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RESEARCH ARTICLE

CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITY OF *FICUS PLATYPODA* (MIQ.) LEAVES

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ABSTRACT

Six compounds were isolated for the first time from the leaves of *Ficus platypoda* and were identified as 3-methoxycarpachromene [1], pheophorbide-a methyl ester [2], pheophorbide-b methyl ester [3], (6*S*, 9*R*)-3-oxo-6-hydroxy-α-ionol [4], β-sitosterol-3-*O*-*β*-D-glucopyranoside [5]and rutin [6] on the basis of their spectroscopic data;UV, IR, ^{1}H- and ^{13}C-NMR in conjunction with 2D experiments (COSY, HSQC, HMBC), ESI/MS and direct comparison with published literatures. The different crude extracts (petroleum ether, ethyl acetate and *n*-butanol) showed selective antimicrobial activities against two Gram-positive bacteria, seven Gram-negative bacteria and two fungi. The different crude extracts also exhibited moderate to weak radical scavenging properties towards DPPH radical. The petroleum ether and ethyl acetate extracts showed remarkable cytotoxic activities against two human tumor cell lines: Human colon carcinoma (HCT-116) and Human breast cancer (MCF-7) cell lines, whereas week activities were detected with the *n*-butanol extract. Compound **2** showed potent antimalarial activity against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* with IC_{50} values in the range of 3.9 µg/mL without showing any cytotoxicity to mammalian cells but showing weak antileishmanial activity with IC_{50} value of 40 µg/mL. This is the first report concerning the biological activities of *Ficus platypoda* leaves.

Key words: *Ficus platypoda*, Cytotoxicity, Antimalarial and Antileishmanial

INTRODUCTION:

The genus *Ficus* (Moraceae) comprises about 1,000 species and is distributed in tropical and subtropical regions (Wagner,*et al.*, 1999). Many are grown as ornamentals in gardens, for shade and as house plants elsewhere, and a few are valued as fruit trees. Many *Ficus* species are commonly used in traditional medicine to cure various diseases. They have long been used in folk medicine as astringents, carminatives, stomachic, vermicides, hypotensives, anthelmintics and anti-dysentery drugs (Trivedi, *et al.*, 1969). They are widely used for human consumption, once it is an excellent source of minerals, vitamins and dietary fiber, being associated with longevity and health benefits (Solomon *et al.*, 2006). Several pheophorbides, alkaloids, terpenoids, steroids, lignans, flavonoids, phenolic acids, volatile compounds and megastigmanes have been reported from the genus *Ficus* (Bafor, *et al.*, 2013, Lansky,*et al.*, 2008, Chawla, *et al.*, 2012, Singh,*et al.*, 2006 and Nascimento, *et al.*, 1999).*Ficus platypoda* (Miq.) is native to northem Australia and grows in monsoon forest and dry scrub in rocky situations. Itis also known as Native Rock Fig.It is small to medium multi-trunked fig to 15m with smooth gray bark and aerial roots. The fruit is orange to red and edible. To date, however, nothing was reported in literatures, so far, concerning the chemical composition and biological activitiesof this plant. Therefore, it deemed of interest to carry out this investigation. In this paper we report the isolation and identification of six compounds from the leaves of *Ficus platypoda*, including 3-methoxycarpachromene[1], pheophorbide-a methyl ester [2], pheophorbide-b methyl ester [3], 3-oxo-6-hydroxy-α-ionol [4], β-sitosterol-3-*O*-*β*-D-glucopyranoside [5] and rutin [6].We have evaluated the antimicrobial, antioxidant and the cytotoxic activities of the petroleum ether, ethyl acetate and *n*-butanol extracts of the titled plant, in addition to the antimalarial and antiprotozoal activities of compound2.

MATERIAL ANDMETHODS:

EXPERIMENTAL:

General experimental procedures:

UV spectra were determined with a Hitachi 340 spectrophotometer. IR spectra were carried out on a Nicolet 205 FT IR spectrometer connected to a Hewlett-Packard Color Pro. Plotter.NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 (^{1}H) and 125 MHz (^{13}C), and a Varian Mercury 400 MHz spectrometer

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at 400 (^{1}H) and 100 MHz (^{13}C)in Pyridine-$d_5$or $CDCl_3$ solutionand chemical shifts were expressed in δ (ppm) with reference to TMS, and coupling constant (*J*) in Hertz. ^{13}C multiplicities were determined by the DEPT pulse sequence (135^o). COSY, HMBC, and HSQC NMR experiments were carried out using a Bruker Avance DRX-500 high field NMR spectrometer. The ESIMS spectra were measured using a Bruker Bioapex-FTMS with electrospray ionization (ESI). Column chromatographic separation was performed on silica gel 60 (Si gel 60, Merck) and Sephadex LH-20 (Pharmacia). TLC was performed on precoated TLC plates with silica gel 60 F254 (0.2 mm, Merck). Developed chromatograms were visualized by spraying with 1% vanillin-H_2SO_4, followed by heating at 100 ^{0}C for 5 min.

Plant material:

The leaves of *Ficus platypoda* were collected from Al-Orman garden in August, 2012, and identified by Mrs. Terase Labib, Taxonomist of Al-Orman Garden, Giza, Egypt. A voucher specimen was deposited at the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

Extraction and isolation:

The dried leaves of *F. platypoda* (3.5 kg) were extracted with MeOH three times till exhaustion to yield 190 g of a dark solid extract, which was then suspended in water (500 ml) and successively partitioned with petroleum ether, ethyl acetate and *n*-butanol to obtain petroleum ether (9.5 g), ethyl acetate (22 g), and *n*-butanol (19 g) extracts after removing solvent in *vacuum*. The ethyl acetate extract was chromatographed on a silica gel column and eluted with *n*-hexane–ethyl acetate gradient (100:0–25:75) to obtain six fractions of A (700 mg), B (900 mg),C (1.5 g), D (600 mg), E (1.3 g) and F (2.0 g). FractionB was further subjected to Si gel column chromatography eluted with *n*-hexane–ethyl acetate gradient (80:20–75:25) to give three sub-fractions of B-1 (70 mg), B-2 (80 mg) and B-3 (350 mg). Sub-fraction B-2 was subjected to Sephadex LH-20 column eluting with methanol to yield compound [1] (16mg). By the same method fractions C, D and E were separatelyapplied to Si gel column chromatography and eluted with *n*-hexane–ethyl acetate gradient (70:30–60:40), (50:50) and (30:70), respectively to give sub-fractions of C-1 (880 mg) and C-2 (400 mg), D-1 (100mg), D-2 (250mg) and D-3 (60mg)and E-1 (110mg), E-2 (130mg) and E-3 (160mg), respectively. Sub-fractions C-1, D-2 and E-3 were subjected separately to Sephadex LH-20 columns eluting with methanol to yield compounds [2] (5 mg), [3] (4 mg), and [4] (15mg), respectively. Fraction F was rechromatographed over Si gel column eluted with *n*-hexane-ethyl acetate: 30-70 to give four sub-fractions of F-1 (90mg), F-2 (120mg), F-3 (80mg) and F-4 (150mg). Sub-fraction F-3 was further purified over Si gel column eluted with$CHCl_3$-MeOH: 95-5 and Sephadex LH 20 column eluted with MeOH to give compound [5] (30 mg).The *n*-butanol (19 g) extract was chromatographed on a silica gel column and eluted with methylene chloride–methanol gradient (100:0–60:40) to gives four fractions of A (400 mg), B (2 g), C (1.0g) andD (1.2 g). Fraction C was further rechromatographed on a silica gel column and eluted with methylene chloride–methanol gradient (75:25) to gives three sub-fractions of C-1 (90mg), C-2 (600mg) and C-3 (130mg).Sub-fraction C-2 was subjected to final purification on a Sephadex LH-20 column eluting with methanol to yield compound [6] (20 mg).

Compound [1]: An amorphous pale yellow powder; UV λ_{max} (MeOH) nm: 230, 300, 350; λ_{max} (MeONa) nm: 395, λ_{max} ($AlCl_3$) nm: 240, 310, 400, λ_{max} ($AlCl_3$/HCl) nm: 240, 309, 401, λ_{max} (AcONa) nm: 230, 300, 360; IR υ_{max} (KBr) Cm^{-1}: 3440 (OH), 1690 (CO) and 1585 (aromatic); ^{1}H NMR (Pyridine-d_5, 500 MHz) and ^{13}C NMR (Pyridine-d_5, 125 MHz) see table 1. ESIMS *m/z*367 $[M+H]^+$, 755 $[2M+Na]^+$ and 1121 $[3M+Na]^+$.

Compound [2]:nworb krad powder; UV λ_{max} (MeOH) nm: 400, 660; IR υ_{max} (KBr) Cm^{-1}: 3325 (NH), 1730 (CO); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see table 2. ESIMS *m/z*607 $[M+H]^+$.

Compound [3]:Brown amorphous solid; UV λ_{max} (MeOH) nm: 430, 650; IR υ_{max} (KBr) Cm^{-1}: 3345 (NH), 1735 (CO), 2735 (CHO); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see table 3. ESIMS *m/z*621 $[M+H]^+$.

Compound [4]: An amorphous colorless solid; UV λ_{max} (MeOH) nm: 235, 315; IR υ_{max} (KBr) Cm^{-1}: 3400 (OH), 1655 (CO); ^{1}H NMR ($CDCl_3$, 500 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) see table 4. ESIMS *m/z*223 $[M-H]^+$, 247 $[M+Na]^+$, 207 $[M-H_2O+H]^+$ and 413 $[2M-2H_2O+H]^+$.

Compound [5]: White amorphous powder; ; IR (KBr) υ_{max} 3432, 1634 cm^{-1};^{1}H NMR (DMSO-d_6, 400 MHz) δ 0.63 (s, Me-18), 0.76 (d, *J*=6.5 Hz, Me-27), 0.78 (t, *J*=7.0 Hz, Me-29), 0.80 (d, *J*=6.5 Hz, Me-26), 0.87 (d, *J*=6.5 Hz, Me-21), 0.93 (s, Me-19), 3.02-3.39 (m, H-2`-H-5`), 3.41 (m, H-6`b), 3.44 (m, H-3), 3.61 (dd, *J*=10.7, 5.8 Hz, H-6`a), 4.18 (d, *J*=6.5 Hz, H-1`), 5.30 (m, H-6); ^{13}C NMR (DMSO-d_6,100.0 MHz) δ 12.10 (C-18), 12.17 (C-29), 19.03 (C-21), 19.35 (C-27), 19.49 (C-19), 20.16 (C-26), 20.91 (C-11), 23.01 (C-28), 24.14 (C-15), 28.50 (C-23), 28.59 (C-16), 29.10 (C-25), 31.10 (C-2), 31.47 (C-7), 31.79 (C-8), 33.74 (C-22), 35.94 (C-20), 36.63 (C-1), 36.76 (C-10), 36.97 (C-4), 38.74 (C-12), 42.26 (C-13), 45.48 (C-24), 49.71 (C-9),

55.46 (C-17), 56.52 (C-14), 61.22 (C-6`), 70.35 (C-4`), 73.61 (C-2`), 76.88 (C-3`, 5`), 77.18 (C-3), 101.16 (C-1`), 121.13 (C-6), 140.0 (C-5); ESI-MS m\z 577 $[M+H]^+$.

Compound [6]:Yellow amorphous powder [MeOH]; UV λ_{max} (MeOH) nm: 260, 366, λ_{max} (MeONa) nm: 273, 315, 412, λ_{max} ($AlCl_3$) nm: 276, 305, 439, λ_{max} ($AlCl_3$/HCl) nm: 275, 303, 409, λ_{max} (AcONa) nm: 277, 320, 395, λ_{max} (AcONa/boric acid) nm: 261, 389; ^{1}H NMR (DMSO-d_6, 500 MHz) δ12.52 (OH), 7.53 (1H, br s, H-2`), 7.50(1H, d, *J*=8.0 Hz, H-6`), 6.87 (1H, d, *J*=8.0 Hz, H-5`), 6.43 (1H, br s, H-8), 6.21 (1H, br s, H-6), 5.38 (1H, d, *J*=7.6 Hz, H-1``), 4.36 (1H, d, *J*=2.5 Hz, H-1```), 3.05-3.36 (10H, m, H-2``-H-6`` of glc and H-2```-H-5``` of rha), 0.95 (1H, d, *J*=6.0 Hz, H-6``); $^{13}$C NMR (DMSO-$d_6$, 125 MHz) δ177.73 (C, C-4), 164.70 (C, C-7), 161.49 (C, C-5), 157.07 (C, C-2),156.78 (C, C-9),148.84 (C, C-4`), 145.16 (C, C-3`), 133.64 (C, C-3), 122.01 (CH, C-6`), 121.48 (C, C-1`), 116.63 (CH, C-5`), 115.74 (CH, C-2`), 104.99 (C, C-10), 101.54 (CH, C-1``), 101.11 (CH, C-1```), 99.22 (CH, C-6), 94.11 (CH, C-8), 76.80 (CH, C-3``), 76.18 (CH, C-5``), 74.41 (CH, C-2``), 72.23 (CH, C-4```), 70.92 (CH, C-3```), 70.69 (CH, C-2```), 70.36 (CH, C-4``), 68.61 (CH, C-5```), 67.40 (CH_2, C-6``), 18.11 (CH_3, C-6```); ESIMS *m/z* 633 $[M+Na]^+$.

Determination of Antimicrobial activity:

The *in vitro*antimicrobial activity was performed by agar cup plate diffusion method (Bauer,*et al*., 1966) at Microbiology and Immunology department, Faculty of Pharmacy, Al-Azhar University. The antibacterial activity was carried out against two Gram-positive strains; *Bacillus cereus* and *Staphylococci aureus* (ATCC 6538), seven Gram-negative strains; *Escherichia coli* (ATCC 8739), *Salmonella typhi*, *Klebsiella pneumonia*, *Psedumonas aeruginosa* (ATCC 27853), *Proteus mirabilis*, *Acinetobacter baumanii* and *Shigella flexneri* and *in vitro* antifungal activity was carried out against two fungal strains; *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). The microbial strains were obtained from the culture collection of Department of Microbiology and Immunology (Faculty of Pharmacy, Al-Azhar University). The results were recorded in Table 5.

Determination of Antioxidant activity:

The free radical scavenging activity of the crude extracts of *F. platypoda* was measured by using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay (Yen and Duh, 1994), at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University. The results were recorded in Table 6.

Determination of Cytotoxic activity:

Cytotoxic activity of petroleum ether, ethyl acetate and *n*-butanol extracts were measured against Human colon carcinoma (HCT-116) and Human breast cancer (MCF-7) cell lines as described by Mosmann, 1983, Gangadevi and Muthumary, 2007 and the results are presented in Table 6. The cells were obtained from the American Type Culture Collection (ATCC, Rockville, MD). The cells were grown on RPMI-1640 medium supplemented with 10% inactivated fetal calf serum and 50µg/ml gentamycin. The cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were sub cultured two to three times a week.

Determination of Antimalarial activity:

Antimalarial activity of compound 2 was determined *in vitro* against chloroquine sensitive (D6, Sierra Leone) and resistant (W2, Indochina) strains of *Plasmodium falciparum* by measuring plasmodial LDH activity as reported previously (Bharate, et al., 2007). Chloroquine was used as positive control.

Determination of Antileishmanial activity:

The antileishmanial activity of the isolated compound 2 was tested *in vitro* against a culture of *L. donovani* Pentamidine and Amphoterecin B were used as positive standards (Ma,*et al*., 2004, Hamid,*et al.*, 2004).

RESULTS AND DISCUSSION:

The ethyl acetate and *n*-butanole xtracts of the leaves of *Ficus platypoda* were subjected to a succession of chromatographic procedures, including silica gel chromatography, gel permeation chromatography using Sephadex LH-20 to afford sixpure isolates **1**-**6**. The structures of the isolated compounds were established using spectroscopic analysis, especially, NMR spectra in conjunction with 2D experiments (COSY, HMQC, HMBC) and direct comparison with published data.

Compound [1]: Was obtained as an amorphous pale yellow powder. Its UV spectrum showed absorption bands at 230,300 and 350 nm. The bathochromic shift with $AlCl_3$, which did not show any change on the addition of HCl, showed the presence of a chelated OH group at C-5. The addition of NaOMe caused a bathochromic shift in band I suggesting that the C-4` phenolic hydroxyl group is free. No significance bathochromic shift occurred in band II after addition of NaOAc indicated that the C-7 phenolic hydroxyl group is not free. IR absorption at 3440, 1690 and 1585 cm^{-1} indicated the presence of hydroxyl function, carbonyl and aromatic ring. Its molecular formula was concluded to be $C_{21}H_{18}O_6$, from the molecular ion peaks in the ESIMS spectrum at *m/z* 367 $[M+H]^+$, 755 $[2M+Na]^+$ and 1121

$[3M+Na]^+$.The ^{13}C NMR spectrum (Table 1) gave 21 carbon signals and the DEPT and HMQC spectra confirmed that ten of these were protonated carbons. The DEPT spectrum further showed one methoxy, two methyls, seven methine and eleven quaternary carbons (including one carbonyl at δ 179.65) in agreement with the molecular formula. This indicated the presence of a prenylated flavonol. The nature and identity of the flavonol was evident from the ^1H NMR spectrum (Table 1) which showed the presence of an AA`BB` system of aromatic protons at δ 8.21 (2H, d, *J*= 8.5 Hz, H-2`, H-6`) and δ 7.33, (2H, d, *J*= 8.0 Hz, H-3`, H-5`) corresponding to a*p*-substituted ringB and one signal at δ 6.60 (1H, s) corresponding to ring A H-8 which showed HMBC correlations with the signals of quaternary carbons at δ 160.11 (C-7), 156.88 (C-9) and 106.77 (C-10). The presence of a methoxy was evident from the sharp 3H signals at δ 3.98 ppm. The methoxy protons showed an HMBC correlation with a carbon resonating at δ 139.21 (C-3) indicated that the methoxy group was located at C-3 position. A chelated OH group at C-5 could be attributed to a lower field signal at δ13.70 (s). The presence of a 2,2-dimethylchromen (cyclized ϒ, ϒ-dimethylallyl unit) was evident from a gem-dimethyl group signal at δ 1.47 (6H, s) and two vinylic protons at δ 5.68 (1H, d, *J*= 12.5 Hz, H-3``) and δ 6.92 (1H, d, *J*= 12.5 Hz, H-4``) (Schwarz *et al.*, 1964). Furthermore, the two methyl groups at δ 1.47 ppm showed HMBC correlations to a quaternary carbon at δ 78.79 (C-2``) and to an olifinic carbon at δ 129.28 (C-3``). The quaternary carbon at δ 78.79 (C-2``) showed HMBC correlations to the two olifinic protons at δ 5.68 (H-3``) and δ 6.92 (H-4``). This pattern confirmed the occurrence of a prenyl moiety. The connectivity of the 2,2-dimethyl pyran ring in ring A was deduced from HMBC data which showed a correlation between the vinylic protons at H-4`` with C-5 and C-7, and H-3`` showed the expected ^3J correlation with C-6, implying that the 2, 2-dimethyl pyran group is connected in ring A at C-6 and C-7 carbons. The rest of the structure was also confirmed through study of direct and long-range heteronuclear CH-coupling interactions. On the basis of the above evidences compound **1** was elucidated as 5, 4`-dihydroxy-3-methoxy-6, 7-(2``, 2``-dimethylchromene) flavonol (3-methoxycarpachromene) and its spectroscopic data were in good agreement with the literature data (Adams,*et al.*, 2009).

Table 1: 1D and 2D NMR data (Pyridine-d_5, 500 and 125 MHz) of Compound [1]

Position	^1H (*J* in Hz)	^{13}C	DEPT	HMBC
2	-	157.18	C	-
3	-	139.21	C	-
4	-	179.65	C	-
5	-	157.20	C	-
6	-	105.90	C	-
7	-	160.11	C	-
8	6.60, s	95.79	CH	C-7, 9, 10
9	-	156.88	C	-
10	-	106.77	C	-
1`	-	121.96	C	-
2`	8.21, d, 8.5	131.53	CH	C-2, 4`, 6`
3`	7.33, d, 8.0	117.12	CH	C-1`, 4`, 5`
4`	-	162.48	-	-
5`	7.33, d, 8.5	117.12	CH	C-1`, 4`, 5`
6`	8.21, d, 8.5	131.53	CH	C-2, 4`, 6`
2``	-	78.79	C	-
3``	5.68, d, 12.5	129.28	CH	C-6, 2``
4``	6.92, d, 12.5	116.21	CH	C-5, 7, 2``
Me-2``	1.47, s	28.67	CH_3	C-2``, 3``, Me-2``
Me-2``	1.47, s	28.67	CH_3	C-2``, 3``, Me-2``
OCH_3	3.98, s	60.54	CH_3	C-3
OH-5	13.70	-	-	-

Compound [2] was isolated as a dark brown powder. The ESIMS analysis of compound **2** gave the observed $[M+H]^+$ peak at m/z 607, consistent with the molecular formula of $C_{36}H_{38}O_5N_4$. The IR spectrum of **2** showed absorptions for amino (3325 cm^{-1}) and ester CO (1730 cm^{-1}) functionalities. The UV/VIS maxima absorptions at 400 and 660 nm evidenced that compound **2** belonged to the chlorophyll a type (Nakatani, *et al.*, 1981, Minguez-Mosquera and Gandul-Rojas, 1995). The ^1H NMR spectrum of compound **2** showed characteristic signals of a porphyrin skeleton, which includes: the two methyl groups directly connected to the conjugated ring system at δ 3.39 (s, H_3-2^1) and 3.68 (s, H_3-12^1),one downfield shifted methyl group at δ 1.82 (d, *J*=7.2 Hz, H_3-18^1), a vinyl group at δ 7.95 (dd, *J*=18.0, 12.0 Hz, H-3^1), 6.25 (d, *J*=18.0 Hz, H-3^2*trans*) and 6.15 (d, *J*=12.0 Hz, H-3^2*cis*) and three meso-olefinic singlets at δ 9.47 (H-10), 9.31 (H-5) and 8.56 (H-20) (Kamarulzaman, *et al.*,2011). In addition to one methyl ester group at δ 3.90 (s, H-13^4), one ethyl group at C-8 was evidenced according to the absorption at δ 3.57 (q, *J*=7.2 Hz, 8^1) and 1.67 (t, *J*=7.2 Hz, 8^2), one methyl propionate group at 3.59 (s, H-17^4), and two amino groups (δ -1.65) similar to that for pheophorbide a methyl ester (Kamarulzaman, *et al.*, 2011) reported in the literature. The ^{13}C- and ^{13}C-DEPT NMR spectra were consistent with the structure assigned above. They revealed the presence of seven methyl, four methylene, seven methine and eighteen quaternary carbons. By using HMQC experiment,for protonated carbons, each proton was assigned to its corresponding carbon and the quaternary carbons were further confirmed by an HMBC experiment. Geminal and vicinal protons were established by their correlations in the COSY spectrum (Table 2).

Table 2: 1D and 2D NMR data (*$CDCl_3$*, 500 and 125 MHz) of Compound [2]

Position	^1H (*J* in Hz)	^{13}C	DEPT	COSY	HMBC
1	-	143.00*	C	-	-
2	-	132.50*	C	-	-
2^1	3.39, s	12.12	CH_3	-	C-1, C-2, C-3
3	-	137.00*	C	-	-
3^1	7.95, dd, 18.0, 12.0	129.02	CH	H-3^2_{cis}, H-3^2_{trans}	
3^2_{cis} 3^2_{trans}	6.15, d, 11.2 6.25, d, 18.0	122.80	CH_2	H-3^1, H-3^2_{trans} H-3^1, H-3^2_{cis}	C-3 C-3
4	-	n.d.	-	-	-
5	9.31, s	97.50	CH		C-3, C-6
6	-	156.00*	C	-	-
7	-	137.00*	C	-	-
7^1	3.18, s	11.19	CH_3	-	C-6, C-7, C-8
8	-	145.0*	C	-	-
8^1	3.57, q, 7.2	19.39	CH_2		
8^2	1.67, t, 7.2	17.43	CH_3	H-8^1	C-8^1, 8
9	-	n.d.	C	-	-
10	9.47, s	104.41	CH		C-12
11	-	138.00*	C	-	-
12	-	129.00*	-	-	-
12^1	3.68, s	12.12	CH_3	-	C-11, C-13
13	-	129.50*	C	-	-
13^1	-	189.75*	C	-	-
13^2	6.27, s	64.70	CH		C-13^1, 13^3, 14, 15
13^3	-	169.50*	C	-	-
13^4	3.90, s	52.92	CH_3	-	C-13^3
14	-	150.00*	C	-	-

15	-	105.00*	C	-	-
16	-	n.d.	-	-	-
17	4.21, d, 8.8	51.71	CH	H-18	
17^1	2.20-2.28, m	29.72	CH_2		
17^2	2.33-2.53, m	31.01	CH_2		
17^3	-	173.50	C	-	-
17^4	3.59, s	51.07	CH_3	-	C-17^3
18	4.47, d, 6.8	50.08	CH	H-17	
18^1	1.82, d, 7.2	23.08	CH_3	-	C-17, C-18, C-19
19	-	173.50*	C	-	-
20	8.56, s	93.12	CH		C-2
NH	-1.65, brs	-	-	-	-

*These quaternary carbons were detected from their correlations in the HMBC spectrum.n.d. (not detectable).

The singlet at δ_H 3.39 corresponding to δ_C 12.12 (C-2^1) showed HMBC correlations at δ 143.00, 137.00 and 132.50 which were assigned to C-1, C-3 and C-2, respectively. Furthermore the correlations between the signals at δ 6.25 and 6.15 and the carbon at δ 137.00 confirmed the chemical shift of C-3. The COSY spectrum was confirmed the vinyl group through that, it showed a correlation between the geminal 3^2_{cis} (δ 6.15) and 3^2_{trans} (δ 6.25) protons and the both were correlated to the vicinal proton 3^1 at δ 7.95. The singlet at δ_H 3.18 corresponding to δ_C 11.19 (C-7^1) showed HMBC correlations at δ 156.00, 145.00 and 137.00 which were assigned to C-6, C-8 and C-7, respectively. The correlation between the triplet at δ 1.67 corresponding to δ_C 17.43 (C-8^2) and the carbons at δ 145.00 (C-8) and 19.39 (C-8^1) confirmed the presence of ethyl moiety at position C-8. This was also confirmed from the cross peak between the quartet at δ 3.57 and the triplet at δ 1.67 in the COSY spectrum. The chemical shifts of C-11, C-12, C-13 were identified through their correlation with the signals at δ 9.47 (H-10) and 3.68 (H-12^1) in the HMBC spectrum. The two methoxy groups at δ 3.90 and 3.59 were located at C-13^3 and C-17^3 from their HMBC correlations at δ 169.50 and 173.50, respectively. Further assignments of ^{1}H and ^{13}C NMR using HMBC spectral data are outlined in Table 2. On the basis of these evidence and on comparison with literature data (Kamarulzaman, *et al.*, 2011), compound **2** was identified aspheophorbide-a methyl ester.

Compound [3] was isolated as a brown amorphous solid. The ESIMS showed a $[M+H]^+$ peak at m/z 621 $[M+H]^+$, which was consistent with the molecular formula of $C_{36}H_{36}O_6N_4$. Compound **3** has additional 14 a.m.u. compared to **2**, in line with the proposed replacement of the methyl group at C-7 in **2** with a CHO group in **3**. Its spectral characteristics revealed a great similarity to **2**. The IR spectrum of **3** revealed the presence of amino (3345 cm^{-1}), aldehyde (2735 cm^{-1}), and ester CO (1735 cm^{-1}) functionalities, and the UV/VIS spectrum showed absorption maxima at 430 and 650 nm, indicating that **3** belonged to the chlorophyll-b type (Nakatani, *et al.*, 1981, Minguez-Mosquera and Gandul-Rojas, 1995).By comparing the ^{1}H- and ^{13}C-NMR data of **3** with those of **2** indicated that **3** has an identical tetrapyrrole moiety to **2**, with the only difference being the presence of 7-formyl group in **3** instead of the 7-Me group in **2**. As in **2** the protonated carbons in **3** were established on the bases of HMQC spectrum. The ^{1}H NMR spectrum of **3** revealed resonances that are typical of pheophorbide-b, including a singlet proton signal at δ 10.95corresponding to δ_C 187.57 in the HMQC spectrum, assignable to a CHO group at position C-7^1, three singlet signals assignable to H-5, H-10 and H-20 at δ 10.14, 9.38 and 8.52 ppm respectively, a vinyl group at C-3 with signals at δ 7.90 (dd, *J*= 11.2, 17.6 Hz), δ 6.30 (d, *J*= 17.6 Hz) and δ 6.18 (d, J= 11.2 Hz), signals for the two methyl groups which are directly connected to the conjugated ring system at δ 3.62 (H-12^1) and 3.35 (H-2^1) and a downfield shifted methyl group at δ1.84 (d, *J*=7.6 Hz, H_3-18^1). An ethyl group at C-8 was evidenced according to the absorption at δ 3.81 (q, 8^1) and 1.71 (t, 8^2). The ^{13}C- and ^{13}C-DEPT NMR spectra of **3** showed 36 carbon signals (Table 3), from which two tertiary methyl groups at δ 12.06 (C-2^1) and 12.20 (C-12^1), one secondary methyl group at δ 23.07 (C-18^1), one vinyl group at δ 128.59 (C-3^1) and 123.52 (C-3^2), one ethyl group at δ 19.20 (CH_2, C-8^1) and 19.33 (CH_3, C-8^2), two methoxy groups at δ 52.98 (C-13^4) and 51.30 (C-17^4) and three olifinic carbon methine at δ 93.33 (C-20), 101.49 (C-5) and 106.85 (C-10). An HMBC correlation observed between H-7^1 at δ 10.95 and C-6 at δ 150.65 confirmed the presence of the formyl group. Other HMBC correlations were observed and were established the

elucidated structure (Table 3). Porphyrin and its derivatives, constructed by four pyrrole rings linked through CH bridges, maintain aromaticity in the macrocycle through an 18 π electron system.

Table 3: 1D and 2D NMR data (*$CDCl_3$*, 500 and 125 MHz) of Compound [3]

Position	^{1}H (*J* in Hz)	^{13}C	DEPT	HMBC
1	-	143.50	C	-
2	-	132.75	C	-
2^1	3.35, s	12.06	CH_3	C-1, 2, 3
3	-	137.91	C	-
3^1	7.90, dd, 17.6, 11.2	128.59	CH	
3^2_{cis} 3^2_{trans}	6.18, d, 11.2 6.30, d, 17.6	123.52	CH_2	C-3 C-3
4	-	137.67	C	-
5	10.14, s	101.49	CH	C-4
6	-	150.65	C	-
7	-	132.17	C	-
7^1	10.95, s	187.57	CH	C-6
8	-	159.0	C	-
8^1	3.81, q, 7.6	19.20	CH_2	
8^2	1.71, t, 7.6	19.33	CH_3	C-8, 8^1
9	-	147.50	C	-
10	9.38, s	106.85	CH	C-8, 11, 12
11	-	137.07	C	-
12	-	132.50*	C	-
12^1	3.62, s	12.20	CH_3	C-11, 12, 13
13	-	129.64	C	-
13^1	-	189.42	C	-
13^2	6.21, s	64.56	CH	C-13^1, 13^3, 14, 15,
13^3	-	169.23	C	-
13^4	3.92, s	52.98	CH_3	C-13^3
14	-	150.50*	C	-
15	-	104.87	C	-
16	-	163.94	C	-
17	4.25, d, 8.8	51.71	CH	
17^1	2.55-2.70, m	29.69	CH_2	
17^2	2.30, m	31.07	CH_2	C-17^3
17^3	-	173.19	C	-
17^4	3.61, s	51.30	CH_3	C-17^3
18	4.50, d, 6.8	50.09	CH	
18^1	1.84, d, 7.6	23.07	CH_3	C-17, 18, 19
19	-	173.91	C	-
20	8.52, s	93.33	CH	C-1, 2
NH	- 1.60 brs	-	-	-

*These quaternary carbons were detected from their correlations in the HMBC spectrum.

The 7-formyl group in **3**, participating in the above conjugated system, enhances the delocalization of π-electrons, which reduced the electron density of the atoms in the tetrapyrrole ring. This caused the slight downfield shift of ^{13}C-NMR resonance of C-19and of the protons surrounding the tetrapyrrole skeleton in **3** than the corresponding resonances of **2** (Cheng, *et al.*, 2001).Based on these data, compound **3** was identified as pheophorbide-b methyl ester and is in good agreement with published data (Kamarulzaman, *et al.*, 2011).

Compound [4]: Was isolated as an amorphous colorless solid and its molecular formula was determined to be $C_{13}H_{20}O_3$ by ESI-MS at m/z 223 $[M-H]^+$, 247 $[M+Na]^+$, 207 $[M-H_2O+H]^+$ and 413 $[2M-2H_2O+H]^+$. The UV spectrum λ_{max} (MeOH) 235 and 315 nm was characteristic of an enone system. The IR band at 1655 cm^{-1} supported this finding. Also IR absorption at 3400 cm^{-1} suggested the presence of a hydroxyl group. The ^{1}HNMR spectrum of **4** showed the three tertiary methyl singlets at δ 0.93 (H_3-11), 0.98 (H_3-12) and 1.82(H_3-13). In addition, the two H-2 α-ketone protons observed asan AB system at δ 2.35 and 2.14 (each 1H, each doublet, *J*=16.0 Hz) and the broad singlet at δ 5.81 (H-4), assignable to the vinyl proton indicated the presence of a substituted double bond. The ^{1}H NMR spectrum of **4**(Table 4) showed also an $ABXY_3$, system, corresponding to a disubstituted (*E*)-olefin at δ 5.71 (1H, *dd*, *J*=16.0, 8.0 Hz, H-8) and 5.66(1H, *d*, *J*=16.0 Hz, H-7), an oxymethine proton at δ 4.31(1H, *t*, *J*=8.0 Hz, H-9) and a secondary methyl group at δ1.19 (3H,*d*, J=8.0 Hz, H-l0). The ^{13}C NMR and DEPT spectra (Table 4) revealed 13 carbon signals, which were assigned to a megastigmanes skeleton. The chemical shifts of these carbons implied that this compound contained four tertiary carbons at δ [23.99 (C-12), 23.57 (C-10), 22.90 (C-11), 29.21 (C-13)], one methylene carbon at δ 49.60 (C-2), three olifinic methine carbon at δ [135.60 (C-7), 128.94 (C-8), 126.46 (C-4)], one oxymethine carbon at δ 67.85 (C-9), three quaternary carbons at δ [164.0 (C-5), 78.91 (C-6), 41.22 (C-1)] and one carbonyl carbon at δ 198.95 (C-3). The absolute configuration at the C-6 and C-9 of **4** was assigned on the basis of comparison to literature data (Galbraith and Horn, 1972; Gonzalez, *et al.*, 1994) to be (6*S*) and (9*R*), respectively. Therefore, on the basis of above and literature data (Galbraith and Horn, 1972; Gonzalez, *et al.*, 1994), compound **4** could be characterized as (6*S*, 9*R*)-3-oxo-6-hydroxy-α-ionol.

Table 4: 1D NMR data (*$CDCl_3$*, 500 and 125 MHz) of Compound [4]

Position	^{1}H (*J* in Hz)	^{13}C	DEPT
1	-	41.22	C
2_α 2_β	2.14, d, 16.0 2.37, d, 16.0	49.60	CH_2
3	-	198.95	C
4	5.81, s	126.46	CH
5	-	164.0	C
6	-	78.91	C
7	5.66, d, 16.0	135.60	CH
8	5.71, dd, 16.0, 8.0	128.94	CH
9	4.31, t, 8.0	67.85	CH
10	1.19, d, 8.0	23.57	CH3
11	0.93, s	22.90	CH3
12	0.98, s	23.99	CH3
13	1.82,s	19.21	CH3

Compound [5] and **[6]** were obtained as a white and yellow amorphous powder, respectively. By comparison their spectral data (UV, IR, NMR and MS) with those of the corresponding data in literature, they were identified as β-sitosterol-3-*O*-*β*-D-glucopyranoside (Daucosterol)(Chaurasia, *et al*., 1987, Voutquenne, *et al*., 1999**)** and quercetin-3-*O*-*α*-L-rhamnopyranosyl-(1→6)-*β*-D-glucopyranoside (rutin) (Harborne, 1994; Markham,*et al*., 1978, Wagner,*et al*., 1976) respectively**.**

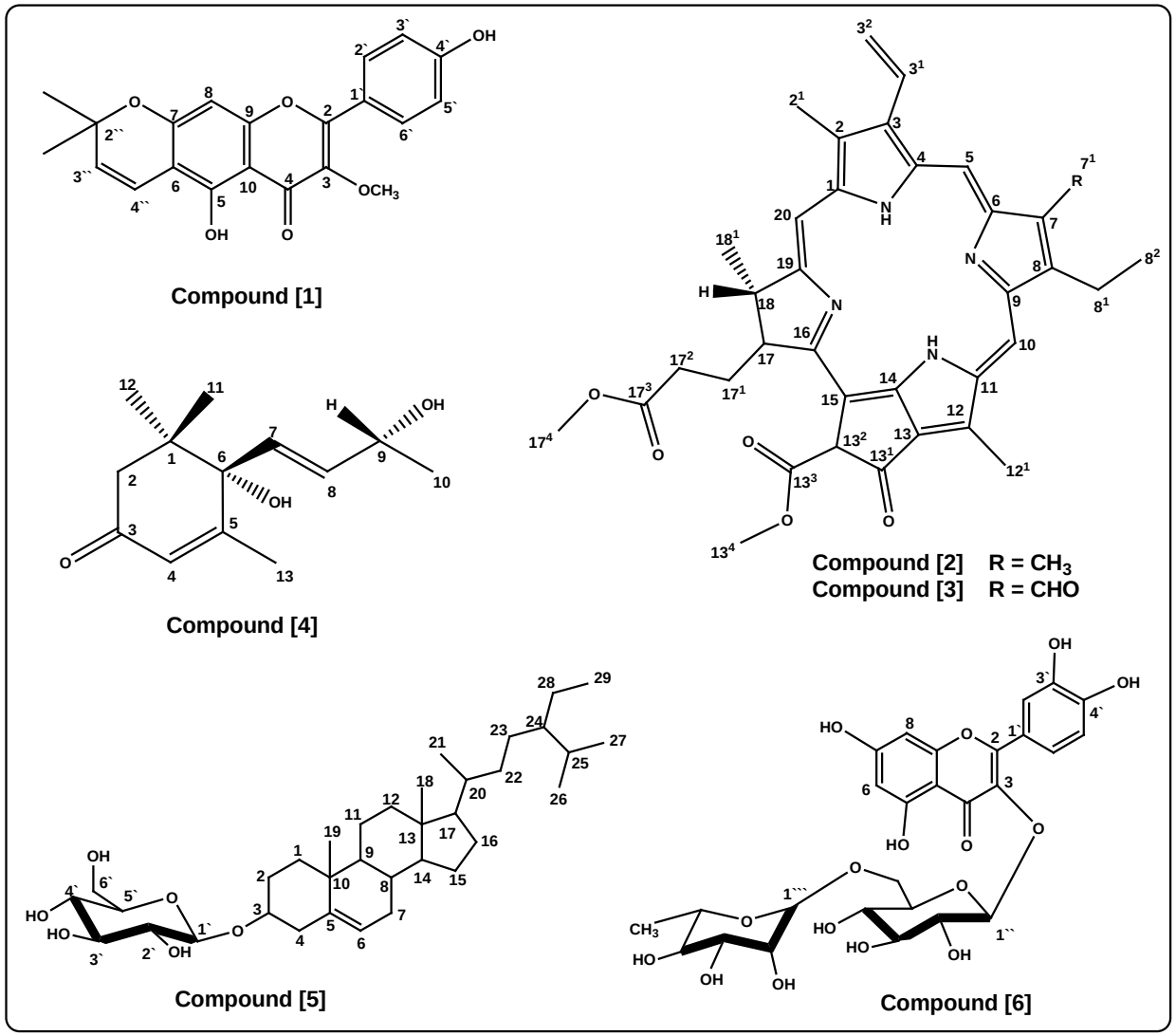


Figure 1: Compounds 1-6

The Antimicrobial activity:

The antimicrobial activities of petroleum ether, ethyl acetate and *n*-butanol extracts of *Ficus platypoda* were systematically investigated against 11 different bacterial and fungal species (two Gram-positive, seven Gram-negative and two fungi) and were compared with standard drugs ciprofloxacin (for bacteria) and nystatin (for fungi). All extracts demonstrated growth inhibitory effect and variable antimicrobial activity against most of the specific organisms tested (Table 5).

The more interesting is that, the ethyl acetate extract showed good activity against the more antibiotic resistant *P. aeruginosa* and the three extracts showed also high activity against the more antibiotic resistant *A. baumanii*. The ethyl acetate and *n*-butanol were the most active extracts as antibacterial agents compared to that of petroleum ether. The *n*-butanol extract showed higher antifungal activity against *A. niger* than that of the petroleum ether and both extracts showed the same activity against *C. albicans*. The ethyl acetate extract did not shows antifungal activity against the tested fungi. In addition, the antimicrobial activity increases with increasing in concentration of the extracts as evident by the zones of inhibition (Table 5).

Table (5): Results of Antimicrobial activity of different extracts of *Ficus platypoda* leaves

Micro-organisms	Diameter of inhibition zone (mm)[1]															
	Butanol extract					Ethyl acetate extract					Petroleum ether extract					Ciprofloxacin 5µg/disc
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	E	
B. cereus	21	16	13	-	-	18	12	-	-	-	20	18	12	-	-	25
S. aureus	22	20	12	10	-	20	8	-	-	-	26	22	15	12	10	26
E. coli	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	27
K. pneumonia	31	27	24	18	-	26	18	12	-	-	27	15	-	-	-	31
S. typhi	22	16	12	-	-	23	21	18	13	-	-	-	-	-	-	25
S. flexneri	22	17	14	11	-	-	-	-	-	-	-	-	-	-	-	29
P. aeruginosa	-	-	-	-	-	18	14	12	10	-	-	-	-	-	-	22
P. mirabilis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28
A. baumanii	20	17	14	-	-	22	21	16	14	-	21	18	10	-	-	22
																Nystatin 30 µg/disc
A. niger	15	12	9	-	-	-	-	-	-	-	12	8	-	-	-	16
C. albicans	17	15	-	-	-	-	-	-	-	-	17	15	8	-	-	19

a, **b**, **c**, **d**, **e**, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml concentrations in DMSO, respectively; [1] average diameter in mm of three measures; - no activity.

The Antioxidant activity:

There are significant variations in the capacity of the test samples to scavenge the DPPH radical with IC_{50} ranging from 79 to 537µg/ml (Table 6). Butanol fraction had the highest anti DPPH radicals activities compared to other fractions with IC_{50} 79 µg/ml followed by ethyl acetate and petroleum ether with IC_{50} 116 and 537µg/ml, respectively. The IC_{50} of these fractions are higher than the IC_{50} 79 of the positive control (ascorbic acid 11.2 µg/ml). As expected the non-polar compounds present in the petroleum ether and ethyl acetate fractions had poor radical scavenging activities compared to the control. From the estimated IC_{50} values, the order of potency is butanol fraction followed by ethyl acetate fraction then petroleum ether fraction.

Table 6: Scavenging effect on DPPH radicals and cytotoxic activity of crude extracts

Extracts	Antioxidant activity/(IC_{50} µg/ml)[1]	Cytotoxic activity (IC_{50} µg/ml)[2]	
		HCT-116	MCF-7
Petroleum ether	537	3.0	2.8
Ethyl acetate	116	9.0	10.8
n-butanol	79	> 50	> 50
Ascorbic acid	11.2	-	-

[1] IC_{50} denotes the concentration of sample required to scavenge 50% of the DPPH free radicals.
[2] IC_{50} is defined as the concentration that resulted in a 50% decrease in cell number.

The Cytotoxic activity:

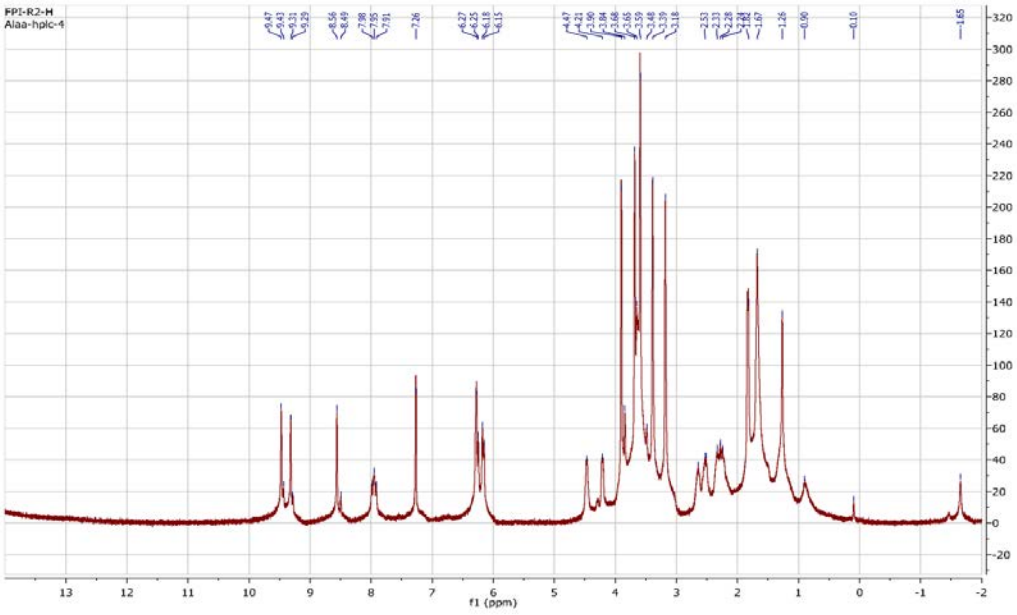
Cytotoxic activities of the petroleum ether, ethyl acetate and *n*-butanol fractions were tested against two cancer cell lines HCT-16 and MCF-7 (Table 6). As a result, petroleum ether fraction exhibited the highest cytotoxic activity against the tested cell lines with values of IC_{50} from 2.8 to 3.0µg/ml. The ethyl acetate fraction showed a moderate cytotoxic activity against the tested cell lines with values of IC_{50} from 9.0 to 10.8µg/ml. On the other hand, the*n*-butanol fraction showed week cytotoxic activities against the tested cell lines.

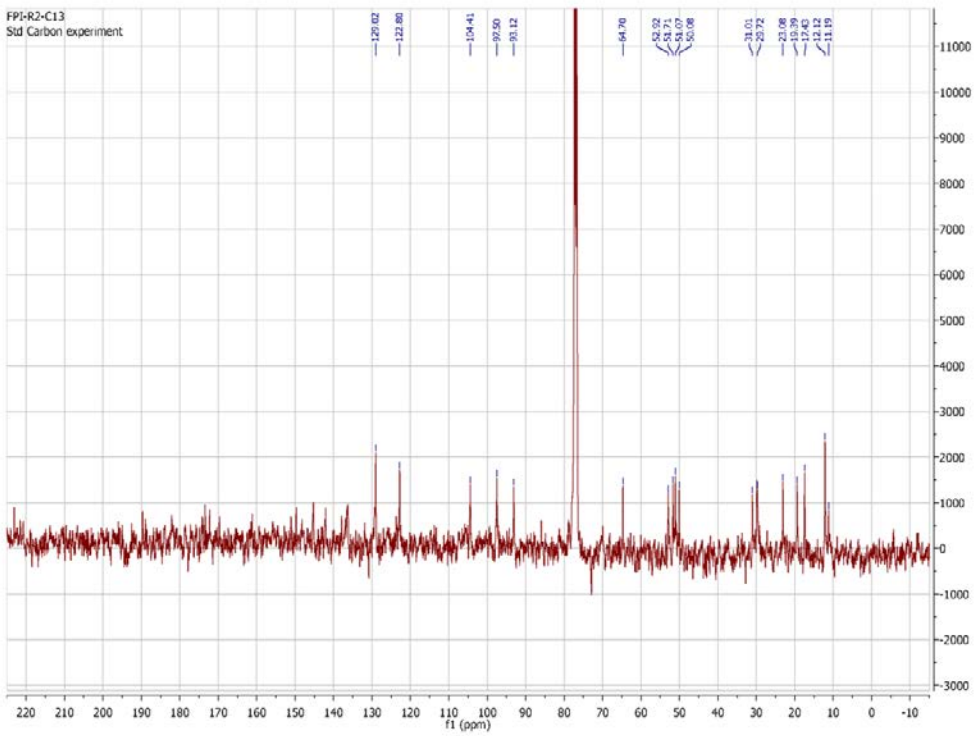
The Antimalarial and the Antileishmanial activities:

Compound 2 appeared to be very active against both chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* with IC_{50} values of 3.9 µg/mL without showing any cytotoxicity to mammalian cells but showing weak activity towards *Leishmania donovani* with an IC_{50} value of 40 µg/mL.

Analysis Supplementary data

Compound 2





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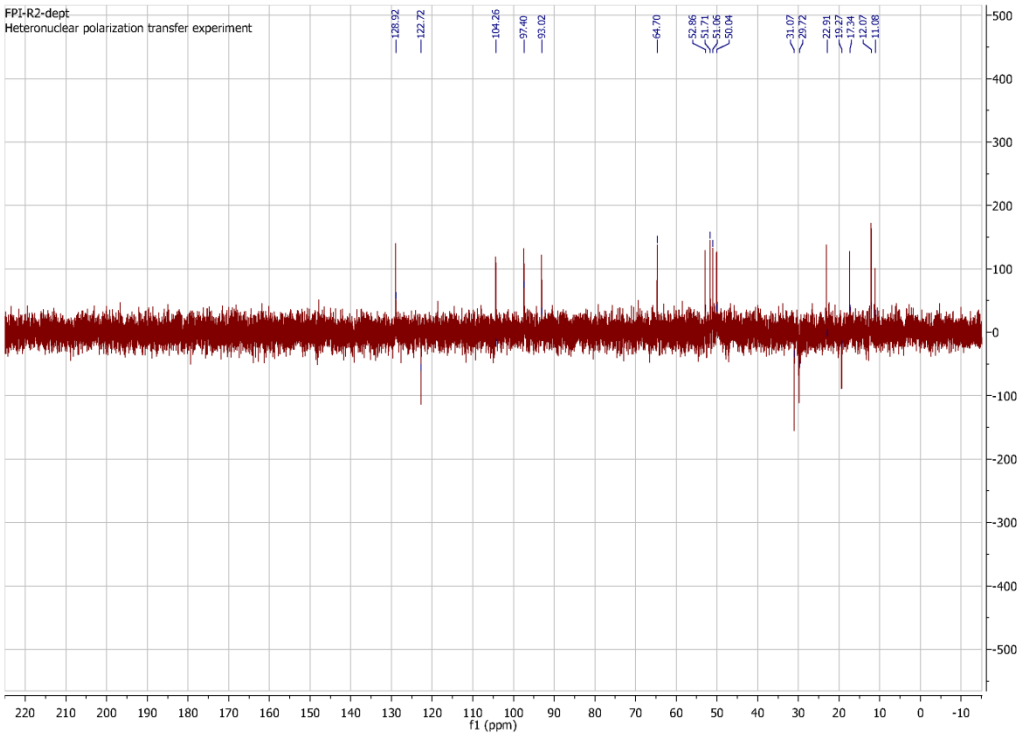
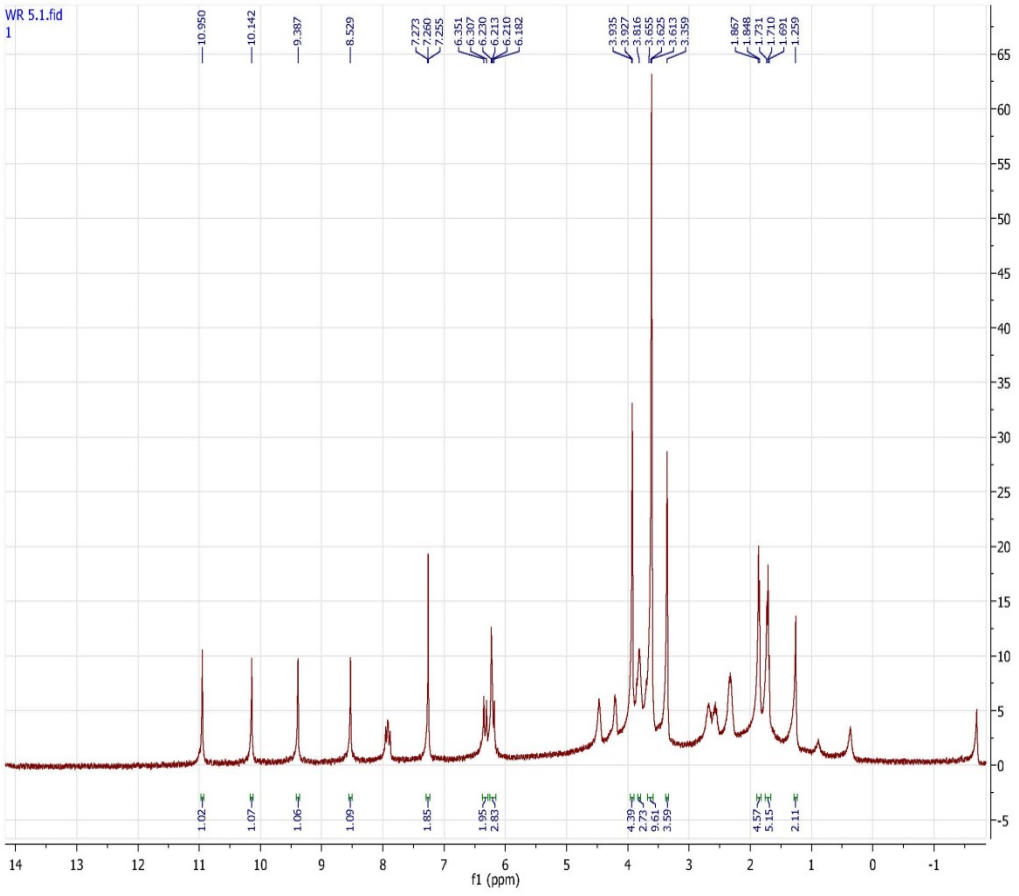
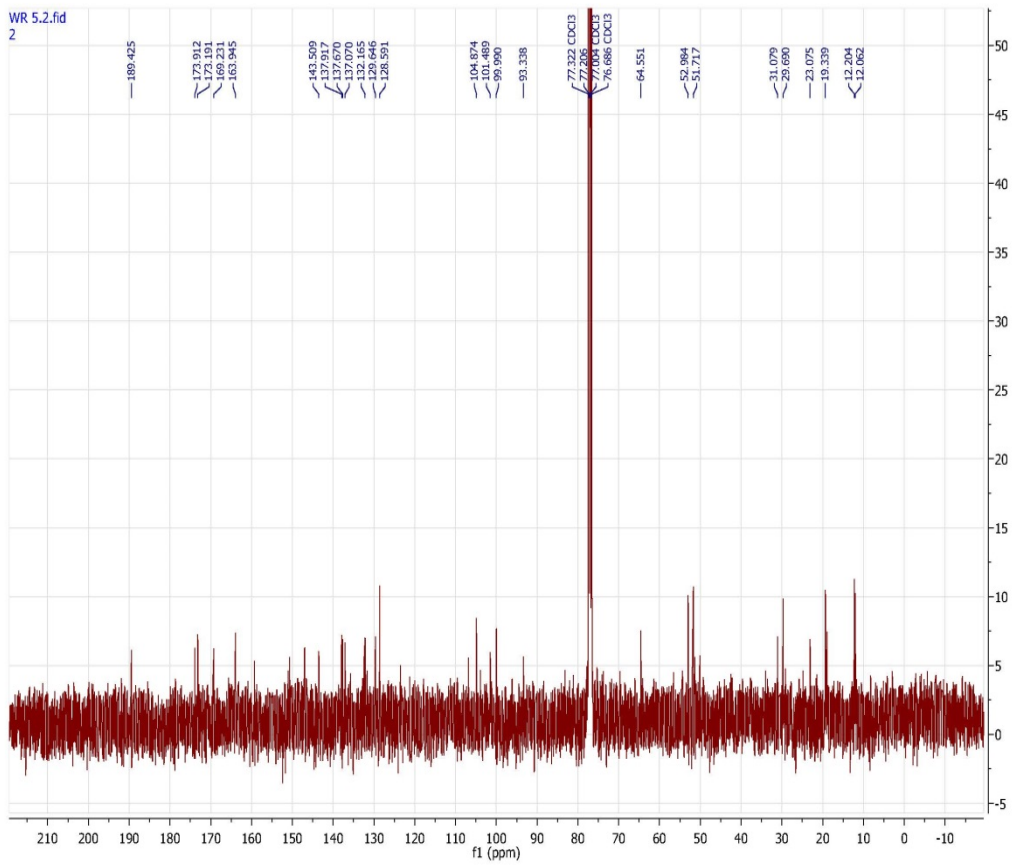
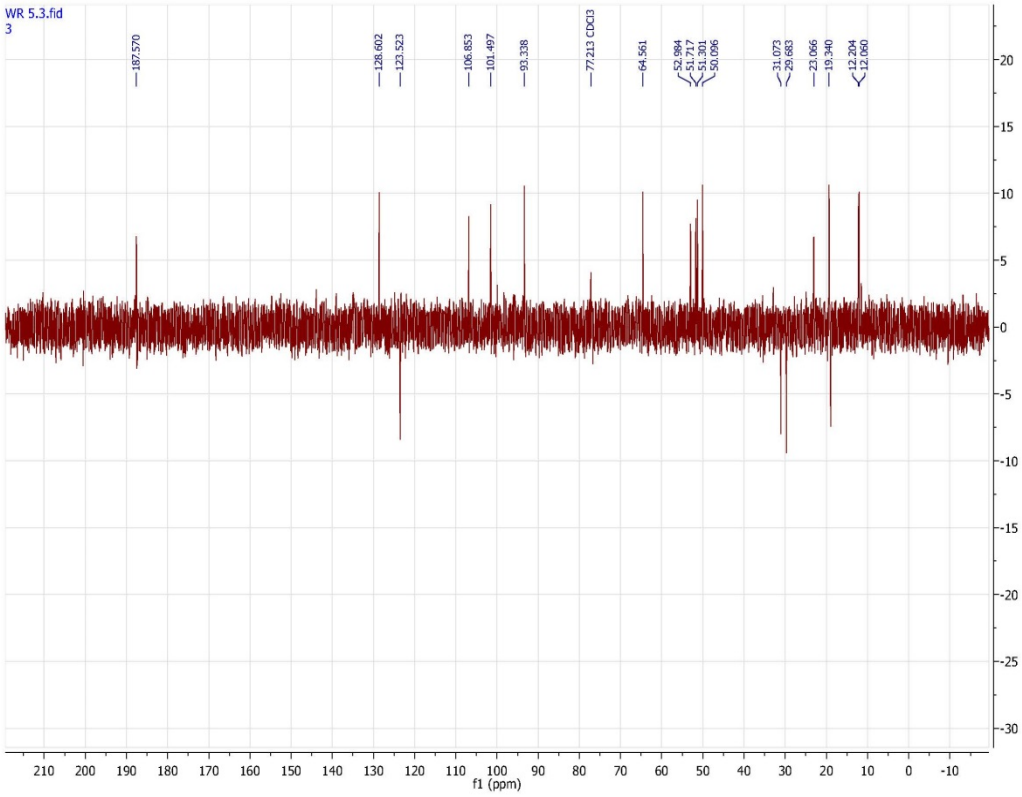
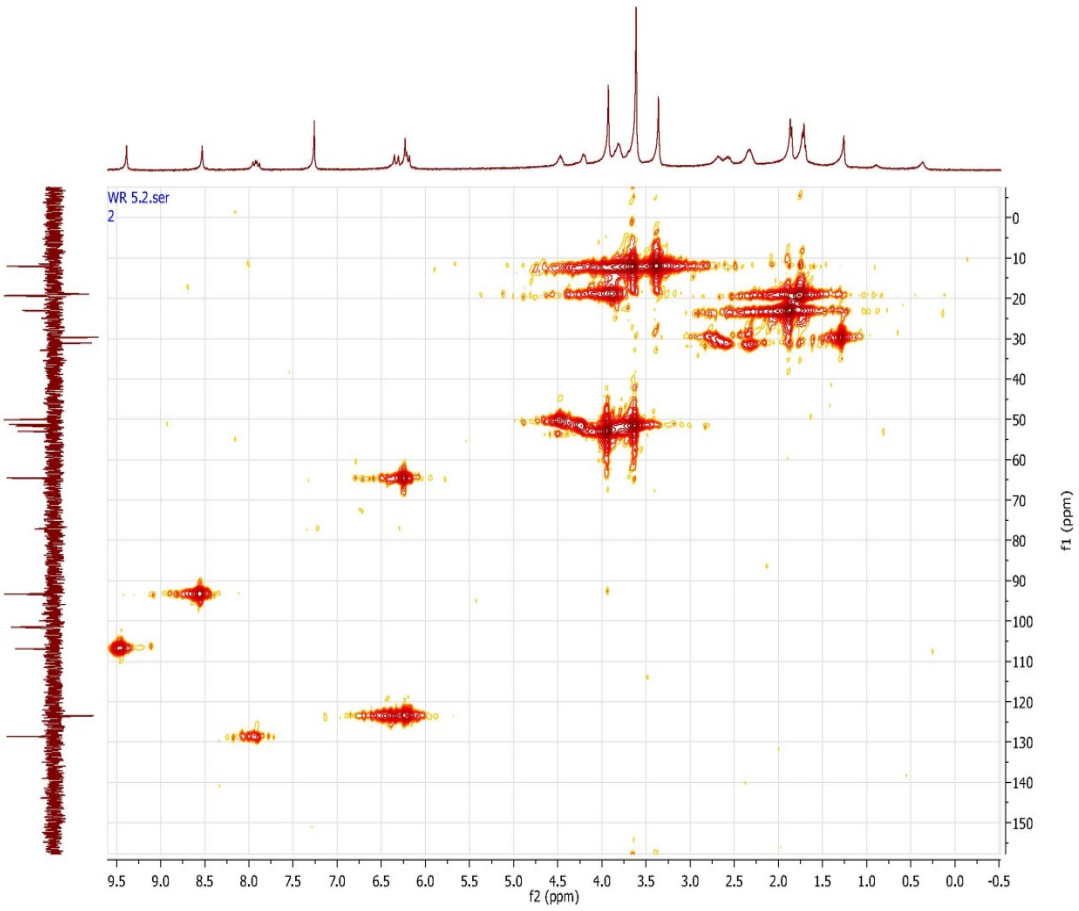


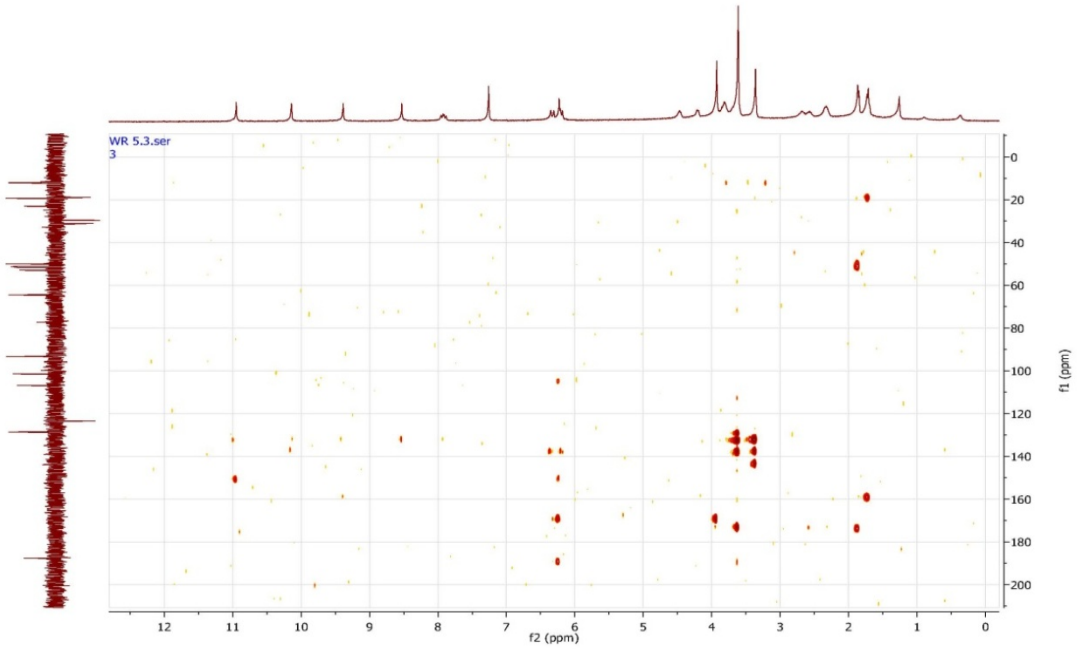
Compound 3











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