# Phytochemical Constituents of *F. Sagittifolia* Warburg ex Mildbraed & Burret Leaves with Antimicrobial Activity

**Olayombo Margaret Taiwo[1,2,*], Olaoluwa Omosalewa Olaoluwa[1], Olapeju Oluyemisi Aiyelaagbe[1], Josphat Clement Matasyoh[2]**

[1]Department of Chemistry, Faculty of Science, University of Ibadan, 200284, Ibadan, Nigeria
[2]Department of Chemistry, Faculty of Sciences, Egerton University, 20115 Egerton, Kenya.

**Corresponding author***
olayombotaiwo@gmail.com

## Abstract

The leaves and bark of *Ficus sagittifolia* have been used as a cure for stomach and pulmonary disorders, respectively. The bark is edible and is taken against colic. From the leaves of *F. sagittifolia*, a steroidal glycoside named Stigmast-5,22-diene-3-*O*-*β*-*D*-glucopyranoside **1** and three isoflavonoids named 5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one **2**, 5-hydroxy-3(4-hydroxylphenyl)-8,8-dimethylpyrano[2,3-f]-chromen-4(8H)-one **3** and 5-hydroxy-3-(4-hydroxyphemyl)-8,8-dimethylpyrano[3,2-g]-chromen-4(8H)-one **4** were isolated, and this is the first report of the isolation of these compounds from this plant. The structural elucidation of the compounds was based on 1D and 2D NMR, IR and MS data analyses. Compounds **1** and **2** inhibited the growth of *Pseudomonas aeruginosa* and *Aspergillus Niger* at 0.0625 mg/mL, respectively while compounds **2** and **4** were active against *Helicobacter pylori* at 0.0625 mg/mL. These findings corroborate the ethno-medicinal use of *F. sagittifolia* leaves as a treatment for stomach disorders.

**Keywords:** Antimicrobial activity; *F. sagittifolia*; Isoflavonoids; Natural products; Steroidal glycoside.

**Abbreviations**: FSL – *Ficus Sagittifolia* leaves; Hex – Hexane; EtOAc – Ethyl acetate; TLC – Thin layer chromatography; $CHCl_3$ – Chloroform; MeOH – Methanol; HPLC – High performance liquid chromatography; *p* –INT – *p*-iodonitrotetrazollium; DMSO – Dimethylsulphoxide; NMR – Nuclear Magnetic Resonance; ^{1}H NMR – Proton Nuclear Magnetic Resonance; ^{13}C NMR – Carbon Nuclear Magnetic Resonance; COSY- Correlation spectroscopy; HSQC- Heteronuclear single quantum correlation spectroscopy; HMBC-Heteronuclear multiple bond correlation spectroscopy; TMS – Tetramethylsilane; HRESIMS – High-resolution electrospray ionization mass spectrometry; LRESIMS – Low-resolution electrospray ionization mass spectrometry; IR – Infrared spectroscopy; UV – Ultraviolet; NA – Nutrient agar; TSB – Tryptic Soy Broth; SDB – Sabouraud dextrose broth; MIC – Minimum inhibitory concentration; MMC – Minimum microbicidal concentration; $(CD_3)_2SO$ – Deuterated dimethylsulpoxide; $(CD_3OD)$ – Deuterated methanol; multi – Multiplet; *J* – Coupling constant; $t_R$ – Retention time; $R_f$ – Retention factor.

## INTRODUCTION

*Ficus sagittifolia* (Warburg ex Mildbraed & Burret) belongs to the family Moraceae and a member of the genus *Ficus*. It is an epiphytic shrub, often on oil palms, becoming a tree to 30 feet high. It is native to Benin, Cameroon, Ghana, Guinea, Guinea-Bissau, Ivory Coast, Liberia, Nigeria, Senegal, Sierra Leone, and Togo (POWO, 2022). *Ficus* species have been used locally as stomach herbs, anthelmintics, vermonicides, astrigents, carminatives, hypotensives, and anti-dysentery drugs (Salem *et al*., 2013). The leaves and bark of *F. sagittifolia* are used as a cure for stomach and pulmonary disorders, respectively. The bark is edible and is taken against colic (Burkill, 1997). Species of *Ficus* are known to possess antioxidant, cytotoxic, antimicrobial, anti-inflammatory, antidiabetic, antiulcer, and anticonvulsant activities (Salehi *et al*., 2021). Phytochemicals such as genistein, protocatechuic acid, stigmatserol, rutin, bergapten, psoralen, alpinumisoflavone, betulinic acid, umbelliferone, apigenin, derrone, β-sistosterol and chlorogenic acid, amongst others, were common compounds reported in this genus (Nawaz, *et al*., 2019; Ateba *et al*., 2019). A recent study on the phytochemical screening of *F. sagittifolia* leaf and stem bark extracts revealed the presence of phenolics, flavonoids, steroids, tannins, saponins, alkaloids, and terpenoids with good antioxidant activities (Taiwo and Olaoluwa, 2020). However, there is little or no information on the isolation and characterization of phytochemicals constituents from *F. sagittifolia* to support its ethno-medicinal use.

Hence, this paper reports four known compounds from *F. sagittifolia* leaves and their antimicrobial activity for the first time.

## MATERIALS AND METHODS

### Plant Collection and Identification
Leaves of *F. sagittifolia* were harvested during the dry season in Ikire, Osun State, Nigeria in April 2018. Identification and authentication were done at the herbarium unit of the Forestry Research Institute of Nigeria (FRIN), Ibadan. A voucher specimen of *F. sagittifolia* (FHI 111988) was documented for reference purposes.

### Plant Preparation and Extraction
*F. sagittifolia* leaves were air-dried and subjected to pulverization. Ground leaves were macerated in ethanol for 72 h and was concentrated under reduced pressure to give ethanol extract. The ethanol extract was partitioned in hexane and subsequently in ethyl acetate to obtain the respective fractions.

### General Experimental Procedure
TLC was performed on precoated silica gel 60GF$_{24}$ by Merck. Column chromatography with a column length – 60 cm; internal diameter of 32.5 mm; external diameter of 36.5 mm and silica gel of 60 – 200 mesh size was used as the stationary phase. Eluates were concentrated at reduced pressure at 40°C using the Buchi Rotavapor R-200 rotary evaporator.

Compounds were purified using a Shimadzu Preparative HPLC equipped with a UV detector, a reversed phase column (Luna®, C18, 5 μm particle size, 10 × 2500 mm, Phenomenex), a Shimadzu LC – 20AP pump equipped with a DGU – 20A5R degassing unit, a Shimadzu SPD – M20A detector, and a Shimadzu SIL – 20ACHT autosampler, using LabSolutions software.

NMR experiments for $^1$H and $^{13}$C were performed on the Bruker Avance 600 and 700 MHz NMR spectrometers for $^1$H; 150 and 175 MHz NMR spectrometers for $^{13}$C. The readings were taken in deuterated methanol and deuterated dimethylsulphoxide with TMS serving as reference. $^1$H and $^{13}$C NMR spectra were elucidated using MestreNova software. HRESIMS were performed on a Bruker Daltonik maXis 4G equipped with ultra-high resolution time-of-flight electrospray ionization in both negative and positive ion modes. LRESIMS were carried out on Bruker Amazone Speed ETD ion trap and 8-Dionex Ultimate 3000 LC in both negative and positive ion modes. In the analysis, Thermo XcaliburQual computer software was used in analysis of the mass chromatogram. IR spectra were recorded on the Perkin- Elmer spectrometer instrument.

### Fractionation
The ethylacetate fraction (10 g) of *F. sagittifolia* was subjected to column chromatography and fractionated by the gradient elution method using a solvent mixture of hexane and ethyl acetate as the mobile phase. A total of 102 fractions (100 mL) were collected from the column, and fractions with similar TLC profiles were pooled together to obtain 16 fractions (FSL 1-16).

### Isolation and Purification of Compounds
Fraction 15 was eluted by Hex/EtOAc (10:90), as a white precipitate to give compound **1** (92.2 mg) which was soluble in DMSO and was confirmed as a spot on the TLC plate ($R_f$ = 0.58). Fractions 13 (Hex/EtOAc, 30:70), 14 (Hex/EtOAc, 20:80) and 16 (Hex/EtOAc, 0:100) were further purified using preparative HPLC analysis. The solvents used were double distilled water with 0.1% formic acid as solvent A and HPLC grade MeOH as solvent B. Gradient elution was carried out with 60% MeOH for 7 min and thereafter, isocratic condition at 100% MeOH for 5 min. The system returned within 0.5 min to the initial condition of 60% MeOH and was equilibrated for 10 min. The eluted fractions were detected by absorbance between 254 - 370 nm and the flow rate was 3 mL/min to obtain compounds **2** ($t_R$ = 15.2 min; 2.1 mg), **3** ($t_R$ = 18.3 min; 5.3 mg) and **4** ($t_R$ = 18.9 min; 4.4 mg). Subsequently, compounds **1** – **4** were subjected to spectroscopic analysis.

### Antimicrobial Assay
#### Microbial cultures
Gram negative bacteria*: Escherichia coli* (ATCC 11175), *Pseudomonas aeruginosa* (ATCC, 27853), *Salmonella typhi* (ATCC, 14028), *Helicobacter pylori*, and fungi: *Aspergillus niger* and *Candida albicans* were maintained on NA and SDA, respectively. A single colony of each organism was inoculated into 5 mL of TSB and SDB for the preparation of bacterial and fungal cultures, respectively. All the microbes were sub-cultured from the original culture and incubated overnight at 37°C for 24 h and at 25°C for 48 h for bacteria and fungi respectively. The bacteria except *H. pylori* were obtained from the Pharmaceutical Microbiology Department, University of Ibadan, Ibadan, Nigeria, and the fungi and *H. pylori* were obtained as clinical isolates from the University College Hospital (UCH), Ibadan. The standard drugs, gentamycin and ketoconazole, were used as the positive control. The negative control used was 1% DMSO.

### Minimum Inhibitory Concentration and Minimum Microbicidal Concentration
The antimicrobial assay was done using the broth micro-dilution method and 96-well plates were used. Compounds **1-4** were dissolved separately in DMSO to obtain a stock solution of 1.0 mg/mL each. This was diluted serially in the microplate wells to obtain a concentration range of 0.5-0.0039 mg/mL. TSB was used, and standard drugs were gentamycin and ketoconazole (10 μg/mL) for the anti-bacterial and anti-fungal assays, respectively. The reference drugs were also diluted to obtain concentration range of 10 – 0.3125

μg/mL. Each of the microplate wells was inoculated with 10 μL of the test organisms and incubated at 37°C and 25°C for 24 h and 48 h for bacteria and fungi, respectively. The least concentrations that showed no growth or turbidity after hours of incubation were streaked on NA and SDA for bacteria and fungi, respectively. The concentration with no trace of growth was taken as the MIC. Also, after incubation, 10 μL of 0.2 mg/mL *p* –INT was added to each well and incubated for another 30 min at 37°C. Wells with a color change from yellow to pinkish red were an indication of microbial growth. The least concentration that showed no trace of color change was taken as the MMC (bacteria/fungi).

## RESULTS AND DISCUSSION

### Phytochemical Investigation

The ethyl acetate fraction of *F. sagittifolia* leaves yielded a steroidal glycoside **1**, and fractions 13, 14, and 16 that were purified by preparative HPLC gave three isoflavonoids, **2** - **4** respectively.

Stigmast-5,22-diene-3-O-β-D-glucopyranoside (stigmasterol glucoside) **1** was isolated as a white powder. LRESIMS gave [M - H] $^-$ ion at m/z 573.75 with the molecular formula $C_{35}H_{58}O_6$ (calcd. for $C_{35}H_{58}O_6$, for 574.4233). In the $^1$HNMR spectrum of **1** (Table 1), the presence of six methyl protons was confirmed by signals at $\delta_H$ 0.67 *s* (H-18), 0.91 s (H-19), 0.96 *d* (H-21), 0.81 *m* (H-26), 0.85, 1.39 *m* (H-27) and 0.82 *d* (H-29); three olefinic protons at $\delta_H$ 5.38 (H-6, *t*, *J*=4.8 Hz), 5.13 (H-22, *dd*, *J*=9.0 Hz, 13.8 Hz) and 5.03 (H-23, *d*, *J*=8.4) and one anomeric proton at $\delta_H$ 4.18 (H-1', *d*, *J*=7.8 Hz). The $^{13}$CNMR spectrum showed six signals in the methyl region ($\delta_C$ 12.2, 12.3, 19.1, 19.4, 20.2 and 23.1). Four methine resonances at $\delta_C$ 73.9, 77.2, 70.6 and 77.1, as well as one methylene resonance at $\delta_C$ 61.6, were due to C-2', C-3', C-4', C-5', and C-6', respectively, of the β-D-glucopyranoside. (Table 1). The olefinic resonances at $\delta_C$ 121.7, 138.5 and 129.3 corresponded to the C-6, C-22, and C-23 methine carbons. An anomeric carbon signal at $\delta_C$ 101.2 indicated the presence of a sugar D-glucose moiety. The value of J = 7.8 on 1' (anomeric proton) reflected an axial-axial position to the C-2' proton, which confirms that the glucopyranoside moiety binds to the sterol moiety in a β-position (Silverstein *et al.*, 1962; Bai *et al.*, 2005). The connectivity of protons and carbon-protons was determined from a combination of COSY, HSQC, and HMBC data as shown in table **1**. The IR band (cm$^{-1}$) at 3589, 2850, and 1104 are typical for hydroxyl, carbon-hydrogen, and carbon-oxygen groups, respectively. This explanation was based on a comparison of $^1$H and $^{13}$CNMR spectra with those previously reported in literature (Valizadeh *et al.*, 2014; Ilango, 2018; Olawumi and Koma, 2019).

5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one (Prunetin) **2**, is pale yellow in color and

powdery, with a molecular formula of $C_{16}H_{12}O_5$, m/z of 285.0756 [M+H] $^+$ in HRESIMS (calcd. for $C_{16}H_{12}O_5$, for 284.0685). Sixteen signals were displayed from the $^{13}$CNMR spectrum of **2** (Table 2). Seven aromatic carbons were observed at $\delta_C$ 91.8 (C-8), 97.8 (C-6), 114.9 (C-3', C-5'), 130.0 (C-2', C-6') and 153.7 (C-2). Four oxygenated aromatic carbons at $\delta_C$ 157.5 (C-4'), 158.2 (C-9), 162.3 (C-5) and 165.9 (C-7). One carbonyl carbon at $\delta_C$ 181.1 (C-4). The $\delta_C$ 55.1 (OCH$_3$, C-7) and $\delta_H$ 3.91 (3H, s, H-7) as well as the HMBC correlations confirmed the presence of a methoxy group at position C-7. The $^1$H NMR also showed two doublets of four aromatic protons with vicinal coupling constants. The $\delta_C$ 153.7 (C-2) is a characteristic of ring B of an isoflavone (Table 2). The COSY correlation of H-6 with H-8 and H2'/3' with H5'/6' supported the positioning of these four aromatic protons. The HMBC correlations from the methoxy protons ($\delta_H$ 3.91) to C-7 supported the attachment of the methoxy group to C-7. The IR spectra indicated the presence of hydroxyl, carbonyl, and aromatic groups (3671, 1717, and 1454 cm$^{-1}$, respectively). This elucidation was in agreement with the literature (Máximoa *et al.*, 2002; Awouafack *et al.*, 2011).

5-hydroxy-3(4-hydroxylphenyl)-8,8-dimethylpyrano[2,3-f]-chromen-4(8H)-one (Derrone) **3**, is a pale yellow powder with a molecular formula of $C_{20}H_{16}O_5$, m/z of 337.1070 [M+H]$^+$ in HRESIMS (calcd. for $C_{20}H_{16}O_5$, 336.0998). The presence of an isoflavone skeleton was confirmed from the $^1$H NMR and $^{13}$C NMR spectra, with proton and carbon signals at $\delta_H$ 7.43 (H-2'), $\delta_C$ 153.4 (C-2), $\delta_C$ 123.6 (C-3) and $\delta_C$ 181.1 (C-4). The The $^1$H NMR showed two doublets of four aromatic protons with ortho-coupling constants and one proton signal at δ 6.23 *s* of aromatic proton in ring A. The 2,2-dimethylpyran substituent was confirmed by signals at $\delta_H$ 5.73 (H-3'') *d* (*J*$_{3''/4''}$ = 9.8 Hz), $\delta_H$ 6.78 (H-4'') *d* (*J*$_{3/4}$, 8.4 Hz), $\delta_H$ 1.50 (H-5''Me,6''Me), $\delta_C$ 127.4 (C-3''), $\delta_C$ 114.9 (C-4'') and $\delta_C$ 27.0 (C-5''/6'') whose location in ring A was established by HMBC correlation of a methine carbon $\delta_{C-6}$ 99.4 with a methine carbon $\delta_{C-5}$ 161.9 and a quaternary carbon $\delta_{C-9}$ 159.5 (Table 3). The IR bands at 3714cm$^{-1}$ and 1635cm$^{-1}$ are typical for hydroxyl and a α,-β unsaturated ketone group respectively. This elucidation was also in agreement with those reported in literature (Maximoa *et al.*, 2002; Ediziri *et al.*, 2012).

5-hydroxy-3-(4-hydroxyphemyl)-8,8-dimethylpyrano[3,2-g}-chromen-4(8H)-one (Alpinumisoflavone) **4,** is pale yellow and powdery, having a m/z of 337.08 [M+H]$^+$ in LRESIMS with a molecular formula of $C_{20}H_{16}O_5$ (calcd. For $C_{20}H_{16}O_5$, 336.0998). A pair of doublets $\delta_H$ 6.87 d (*J*=7.7 Hz) and 7.41 d (*J*=7.0 Hz) integrated for two protons and were allocated to the H-3'/H-5, H-2'/H-6' of the parasubstituted aromatic ring. The high chemical shift of $\delta_H$ 6.87 (*J*=7.7 Hz) signified the presence of an oxygened substituent (OH) at C-4'of the aromatic

nucleus. The ring C of the isoflavone was also confirmed with signals at $\delta_H$ 7.41 (H-2), $\delta_C$ 153.5 (C-2), $\delta_C$ 121.7 (C-3) and $\delta_C$ 181.0 (C-4). The evidence of proton signals at $\delta_H$ 5.75 (H-3'') $d$ ($J_{3''/4''}$ = 9.8 Hz), $\delta_H$ 6.71 (H-4'') $d$ ($J_{3/4}$, 10.5 Hz), $\delta_H$ 1.48 (5''Me/6''Me) and carbon signals at $\delta_C$ 128.2 (C-3''), $\delta_C$ 114.7 (C-4'') and $\delta_C$ 27.1 (5''/6'') confirmed the attachment of the 2,2-dimethylpyran substituent on ring A. Compounds **3** and **4** have the same molecular formula and mass but different structures due to the position of the 2,2-dimethylpyran substituent on ring A. The positon was further confirmed using HSQC and HMBC, which revealed different chemical shifts for $\delta_C$ (C-6) and $\delta_C$ (C-8) and different HMBC correlations as shown on table 4. The $\delta_C$ (C-6) is a quaternary carbon in 4, whereas in 3, $\delta_C$ (C-6) is a methine carbon and vice versa for $\delta_C$ (C-8). The IR spectra indicated the presence of hydroxyl, carbonyl, and aromatic groups (3571, 1717, and 1454 cm$^{-1}$). This spectral data was compared to those previously published for alpinumisoflavone (Rahman *et al.*, 2007; Hussain *et al.*, 2011 Tjahjadarie *et al.*, 2016).
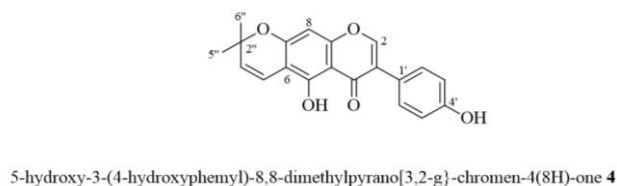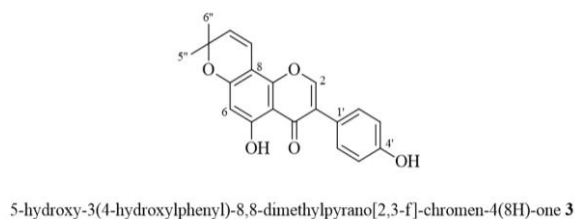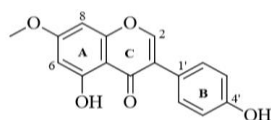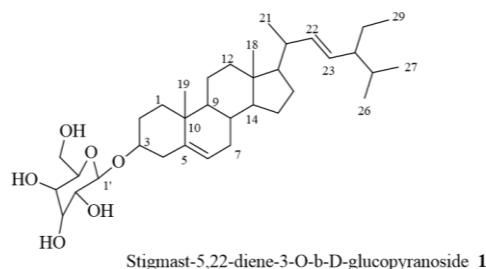


Stigmast-5,22-diene-3-O-b-D-glucopyranoside **1**



5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one **2**



5-hydroxy-3(4-hydroxylphenyl)-8,8-dimethylpyrano[2,3-f]-chromen-4(8H)-one **3**



5-hydroxy-3-(4-hydroxyphemyl)-8,8-dimethylpyrano[3,2-g]-chromen-4(8H)-one **4**

**Table 1.** $^1$H NMR (600 MHz) and $^{13}$C NMR (150 MHz), HMBC, COSY assignment of Compounds **1** in (CD$_3$)$_2$SO.

| Position | $\delta_{C, Type}$ | $\delta_H$ *mult.* (*J* in Hz) | HMBC (H/C) | COSY |
|---|---|---|---|---|
| 1 | 37.3, CH$_2$ | 0.99, *m*;1.78, *m* | C-14, 4 | |
| 2 | 29.7, CH$_2$ | 1.23, *m*; 1.81, *m* | | |
| 3 | 77.4, CH | 3.46, *m* | | |
| 4 | 42.2, CH$_2$ | 1.96, *m* | | |
| 5 | 140.9, C | | | |
| 6 | 121.7, CH | 5.38, *d* (*J*=4.8) | C-8, 10 | H-8 |
| 7 | 31.8, CH$_2$ | 1.39, *m* | | |
| 8 | 31.9, CH | 1.53, *s* | C-6,7,13,14 | H-6 |
| 9 | 50.1, CH | 0.88, *m* | | |
| 10 | 36.7, C | | | |
| 11 | 21.1, CH$_2$ | 0.97, *m* | | |
| 12 | 38.5, CH$_2$ | 2.11, *m* | | |
| 13 | 42.3, C | | | |
| 14 | 56.6, CH | 0.99, *m* | | |
| 15 | 39.6, CH$_2$ | 1.95, *m* | C-13 | |
| 16 | 24.3, CH$_2$ | 1.54, *m* | C-5,6,8,14 | |
| 17 | 55.9, CH | 1.10, *m* | | |
| 18 | 12.2, CH$_3$ | 0.67, *s* | C-4,14 | |
| 19 | 19.1, CH$_3$ | 0.91, *s* | C-17, 20 | |
| 20 | 36.0, CH | 1.30, *m* | | |
| 21 | 19.4, CH$_3$ | 0.96, *d* (*J*=7.2) | | H-29 |
| 22 | 138.5, CH | 5.13, *dd* (*J*=9.0, 13.8) | | |
| 23 | 129.3, CH | 5.03, *dd* (*J*=8.4, 15.0) | | |
| 24 | 45.6, CH$_2$ | 0.92, *m* | | |
| 25 | 29.1, CH | 1.63, *m* | | |
| 26 | 19.6, CH | 0.81, *m* | | |
| 27 | 20.2, CH$_3$ | 0.85, *m*; 1.39, *m* | | |
| 28 | 23.1, CH$_3$ | 0.76, *m* | | |
| 29 | 12.3, CH$_3$ | 0.82, *d* (*J*=7.2) | C-24, 25, 26 | H-21 |
| 1' | 101.2, CH$_2$ | 4.18, *d* (*J*=7.8) | C-3' | |
| 2' | 73.9, CH | 2.89, *m* | | |
| 3' | 77.2, CH | 3.12, *m* | | |
| 4' | 70.6, CH | 3.01, *m* | C-5',6' | |
| 5' | 77.1, CH | 3.06, *m* | | |
| 6' | 61.6, CH$_2$ | 3.39, *m*; 3.63, *m* | C-5' | |

**Table 2.** ¹H NMR (700 MHz) and ¹³C NMR (175 MHz) assignment of Compounds 2-4 in (CD₃OD).

| Position | 2 | | 3 | | 4 | |
|---|---|---|---|---|---|---|
| | $\delta_{C, Type}$ | $\delta_H$ mult. (*J* in Hz) | $\delta_{C, Type}$ | $\delta_H$ mult. (*J* in Hz) | $\delta_{C, Type}$ | $\delta_H$ mult. (*J* in Hz) |
| 2 | 153.7, CH | 8.15, *s* | 153.4, CH | 8.17, *s* | 153.5, CH | 8.10, *s* |
| 3 | 121.8, C | | 121.6, C | | 121.7, C | |
| 4 | 181.1, C | | 181.1, C | | 181.0, C | |
| 5 | 162.3, C | | 161.9, C | | 157.5, C | |
| 6 | 97.8, CH | 6.40, *d* (*J*=2.8) | 99.4, CH | 6.23, *s* | 105.2, C | |
| 7 | 165.9, C | | 152.2, C | | 156.3, C | |
| 8 | 91.8, CH | 6.58, *d* (*J*=2.1) | 105.6, C | | 94.4, CH | 6.38, *s* |
| 9 | 158.2, C | | 159.5, C | | 159.4, C | |
| 10 | 105.7, C | | 101.1, C | | 105.6, C | |
| 1' | 123.6, C | | 123.6, C | | 123.4, C | |
| 2', 6' | 130.0, CH | 7.42, *d* (*J*=8.4) | 130.0, CH | 7.43, *d* (*J*=11.9) | 130.0, CH | 7.41, *d* (*J*=7.0) |
| 3',5' | 114.9, CH | 6.88, *d* (*J*=9.1) | 114.9, CH | | | 114.9, CH | 6.87, *d* (*J*=7.7) |
| 4' | 157.5,C | | 157.5, C | | 157.4, C | |
| 2'' | | | 78.0, C | | 77.9, C | |
| 3'' | | | 127.4, CH | 5.73, *d* (*J*=9.8) | 128.2, CH | 5.75, *d* (*J*=9.8) |
| 4'' | | | 113.9, CH | 6.77, *d* (*J*=8.4) | 114.7, CH | 6.71, *d* (*J*=10.5) |
| 5'', 6'' | | | 2.70, CH₃ | 1.50, *s* | 27.2, CH₃ | 1.48, *s* |
| OCH₃ | 55.1, CH₃ | 3.91, *s* (*J*=2.8) | | | | |

## Antimicrobial Assay

The broth dilution method was used to determine the MIC and MMC of compounds **1-4** against different bacteria and fungi strains: *Escherichia coli* (ATCC 11175), *Pseudomonas aeruginosa* (ATCC, 27853), *Salmonella typhi* (ATCC, 14028), *Helicobacter pylori*, *Aspergillus niger* and *Candida albicans*. Compounds **1-4** showed good antimicrobial activity as demonstrated by their MIC values (0.0625-0.25 mg/mL), in table 3a. The lowest MIC value (0.0625 mg/mL) was obtained from compound **1** and **2** against *P. aeruginosa* and *A. niger*. At the same concentration, compounds **2** and **4** inhibited the growth of *H. pylori*, among others. Compounds **2**, **3**, and **4** showed moderate inhibition (MIC =0.125 mg/mL) against the growth of *E. coli* while for compound **1**, 0.25 mg/mL was required. Compounds **1**, **3** and **4** inhibited the growth of *C.*

*albicans* at a concentration of 0.125 mg/mL. Compound **2** had the lowest MIC and MMC values against *S. typhi* (0.125 mg/mL) and *H. pylori* (0.0625 mg/mL), respectively in table 3a. Gentamicin had an MIC value ranging from 10 to > 10 µg/mL while ketoconazole had an MIC values ranging from 1-0.5 µg/mL. Overall, compounds **2** and **4** showed very good antimicrobial activity against all the test organisms among others. Flavonoids and isoflavonoids are well-known natural products with extensive pharmacological activities and extremely low toxicity (Wang *et al*., 2020). More importantly, they possess a wide range of biological activities, such as antibacterial, antifungal, antiviral (Dastidar *et al*., 2004; Orhan *et al*., 2010), antitumour (Kopustinskiene *et al*., 2020), anti-inflammatory (Sychrová *et al*., 2020) and antiaging activities (Gupta *et al*., 2014).

**Table 3a.** MIC and MMC of compound **1-4**.

| Organisms | Compound 1 | | Compound 2 | | Compound 3 | | Compound 4 | |
|---|---|---|---|---|---|---|---|---|
| | MIC[a] | MMC[a] | MIC[a] | MMC[a] | MIC[a] | MMC[a] | MIC[a] | MMC[a] |
| *H. pylori* | 0.125 | 0.125 | 0.0625 | 0.0625 | 0.125 | 0.25 | 0.0625 | 0.0625 |
| *E. coli* | 0.25 | 0.25 | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 | 0.125 |
| *P. aeruginosa* | 0.0625 | 0.125 | 0.25 | 0.25 | 0.25 | 0.25 | 0.125 | 0.125 |
| *S. typhi* | 0.25 | 0.25 | 0.125 | 0.125 | 0.25 | 0.25 | 0.25 | 0.25 |
| *A. niger* | 0.25 | 0.25 | 0.0625 | 0.25 | 0.125 | 0.125 | 0.125 | 0.125 |
| *C. albicans* | 0.125 | 0.25 | 0.25 | 0.25 | 0.125 | 0.125 | 0.125 | 0.25 |

Note: [a]: mg/mL

**Table 3b.** MIC and MMC of bacteria and fungi standards.

| Organisms | Gentamycin | | Ketoconazole | |
|---|---|---|---|---|
| | MIC[a] | MMC[a] | MIC[a] | MMC[a] |
| *H. pylori* | 10 | >10 | NA | NA |
| *E. coli* | 10 | 10 | NA | NA |
| *P. aeruginosa* | >10 | >10 | NA | NA |
| *S. typhi* | 10 | 10 | NA | NA |
| *A. niger* | NA | NA | 1 | 1 |
| *C. albicans* | NA | NA | 0.5 | 0.5 |

Note: [a]: µg/mL

## CONCLUSIONS

A steroidal glucoside namely, stigmast-5, 22-diene-3-O-β-D-glucopyranoside (Stigmasterol glucoside) **1** and three isoflavanoids, namely 5-hydroxy-3-(4-hydroxyphenyl)-7-methoxy-4H-chromen-4-one (Prunetin) **2**, 5-hydroxy-3(4-hydroxylphenyl)-8,8-dimethylpyrano[2,3-f]-chromen-4(8H)-one (Derrone) **3**, and 5-hydroxy-3-(4-hydroxyphemyl)-8,8-dimethylpyrano[3,2-g}-chromen-4(8H)-one (Alpinumisoflavone) **4,** were isolated from the ethylacetate fraction of *F. sagittifolia* leaves for the first time. Compounds **1** and **2** showed inhibition against *P. aeruginosa* and *A. niger*; **2** and **4** inhibited the growth of *H. pylori*. Therefore, compounds **1**, **2**, and **4** could be antimicrobial agents for diseases related to stomach disorders. This study also provided a scientific justification for the ethnomedicinal use of *F. sagittifolia* leaves for the treatment of stomach disorders, particularly those caused by microbes.

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