ISOLATION AND CHARACTERIZATION OF CHEMICAL CONSTITUENTS FROM *CHRYSOPHYLLUM ALBIDUM* G. DON-HOLL. STEM-BARK EXTRACTS AND THEIR ANTIOXIDANT AND ANTIBACTERIAL PROPERTIES

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Abstract

**Background:** The plant, *Chrysophyllum albidum* is indigenous to Nigeria and its stem-bark has wide application in traditional medicine for the treatment of infections and oxidative stress related diseases. The aim of the study was to isolate the chemical constituents responsible for the antioxidant and antibacterial activity from the stem-bark of the plant in order to justify some of its ethnomedicinal uses.

**Materials and Methods:** Crude extract of stem-bark of *Chrysophyllum albidum* obtained from 80% ethanol was successively partitioned with ethyl acetate (EtOAc) and n-butanol. The solvent fractions and isolated compounds were tested for antioxidant property using 2,2-diphenyl-1-picrylhydrazyl. Antibacterial activities were also assessed by means of agar-diffusion and broth micro dilution methods. EtOAc fraction was repeatedly fractionated on column chromatography to afford four compounds and their chemical structures were established using NMR (1D and 2D) and MS.

**Results:** Chromatographic fractionation of EtOAc fraction with the highest antioxidant and antimicrobial activities afforded stigmasterol (1), epicatechin (2), epigallocatechin (3) and procyanidin B5 (4). Procyanidin B5 isolated for the first time from genus *Chrysophyllum* demonstrated the highest antioxidant activity against *Escherichia coli* (MIC 156.25 µg/mL), *Staphylococcus aureus* (MIC 156.25 µg/mL), *Pseudomonas aeruginosa* (MIC 625 µg/mL) and *Bacillus subtilis* (MIC 156.25 µg/mL).

**Conclusion:** The antibacterial and antioxidant activities of epicatechin, epigallocatechin and procyanidin B5 isolated from *Chrysophyllum albidum* stem-bark validate the folkloric uses.

**Keywords:** *Chrysophyllum albidum*, free-radical scavenging, antioxidant, antibacterial, isolation, characterization, procyanidin B5

Introduction

*Chrysophyllum albidum* G. Don-Holl. (Sapotaceae) is a tree with edible fruits that grows widely throughout tropical West Africa (Iwu, 1993). Different parts of the plant including the fruits, leaves, root-bark and stem-bark are used for medicinal purposes (Adeewusi, 1997, Adewoye et al., 2010). A decoction of the leaves is used for diarrhoea and stomach ache while the leaves are used as emollient and for treatment of skin eruptions (Idowu et al., 2006, Adewoye et al., 2010). The cotyledons from the seeds are used in the treatment of vaginal and dermatological infections while the fruit pulp is taken by pregnant women to prevent nausea.in Western Nigeria (Iwu, 1993; Obanjo et al., 1979). The stem-bark is used as a remedy for sleeping sickness, yellow fever and malaria (Postma et al., 2010; Adewusi, 1997, Adewoye et al., 2010). A decoction of the leaves is used for diarrhoea and stomach ache while the leaves are used as emollient and for treatment of skin eruptions (Idowu et al., 2006, Adewoye et al., 2010). The cotyledons from the seeds are used in the treatment of vaginal and dermatological infections while the fruit pulp is taken by pregnant women to prevent nausea.

Previous investigation of the chemical constituents of *C. albidum* showed that the leaves and stems contain, myricetin rhamnoside, β-amyrin acetate and gentisic acid (Iwu, 1993; Onabanjo et al., 1979). The stem-bark was used as a remedy for sleeping sickness, yellow fever and malaria (Postma et al., 1996, Iyamah et al., 2014). The methanol extract of the seed and root had been reported to exhibit antihistamine and anti-inflammatory activities (Onabanjo et al., 1979), while the exocarp fruit extract of *C. albidum* had been shown to demonstrate free radical scavenging activity (Orijajogun et al., 2013). The ethanol extract of the root of the *C. albidum* was also reported to demonstrate antioxidant and antifertility activities (Onyeka et al., 2012a, Onyeka et al., 2012b).

The leaves extract has also been reported to show antioxidant and antimicrobial activities (Adebayo et al., 2011, Ajetunmobi and Towolawi 2014) while the methanolic extract of the stem-bark had been reported to exhibit anti-plasmodial activity (Adewoye et al., 2010),

**Keywords:** *Chrysophyllum albidum*, free-radical scavenging, antioxidant, antibacterial, isolation, characterization, procyanidin B5
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and characterising the constituents responsible for the antioxidant and antibacterial principles from it. We now report the isolation of stigmasterol, epicatechin, epigallocatechin and epicatechin dimer (procyanidin B5), all of which were isolated for the first time from the stem-bark of <i>C. albidum</i>.

### Materials and Methods

#### Instruments

Column chromatography (cc) was performed using the Accelerated Gradient Chromatography (AGC), a modification of conventional medium pressure liquid chromatography (Dunstan, 1995). The equipment for the AGC work station was from Baeckstrom Separo AB, Lidingo, Sweden and used at Department of Chemistry, University of Botswana, Gaborone, Bostswana and Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria. The 1H-NMR spectra were recorded at 300 MHz and 13C-NMR at 75 MHz respectively, on Bruker Avance DPX 300 spectrometer, the EIMS was done on Finnigan MAT SSQ 7000 Single Quadrupole Instrument at 70 eV while the ESI were done on Finnigan LQC Deca and Optical rotation was done on AUTOPOL (R) IV optical rotation meter using methanol and acetone at 20 °C while UV spectra were measured in methanol, on a Shimadzu UV-2101PC spectrometer at Department of Chemistry, University of Botswana. Absorbance for antioxidant experiment was measured on spectrophotometer (Pharmacia Biotech Novaspec II) at the Department of Biochemistry, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria.

#### Solvents and reagents

Anisaldehyde, Kieselgel 60 (ASTM 230–400 mesh, 0.040–0.063 mm particle size, Merck), quercetin, gallic acid, thiobarbituric acid, sodium dodecyl sulphate, ascorbic acid, sodium nitroprusside, precoated silica gel GF254, (Merck, Germany), vanillin and sulfuric acid were purchased from Sigma –Aldrich. Sephadex LH-20 was purchased from Amersham Pharmacia Biotech (Uppsala, Sweden). Solvents used for extraction and chromatography included hexane, dichloromethane, chloroform, ethyl acetate n-butanol, ethanol and methanol and were redistilled before use.

#### Collection and preparation of plant extract

The stem-bark of <i>C. albidum</i> was collected at Ede road, Ile-Ife in February 2009. A voucher specimen (FPH/S/001) identified by Mr. A. T. Oladele was deposited at the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife Nigeria.

#### Extraction and Isolation

The dried stem-bark (1.2 kg) was extracted with 80% ethanol in water (3 x 5 L) at room temperature for 72 hours and the pooled extract was concentrated in vacuo to obtain a crude ethanolic extract (132 g). About 120 g of this was suspended in distilled water and partitioned with ethyl acetate (3 x 500 mL) and n-butanol (3 x 500 mL) successively. The solvent fractions were concentrated in vacuo to obtain the ethyl acetate fraction (26 g) and n-butanol fraction (31 g) respectively. A portion of the ethyl acetate fraction (20 g) was subjected to separation on silica gel using a gradient of petroleum ether (PE), CHCl3 and MeOH to give six fractions F1-F6. Fraction F2 (300 mg) was further fractionated with PE and CHCl3 resulting in five fractions coded F2a-F2d. Fraction F2d (90 mg) was subjected to preparative TLC for purification using PE-chloroform (6:4) solvent system, which afforded 1 (31 mg). Fraction F4 (1.3 g) was dissolved in a minimum amount of CHCl3-MeOH (70:30) solvent mixture and loaded on a Sephadex LH-20 column previously equilibrated with the same solvent mixture and elution was effected isocratically to obtain 2 (280 mg). Fraction F5 (2.2 g) was also dissolved in a minimum amount of CHCl3-MeOH (70:30) solvent mixture and loaded on a Sephadex LH-20 column previously equilibrated with the same solvent mixture and elution was effected isocratically in five fractions F5a-F5f. Fraction F5e (360 mg) was fractionated on silica gel using a gradient of n-hexane, ethyl acetate and methanol leading to obtain 3 (145 mg). Fraction F6 was loaded on a Sephadex LH-20 column and elution was effected isocratically using CHCl3-MeOH (70:30) thereby resulting in five fractions F6a-F6e. Thereafter, a repeated fractionation of F6e (840 mg) on silica gel using a gradient of PE, CHCl3 and MeOH afforded 4 (71 mg).

#### Evaluation of antioxidant activity

TLC autography assay was conducted as follows. A small amount of the sample was dissolved in an appropriate organic solvent and spotted on silica gel sheet, dried and developed using a suitable solvent system. The dried plate was sprayed with 0.2% methanolic solution of stable radical DPPH•. The spray reagent was used to confirm the presence of antioxidant spots (Burits and Bucar, 2000). Quantitative antioxidant assays were done as follows: the hydrogen donating or radical scavenging properties of the isolated compounds, was determined using the stable radical DPPH• as described by Brand-Williams et al., (1995). All samples were analysed in triplicate and the percentage free radical inhibition was calculated as expressed in the equation below.

\[
\text{DPPH} \% \text{ scavenging capacity} = \left( \frac{A_0 - A_s}{A_0} \right) \times 100
\]

or

\[
\left( 1 - \frac{A_s}{A_0} \right) \times 100
\]

Where
Lipid peroxidation assay

The lipid peroxidation inhibition potential of the isolated compounds was determined using a modified thiobarbituric acid reactive species (TBARS) assay of Ohkowa et al., (1979) as described by Nabasree & Bratati, (2004). Percent inhibition of lipid peroxidation was calculated as expressed above with DPPH radical scavenging.

Nitric oxide radical inhibition activity

The nitric oxide radical inhibiting activity of the extracts was carried out according to the method of Green et al., (1982) as described by Marcocci et al., (1994). Percentage inhibition nitric oxide radical formation was calculated as expressed above with DPPH radical scavenging.

Determination of Antibacterial Activity

Agar-diffusion method

The antibacterial activities of the test samples (crude/fractions/pure compounds) obtained from the stem-bark of C. albidum and the standard agent (Streptomycin) were determined using the agar diffusion method (Agyare et al., 2013). Broth cultures (18 hr) of the test organisms; Staphylococcus aureus (NCTC 6571), Escherichia coli (ATCC 25922), Bacillus subtilis (NCTC 8263) and Pseudomonas aeruginosa (ATCC 10145) were used to seed molten nutrient agar and allowed to set. Thereafter, wells (8 mm diameter) were cut out using a sterile cork borer. The test solutions, 20 mg/mL (100 μL), Streptomycin, 1 mg/mL (100 μL) and the solvent, 50% methanol (100 μL), were introduced into each of the wells and allowed to diffuse for 1 hr before incubation at 37°C for 24 hr. The diameter of the zones of inhibition were measured to the nearest mm.

Determination of minimum inhibitory concentrations (MICs) using the broth microdilution method

The MICs were determined using the broth microdilution method as described by Amsterdam (1996). Mueller-Hinton broth (100 μL) was dispensed into the wells of a 96-well microtitre plate. 100 μL each of the test samples (20 mg/mL) and the standard agents, ciprofloxacin and streptomycin (1 mg/mL) were added to the first well in each row with adequate mixing. The test sample in each row was serially diluted two-fold to obtain concentration ranges of 10 – 0.0390625 mg/mL and 500 – 0.9765625 μg/mL for the standard agents. 5 μL of an overnight broth culture (2×10^4 cfu/mL) of the test bacteria was added to each well and the plate was incubated at 37°C for 24 hr. Each well was examined for the presence or absence of growth by addition of 20 μL of 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) and incubation at 37°C for 30 min. The MIC is the lowest concentration that prevents the change in colour from yellow to blue-indicating inhibition of growth. The above experiments were performed in triplicate.

Results

The detailed chromatographic separation of the ethyl acetate fraction from the stem-bark of C. albidum led to the isolation of the two main types of chemical constituents viz: a steroid and flavan-3-ol derivatives. The physical and spectroscopic data of the isolated compounds are as recorded below while the structures are as in Figure 1. Table 1 shows the antioxidant activities of the isolated compounds while Tables 2 and 3 depict the antibacterial activities of various fractions and the isolated compounds respectively. Table 4 shows the minimum inhibitory concentrations (MICs) of various fractions and two isolated compounds against selected typed bacterial strains using the broth microdilution method.

Table 1: The in vitro antioxidant activities of compounds 2, 3 and 4 isolated from Chrysophyllum albidum in different antiradical test systems

| Tested compounds | DPPH radical scavenging | Lipid peroxidation inhibition | Nitric oxide inhibition |
|------------------|-------------------------|-------------------------------|------------------------|
|                  | IC₅₀ (µM)               | IC₅₀ (µM)                     | IC₅₀ (µM)              |
| 1                | ND                      | ND                            | ND                     |
| 2                | 19.02±0.17              | 436.98±4.73                   | 27.34±1.02             |
| 3                | 15.88±0.13              | 437.61±5.61                   | 22.54±0.41             |
| 4                | 8.80±0.11               | 244.36±4.33                   | 11.20±0.09             |
| (Gallic acid)    | 12.82±0.09              | ND                            | 45.15±1.46             |
| (Quercetin)      | ND                      | 201.29±3.58                   | ND                     |

Values are expressed as mean ± SD of triplicate experiments. IC₅₀ values were calculated from regression equations of compound’s concentrations against % inhibition of free radical formation/prevention of lipid peroxidation. ND – not determined.
Table 2: Antibacterial activity of the crude ethanol extract, ethyl acetate, n-butanol, and the aqueous fractions obtained from the stem-back of *Chrysophyllum albidum*

| Test Samples / Standard | Zones of Inhibition (mm)* |
|-------------------------|---------------------------|
|                         | Conc. (mg/mL) | *E. coli* (ATCC 25922) | *S. aureus* (NCTC 6571) | *P. aeruginosa* (ATCC 10145) | *B. subtilis* (NCTC 8263) |
| Crude ethanolic extract | 20            | 0                       | 10                        | 4.5                         | 12                         |
| Ethyl acetate fraction  | 20            | 4                       | 7.5                       | 2                           | 7.5                        |
| n-butanol fraction      | 20            | 0                       | 7                         | 3                           | 7                          |
| Aqueous Fraction        | 20            | 0                       | 4.5                       | 0                           | 5.5                        |
| Streptomycin sulphate   | 1             | 17.5                     | 15                        | 4                           | 19                         |
| Solvent MeOH:H₂O (1:1)  | 0             | 0                       | 0                         | 0                           | 0                          |

MeOH: H₂O (1:1) - The zones of inhibition recorded are less the diameter of the cup, which is 8 mm.

* Represents the mean of three determinations.

Table 3: Antibacterial activity of the isolated compounds from *Chrysophyllum albidum* stem-bark.

| Test Samples / Standard | Zones of Inhibition (mm)* |
|-------------------------|---------------------------|
|                         | Conc. (mg/mL) | *E. coli* (ATCC 25922) | *S. aureus* (NCTC 6571) | *P. aeruginosa* (ATCC 10145) | *B. subtilis* (NCTC 8263) |
| 1                       | 20            | 0                       | 0                         | 0                           | 0                          |
| 2                       | 20            | 5                       | 0                         | 0                           | 5                          |
| 3                       | 20            | 0                       | 0                         | 0                           | 0                          |
| 4                       | 20            | 0                       | 4                         | 0                           | 9                          |
| Streptomycin sulphate   | 1             | 17.5                     | 18                        | 3.5                         | 18.5                       |
| Solvent MeOH:H₂O (1:1)  | 0             | 0                       | 0                         | 0                           | 0                          |

MeOH: H₂O (1:1) - The zones of inhibition recorded are less the diameter of the cup, which is 8 mm.

* Represents the mean of three determinations.

Table 4: Minimum Inhibitory Concentrations (MICs) of various fractions and isolated compounds from *Chrysophyllum albidum* stem-bark.

| Test Samples / Standard | Minimum Inhibitory Concentrations (MICs) in µg/mL against |
|-------------------------|----------------------------------------------------------|
|                         | *E. coli* (ATCC 25922) | *S. aureus* (NCTC 6571) | *P. aeruginosa* (ATCC 10145) | *B. subtilis* (NCTC 8263) |
| Crude ethanolic extract | 625 | 625 | 1250 | 625 |
| Ethyl acetate fraction  | 156 | 312 | 625 | 156 |
| n-butanol fraction      | 312 | 312 | 625 | 312 |
| Aqueous Fraction        | 1250 | 1250 | 1250 | 1250 |
| Epicatechin (2)          | 625 | 312 | 1250 | 625 |
| Procyanidin B5 (4)      | 156 | 156 | 625 | 156 |
| Ciprofloxacin           | 3.91 | < 0.977 | < 0.977 | < 0.977 |
| Streptomycin            | 7.81 | < 195 | 500 | 15.6 |

Characterization of stigmasterol (1)

White crystal. ESI-MS *m/z* (rel. int. %): [M + H]+: 413 (10), 395 (12), 369 (6), 327 (7). IR *ν* max KBr cm⁻¹: 3416.93, 2956.78, 1634.83, 1458.19, 1375.93, 1043.12; UV *λ* max MeOH nm: 200.80, 209.20, 221.80; ¹H- and ¹³C-NMR (CDCl₃):

¹Hnmr (300MHz, CDCl₃) δ ppm: 1.80 (2H, m), 3.60 (1H, m), 1.70 (1H, m, H-4a), 1.32 (1H, m, H-4b), 5.17 (1H, t, H-6), 1.29 (1H, H-17), 0.55 (3H, s, 18-CH₃), 0.81 (3H, s, 19-CH₃), 1.03 (3H, d, J = 6.64, 21-CH₃), 5.20 (1H, d, J=15.14, H=22), 5.06 (1H, dd, J=15.16, H=23), 0.86 (3H, 26-CH₃), 0.83 (3H, 27-CH₃), 0.79 (3H, 29-CH₃).
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13Cnmr (75 MHz, CDCl3) δ ppm: 30.05 (C-1), 31.90 (C-2), 71.45 (C-3), 38.42 (C-4), 139.96 (C-5), 117.86 (C-6), 37.56 (C-7), 41.20 (C-8), 49.88 (C-9), 34.63 (C-10), 21.96 (C-11), 39.88 (C-12), 43.70 (C-13), 56.33 (C-14), 23.41 (C-15), 28.88 (C-16), 55.54 (C-17), 12.62 (C-18), 13.42 (C-19), 40.68 (C-20), 21.46 (C-21), 138.55 (C-22), 129.88 (C-23), 51.65 (C-24), 32.27 (C-25), 21.76 (C-26), 19.37 (C-27), 25.78 (C-28), 12.44 (C29).

Figure 1: Structures of compounds 1-4 isolated from *Chrysophyllum albidum* stem-bark.

**Characterization of epicatechin (2)**

Brown amorphous powder, [α]D20 -70° (MeCO, c=1.00); ESI-MS [M-H] 289.1, IR v max KBr cm⁻¹: 3454.93, 2927.45, 1620.74, 1522.08, 1468.43, 1386.61, 1259.37, 1144.73; 1H- and 13C-NMR (CD3OD).

1Hnmr (75 MHz, CDCl3) δ ppm: 30.05 (C-1), 31.90 (C-2), 71.45 (C-3), 38.42 (C-4), 139.96 (C-5), 117.86 (C-6), 37.56 (C-7), 41.20 (C-8), 49.88 (C-9), 34.63 (C-10), 21.96 (C-11), 39.88 (C-12), 43.70 (C-13), 56.33 (C-14), 23.41 (C-15), 28.88 (C-16), 55.54 (C-17), 12.62 (C-18), 13.42 (C-19), 40.68 (C-20), 21.46 (C-21), 138.55 (C-22), 129.88 (C-23), 51.65 (C-24), 32.27 (C-25), 21.76 (C-26), 19.37 (C-27), 25.78 (C-28), 12.44 (C29).

**Characterization of procyanidin B5 (4)**

Brown amorphous powder, [α]D20 +15° (MeCO, c=1.00); ESI-MS m/z (rel. int. %) [M-H] -577.2 (39), 451.2 [M+H-126]+ (3), 425.2 [M+H-152]+ (12), 407.3 [M+H-126-152-18]+ (7), 289.2 (11); IR v max KBr cm⁻¹: UV λmax MeCO nm 210.00, 323.50, 325.50; 1H- and 13C-NMR (CD3OD).

1Hnmr (75 MHz, CDCl3) δ ppm: 30.05 (C-1), 31.90 (C-2), 71.45 (C-3), 38.42 (C-4), 139.96 (C-5), 117.86 (C-6), 37.56 (C-7), 41.20 (C-8), 49.88 (C-9), 34.63 (C-10), 21.96 (C-11), 39.88 (C-12), 43.70 (C-13), 56.33 (C-14), 23.41 (C-15), 28.88 (C-16), 55.54 (C-17), 12.62 (C-18), 13.42 (C-19), 40.68 (C-20), 21.46 (C-21), 138.55 (C-22), 129.88 (C-23), 51.65 (C-24), 32.27 (C-25), 21.76 (C-26), 19.37 (C-27), 25.78 (C-28), 12.44 (C29).
Repeated chromatography of the ethyl acetate fraction from the stem-bark of *C. albidum* on silica gel and Sephadex LH-20 yielded compounds 1–4 (Figure 1).

Compounds 1 was identified as stigmasterol by comparison of the recorded spectral characteristics with those reported for the same compound in the literature (Grega et al., 1990; Holland et al., 1978; Wright et al., 1978). Compound 2 was obtained as amorphous brown powder, and was identified as epicatechin by comparison of its spectral characteristics with literature (Porter et al, 1991 and Agrawal, 1989). Compound 3 obtained as brown amorphous powder gave a molecular ion peak at m/z 306.07 in EIMS which is compatible with the molecular formula C15H10O7. Other fragment ions were found at m/z 168.04, 139.04 and 126.03 respectively. The fragment ion [M-H - 126] at m/z 179.0 corresponded to the elimination of a phloroglucinol molecule from epigallocatechin. The benzofuran ring forming fragmentation (BFF) produced the fragment ion at 168.04 while the ion at m/z 139.4 resulted from a retro-Diels–Alder (RDA) fragmentation. 1H- and 13C-NMR experiments, especially HMQC and HMBC, confirmed proton and carbon correlations of 3 and the DEPT spectrum affirmed its carbon multiplicities, which are similar to the physical and spectra data reported in the literature for epigallocatechin (Agrawal, 1989; Luo et al., 2002; Cândida da Silva et al., 2006). Compound 4 obtained as a brown amorphous powder was determined as C15H20O12 by ESI-MS. The ESI-MS (negative-ion mode) of 4 displayed a pseudomolecular ion peak [M-H] at m/z 577 and was assumed to be a dimeric proanthocyanidin. The presence of the AB coupling system due to H-2 and H-3 at δ 3.94 and 4.60 of the first (upper) monomer unit, the meta-coupled doublets δ 6.03 and 6.09 (each, d, J=2.19 Hz and 2.18 Hz) respectively, one aromatic singlet at δ 5.98 (ring D), and the presence of two ABX systems in the aromatic region (δ 6.73 - 7.01) due to rings B and E confirmed the B-type proanthocyanidin dimeric structure. The 13C-NMR spectrum indicated the presence of two flavan-3-ol units from the signals at δ 72.53 and 66.02 attributable to C-3 in the heterocyclic rings C and F and two carbon signals at δ 76.11 and 78.78 due to the C-2 in the heterocyclic rings C and F respectively. The upper and lower units were determined to be epicatechin from the C-2 and C-3 carbon signals of each unit.

The 13Cnmr and DEPT NMR spectra showed 30 carbon signals composed of CH2 (1), non-aromatic CH (5), aromatic CH (9), and quaternary carbons (15). In the HMBC spectrum, particularly diagnostic was the correlation from the proton signal at δ 4.60 attributed to H-4, C-ring of upper monomer which showed cross peaks with δ 106.07, 154.47, 157.03 (C-6, C-5, C-7) of A-ring of lower monomer respectively. The identity of this proanthocyanidin as epicatechin- (4β→6) epicatechin (Procyanidin B5) was confirmed by comparison of its physical and spectra data with those reported in the literature, especially the C-2, C-3, C-4 and C-8 13Cnmr signals (Agrawal et al., 1989; Porter et al., 1982) which is isolated for the first time from genus *Chrysophyllum*.

**Antioxidant activities**

The antioxidant activities of compounds 1–4 were evaluated using the DPPH, inhibition of lipid peroxidation and nitric oxide radical scavenging assays as IC50 values. Qualitative determination on TLC revealed that all the compounds demonstrated varying antioxidant activity as they rapidly bleached the purple colour background of DPPH on TLC within minutes except for stigmasterol (1), which had no antioxidant activity. Quantitatively, the results for the three antioxidant test systems for compounds 2, 3 and 4 are reported in Table 1. In all the three in vitro complementary assays, the isolated compounds expressed their antioxidant effects in a concentration-dependent manner and the antioxidant activities in DPPH parallel those of lipid peroxidation inhibitory and nitric oxide radical scavenging assay. The highest free radical scavenging activity was shown by procyanidin B5 (4) in DPPH and nitric oxide test systems with IC50 of 8.80 μM and 11.20 μM when compared with corresponding IC50 values of 12.82 μM and 45.15 μM for gallic acid that was used as standard. The free radical scavenging activity of the isolated compounds decreased in the following order: procyanidin B5 (4) with IC50 of 8.80 μM, 244.36 μM and 11.20 μM > epigallocatechin (3) with IC50 of 15.88 μM, 437.61 μM and 22.54 μM > epicatechin with IC50 of 19.02 μM, 236.98 μM and 27.34 μM in DPPH, lipid peroxidation, nitric oxide test systems respectively except in lipid peroxidation in which 2 is marginally higher than 3.

Structural activity relationship study in published literatures have shown that apart from 3-OH of ring-C and B-ring catechol groups in flavan-3-ol class of compounds, 4 → 8 and 4 → 6 linkages in their dimers, trimers and polymers had been shown to increase the stability of its radical and thereby endows a polymer with significant free radical scavenging properties (Morimoto et al., 1986, Castillo et al., 2000, Heim et al., 2002, Muselik et al., 2007) This explains why 4 demonstrated higher antioxidant property compared to 3. Compound 3 in DPPH and nitric oxide assays likewise demonstrated stronger antioxidant activity compare to 2. This observation is not surprising as 3′, 4′, 5′-triOH hydroxyl substitution pattern of ring–B (galloyl group) in a molecule has been reportedly shown to further enhance / increase free radical scavenging property of such molecule (Rice–Évans et al., 1996, Wang et al., 2007, Seyoum et al., 2006). This is a characteristic that compound 2 lacked.

However, the reduced inhibitory effects of epigallocatechin (3) on lipid peroxidation which was somewhat different (unusually low and marginally lower than that of epicatechin), in contrast to what was observed in DPPH radical scavenging activity where the antiradical activity was significantly high almost comparable with the standard gallic acid used had been attributed to the presence of galloylation which had been reported in literatures to reduce the ability of a molecule to prevent peroxidation (Plumb et al., 1998, 2006). This is a characteristic that compound 2 lacked.

**Antibacterial Activity**

The results of the antibacterial activity tests of the crude extract, ethyl acetate, n-butanol and aqueous fractions at 20 mg/mL are as summarised in Table 2. Solvent partitioning of the crude extract revealed that the n-butanol and ethyl acetate fractions possessed almost equal antibacterial activity while the residues in the mother liquors showed little or no activity against test bacteria. However, the
E. coli

All the isolated compounds, stigmasterol (1), epicatechin (2), epigallocatechin (3) and procyanidin B5 (4) were equally subjected to antibacterial test at 20 mg/mL as shown in Table 3. However only two of them epicatechin (2) and procyanidin B5 (4) demonstrated inhibitory activity. While epicatechin (2) was active against E. coli and B. subtilis, procyanidin B5 (4) was active against B. subtilis and S. aureus as revealed in Table 3. The lack of activity in epigallocatechin (3) is in line with published literature that galloyled molecules lack antibacterial activity except when alkylated with long chain aliphatic group such C8 (octanyl) and C10 (decanyl) (Stapleton et al., 2004, Tsuchiya et al., 1996).

The ethyl acetate fraction demonstrated a broad spectrum of activities against the selected bacteria and the most promising with an MIC of 156 μg/mL against E. coli and B. subtilis. The MIC of the aqueous fraction was the same (1250 μg/mL) for all the test organisms while the MICs of all the fractions and isolated compounds against P. aeruginosa were the highest (1250 μg/mL). However, the procyanidin B5 isolated from the plant for the first time demonstrated the highest inhibitory activity against E. coli (MIC 156.25 μg/mL), S. aureus (MIC 156.25 μg/mL), P. aeruginosa (MIC 625 μg/mL) and B. subtilis (MIC 156.25 μg/mL) when compared with crude and other fractions as shown in Table 4. Procyanidin B5 demonstrated activity which was 4 times better against the strains of bacteria tested when compared with the crude. Though, these activities were much lower compared to that observed for the standard, however the moderately good MIC values ranging from 156 to 625 μg/mL exhibited by procyanidin B5 against the tested bacterial strains were noteworthy.

Conclusions

Three of the isolated compounds from C. albidum stem-bark, procyanidin B5 (4), epigallocatechin (3) and epicatechin (2) demonstrated moderate antibacterial and strong free radical scavenging activities, thus justifying some of its ethnomedicinal uses.

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