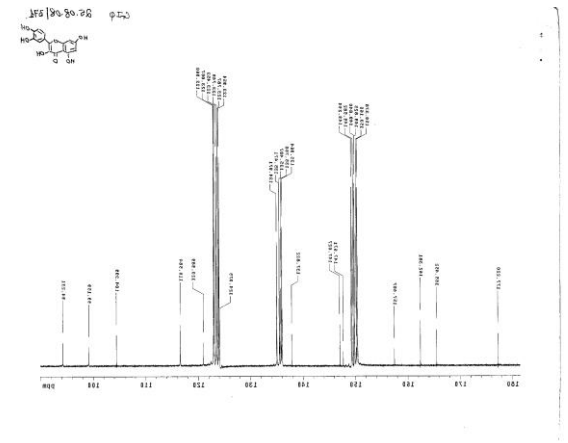

Figure 3: ^{13}C NMR for C1.

^{1}H NMR: δ 2.14 (3H, s, Me-6), 3.74 (3H, s, OMe-3), 3.96 (3H, s, OMe-3'), 4.01 (3H, s, OMe-7); 4.56 (3H, s, OMe-8), 6.91 (1H, d, J= 8.5 Hz, H-5'), 7.60 (1H, *dd,* J = 2.3, 8.4 Hz, H-6'), 7.70 (1H, d, J= 2.3 Hz, H-2'), 10.90 (1H, s, 4'-OH), 12.92 (1H, s, 5-OH). ^{13}C NMR (Figures 4 and 5; Table 6). The C2 compound was suggested to be 3,7,8,3'–Tetramethoxy-6–C-methyl-5,4'–dihydroxyflavone(6–C–methylquercetin 3, 3', 7, 8 – tetramethyl ether) (Figure 6).

The spectra properties for C3 were UV λ_{max} nm (log ε): 260 (4.62), 351 (4.60); +NaOAc: 260, 355; +NaOH: 270, 405; +A1C1$_3$: 277, 435. IR (v cm^{-1}) pronounced peaks: 3429, 1652, 1613, 1568, 1556 EIMS: molecular ion peak $(M)^{+\cdot}$ *m/z* 346.0752. (calculated mol. formula $C_{17}H_{14}O_8$). ^{1}H NMR: δ 2.11 (3H, s, Me-6), 3.87 (3H, s, OMe-3), 7.03 (1H, d, J=8.6Hz, H-5'), 7.62 (1H, dd, J= 2.3, 8.6 Hz, H-6'), 7.75 (1H, d, J= 2.3Hz, H-2'), 10.23 (1H, s, 4'-OH), 11.15 (1H, s, 3'-OH).12.05 (1H, s, 8-OH), 12.90 (1H, s, 7-OH), 13.05 (1H, s, 5-OH). ^{13}C NMR (Table 6 ; Figures 7 and 8). The molecular formula of C3 was assigned as $C_{17}H_{14}O_6$ on the basis of the molecular ion peak at m/z 346.0752. C3 was consequently assigned the structure3–methoxy–6–C–methyl–3',4',5,7,8 pentahydroxyflavone(6–C–methylquercetin–3–methylquercetin– 3 - methyl ether) (Figure 6).

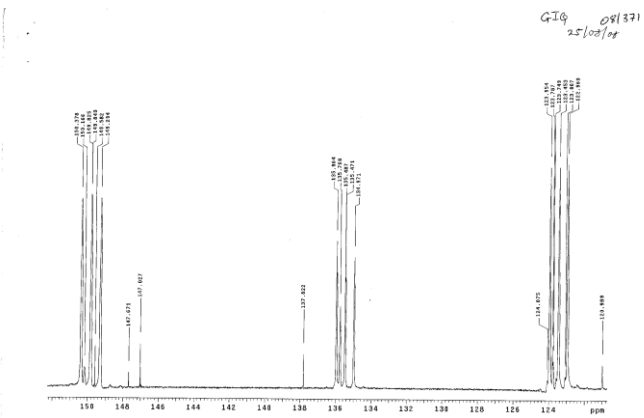

Figure 4: ^{13}C NMR for C2.

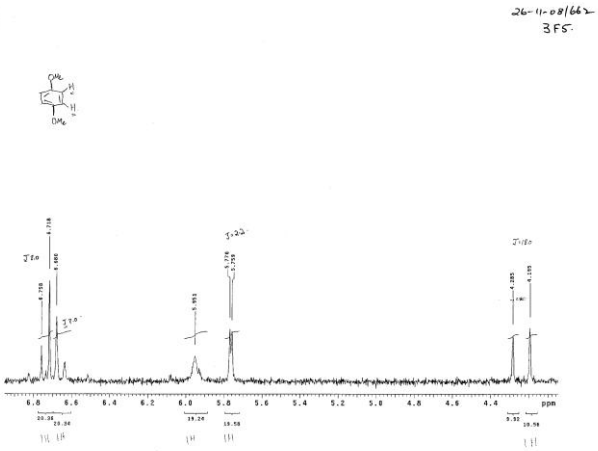

Figure 5: ^{1}H NMR for C2.

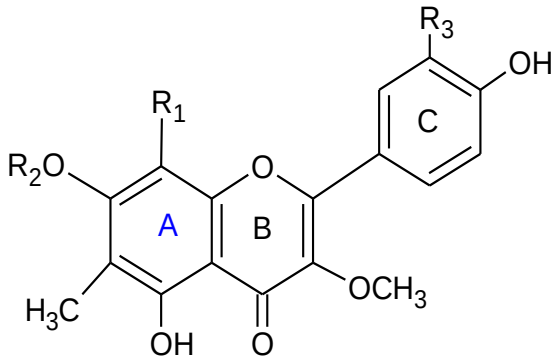

Figure 6: Compound C2 - 3, 7, 8, 3'–Tetramethoxy-6–C-methyl-5, 4'–dihydroxyflavone (6–C–methylquercetin 3, 3', 7, 8 – tetramethyl ether):
C3 - 3–methoxy–6–C–methyl–3',4',5,7,8–pentahydroxyflavone (6–C– methylquercetin– 3 – methylquercetin– 3 - methyl ether)

	R_1	R_2	R_3
C2	OCH_3	OCH_3	OCH_3
C3	OH	OH	OH

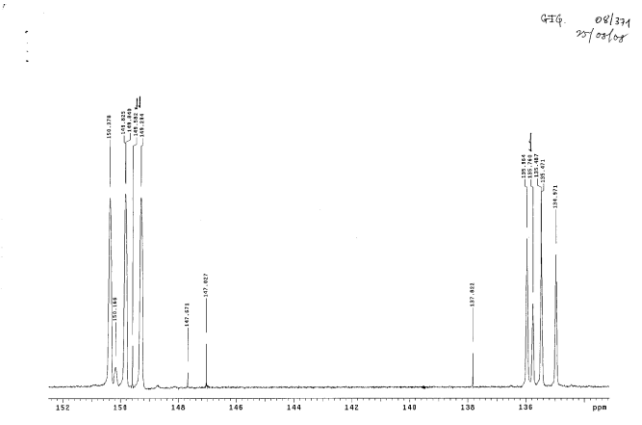

Figure 7: ^{13}C NMR for C3.

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Table 6: ^{13}C NMR (100 MHz) data for compounds C2 and C3 in C_5D_5N

Position	^{13}C NMR	
	C2	**C3**
2	155.5	155.0
3	137.5	137.3
4	178.8	177.9
5	156.3	157.1
6	106.6	107.3
7	145.4	159.2
8	149.5	162.8
9	151.4	154.4
10	103.9	104.8
1'	121.6	120.4
2'	115.3	115.4
3'	147.2	163.6
4'	148.5	164.5
5'	115.8	115.4
6'	120.6	120.8
3-OMe	59.5	59.2
7-OMe	60.2	-
8-OMe	63.5	-
3'-OMe	56.5	-
6-Me	8.1	7.2

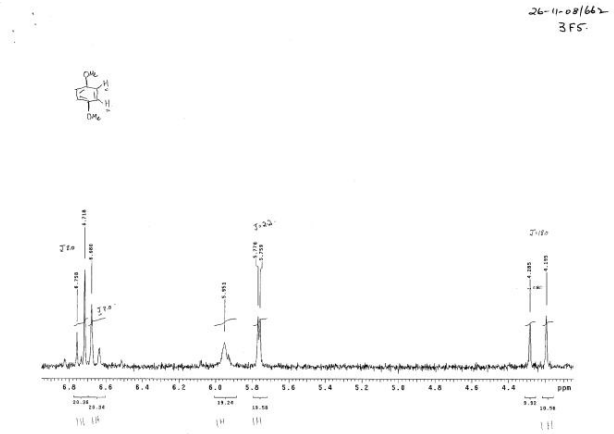

Figure 8: ^{1}H NMR for C3.

The extractable organic compounds naturally deposited in the heartwood are more important contributors to the natural resistance of wood to biodegradation (Carey *et al.,* 1984 and Martinez-Inigo *et al.,* 2005). The major wood preservatives used today are water-borne chromated copper arsenate (CCA). However, there are some concerns with CCA including a public perception of possible arsenic exposure, leaching of the metal oxide and the question of the ultimate disposal of CCA-treated wood. Consequently, the use of biocontrol of wood decay which is more environmentally friendly and will make treated wood less expensive is a better alternative to chemical preservatives.

ACKNOWLEDGEMENT

We hereby acknowledge the assistance rendered by Dr. Babajide of the department of Chemistry, Adeyemi College of Education, Ondo, Nigeria, in whose laboratory we conducted the chemistry aspect of this work and who also helped us to interpret the spectra.

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