

# Antimethicillin-resistant *Staphylococcus aureus* and Biological Activities of Metabolites from *Digitaria Sanguinalis* L.

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Ibrahim *et al.*: Biological Activities of *Digitaria Sanguinalis* L.

The present research was aimed to evaluate the antimicrobial, antimethicillin-resistant *Staphylococcus aureus*, antiviral and cytotoxic activities of active fractions of the alcohol extract and seven isolated compounds from the aerial parts of *Digitaria sanguinalis*. Bioassay-guided fractionation of the ethanol extract of the aerial parts of *Digitaria sanguinalis* L. (Poaceae) followed by several chromatographic purification steps resulted in the isolation of seven compounds. Structural elucidation of isolated compounds was achieved on the basis of spectroscopic analysis (ultraviolet, infrared, <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance and mass spectra). Antimicrobial, antimethicillin-resistant *Staphylococcus aureus*, antiviral and cytotoxic activities were determined for the n-hexane, ethyl acetate, n-butanol fractions of the ethanol extract, in addition to the isolated compounds. Seven compounds were isolated, p-coumaric acid (1) triclin (2), p-hydroxybenzoic acid (3), stigmasterol (4),  $\beta$ -sitosterol-3-O- $\beta$ -D-glucoside (5), triclin-7-O- $\beta$ -D-glucopyranoside (6) and isoorientin (7). The results revealed that most of the isolated compounds exhibited marked antimicrobial, antiviral and cytotoxic activities, while compounds 1, 2, 5 and 6 showed a significant activity against methicillin-resistant *Staphylococcus aureus*. This study revealed that several fractions and isolated compounds from *Digitaria sanguinalis* appear to be potential new therapeutic agents, due to their significant antimicrobial, antimethicillin-resistant *Staphylococcus aureus*, antiviral and cytotoxic activities.

**Key words:** Cytotoxic, *Digitaria sanguinalis*, MRSA, p-coumaric acid, triclin

Poaceae (Gramineae) is one of the largest vascular plant families, containing between 650 and 785 genera and 10 000 species. Poaceae is also one of the most ecologically and economically important plant families<sup>[1]</sup>. Some Poaceae plants are used in folk medicine as antihypertensive, antidiabetic, antiinflammatory, anthelmintic, astringent, antiulcer, diuretic and antioxidant agents<sup>[2]</sup>. This family is of great economic and medicinal importance as it includes all cereals, bamboos and sugar cane. Steroids, terpenoids, volatile oils, saponins, flavonoids, phenolic acids, fatty acids, hydroxamic acids and alkaloids are the main classes of phytoconstituents in Poaceae<sup>[3]</sup>. *Digitaria* belongs to Poaceae family and is found in Egypt as three species under the local name *Dafraa*<sup>[4]</sup>.

One of the main causes of hospital-related mortality and morbidity is pathogenic bacterial infection<sup>[5]</sup>. The pathogen *Staphylococcus aureus* often colonizes the skin

surface, the upper respiratory tract, and the nasal tract<sup>[6]</sup>. *S. aureus* accounts for high mortality rates in patients with pneumonia, endocarditis, sepsis and urinary tract infections<sup>[7]</sup>. Methicillin has been used to treat *S. aureus* infections since the 1950s. Tragically, *S. aureus* acquired resistance towards methicillin after several years<sup>[8]</sup>. Methicillin is a  $\beta$ -lactam antimicrobial that alters the penicillin-binding proteins required for blending of the peptidoglycans of *S. aureus*<sup>[9]</sup>. The occurrence of the MecA gene that encodes for PBP2a were utilized as benchmarks to identify the proximity of methicillin resistant *S. aureus* (MRSA)<sup>[10]</sup>. The

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development of MRSA infections is serious, as MRSA renders medicines ineffective and contributes to increased morbidity, mortality and healthcare costs<sup>[11]</sup>. Moreover, MRSA has high resistance rates against tetracycline, clindamycin, cotrimoxazole, rifampicin, macrolides and fluoroquinolones<sup>[12]</sup>. Several studies have identified alternative medications to respond MRSA, particularly by using natural products. Plants offer an assorted supply of biologically active components as potential healing compounds, including antimicrobials.

*Digitaria sanguinalis* L., known as hairy crabgrass, is a perennial, densely tufted grass with rhizomiferous main stems that is native to tropical Asia, the Middle East and parts of Africa. *D. sanguinalis* has been introduced to many other parts of the world for cultivation and has become naturalized in other locations, including Australia<sup>[13]</sup>. Some *Digitaria* species were proven to possess antimicrobial and antioxidant activities<sup>[14]</sup>. The methanol leaf extract of *Digitaria sanguinalis* was proven to have moderate antiMRSA activity<sup>[15]</sup>. Veratric acid, maltol, and (–)-loliolide were isolated from *Digitaria sanguinalis* roots and showed changes in soil microbial community structures<sup>[16]</sup>. It is worth noting that there are no reports available regarding the phytochemical and biological properties of the aerial parts of *Digitaria sanguinalis* L. Therefore, it is important to examine the phytochemical and biological properties of different extracts and isolated compounds of the aerial parts of *Digitaria sanguinalis*.

## MATERIALS AND METHODS

The aerial parts of *Digitaria sanguinalis* L. were collected from the garden of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt in June 2013. The plant material was authenticated in the Botany Department, Faculty of Science, Al-Azhar University, Cairo, Egypt. The aerial parts of the plant were air dried, powdered and preserved at room temperature for chemical and biological investigation. A specimen of the aerial parts of *Digitaria sanguinalis* was deposited in the Pharmacognosy Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt.

### General experimental procedures:

Melting points (mp, uncorrected) were determined using the electrothermal capillary melting point apparatus. UV spectra were determined on a Pye-Unicam spp. 1750 spectrophotometer (Cambridge, UK). IR spectra were obtained using KBr on a Philips PU-9712

infrared spectrophotometer and a Shimadzu IR-435. Electrospray ionization mass spectrometry (ESIMS) was carried out on a Xevo TQD triple quadrupole (Waters Corporation, Milford, MA 01757, USA) mass spectrometer. EIMS was carried on a Scan EIMS-TIC (158VG-ZAB-HF, X-.64, 800.00) mass spectrometer (VG Analytical, Inc.). The <sup>1</sup>H and <sup>13</sup>C nuclear magnetic resonance (NMR) measurements were obtained with a Bruker Avance III (400) NMR spectrometer operating at 400 MHz (for <sup>1</sup>H) and 100 MHz (for <sup>13</sup>C) in CD<sub>3</sub>OD or dimethyl sulfoxide (DMSO)-*d*<sub>6</sub> solution; chemical shifts were expressed in δ (ppm) with reference to tri methyl silane (TMS) and with coupling constant (*J*) in Hertz. Silica gel (Si gel 60, Merck, Germany) and Sephadex LH-20 (Pharmacia, Biotech, Sweden) were used for column chromatography. Solid phase extraction (SPE) was carried out on SPE-C18 cartridges (Strata column, Phenomenex, USA). Thin-layer chromatography (TLC) was carried out on pre-coated silica gel 60 F254 (Merck, Germany) plates. Chromatograms were visualized under UV light (254 nm) after spraying with ammonia, aluminium chloride solutions, or 1% vanillin/H<sub>2</sub>SO<sub>4</sub>, followed by heating at 100° for 5 min. Shift reagents<sup>[17]</sup>, sodium metal, boric acid, hydrochloric acid, sodium acetate reagents and other chemicals used were of analytical grade.

### Extraction and isolation from plant material:

Air-dried, powdered aerial parts of *Digitaria sanguinalis* (2 kg) was subjected to exhaustive extraction at room temperature with 70 % ethanol (8 l×5, 24 h each). The combined ethanol extract was concentrated under vacuum at 40° to dry. The concentrated ethanol extract (140 g) was then suspended in distilled water (500 ml) and partitioned successively with *n*-hexane (7×500 ml, 30 min each), ethyl acetate (5×500 ml, 30 min each) and *n*-butanol (7×500 ml, 30 min each) to give 18.8 g, 13.2 g and 16.5 g, respectively. Aliquots of *n*-hexane fraction (12 g) were subjected to a silica gel column (5 cm, 75 cm, 500 g) using gradient elution with *n*-hexane-ethyl acetate 100:0 v/v (2 l), 90:10 v/v (3 l), 80:20 v/v (2 l), 70:30 v/v (3 l), 60:40 v/v (2 l) and 50:50 v/v (2 l) with a flow rate of 3 ml/min; fractions of 100 ml were collected and monitored by TLC. Six fractions were obtained: A (510 mg), B (730 mg), C (450 mg), D (850 mg), E (933 mg) and F (810 mg). Fraction B was re-chromatographed on a silica gel column (50 cm, 1 cm, 50 g) eluted with *n*-hexane:ethyl acetate (90:10) v/v to give compound 1 (45 mg). Fraction D was chromatographed on a Sephadex

LH-20 column (50 cm, 1 cm, 30 g) and eluted with  $\text{CH}_2\text{Cl}_2$ :MeOH (50:50 v/v, 500 ml) to give compound 2 (20 mg) and subfraction D-1 (90 mg). Subfraction D-1 was further subjected to SPE (RP-C18) using 50:50; 60:40 MeOH:water v/v (500 ml, each) to obtain compound 3 (25 mg). Fraction E was subjected to a Sephadex LH-20 column (50 cm, 1 cm, 30 g) and eluted with MeOH (500 ml) to afford compound 4 (50 mg). From ethyl acetate fraction (8 g), compounds 5 (8 mg) and 6 (30 mg) were isolated using SPE-C18 cartridges eluted with methanol and increasing polarity with distilled water. The *n*-butanol fraction (10 g) was subjected to VLC (75 cm, 5 cm, 350 g) eluted with  $\text{CH}_2\text{Cl}_2$ : MeOH 95:5 to 30:70 v/v (500 ml, each) to give five fractions: G (1.5 g), H (2 g), I (1.7 g), J (3 g) and K (2.2 g). Fraction G was rechromatographed on a silica gel column eluted with  $\text{CH}_2\text{Cl}_2$ : MeOH (95:5 v/v; 300 ml) to give three subfractions, G-1 (300 mg), G-2 (250 mg) and G-3 (430 mg). Subfraction G-2 was purified on a Sephadex LH-20 column (50 cm, 1 cm, 30 g) and eluted with MeOH (200 ml) to obtain compound 7 (20 mg).

#### Acid hydrolysis of compound 6:

Five milligrams of compound 6 was refluxed with 2 M HCl in MeOH (5 ml) for 6 h at 80°. The reaction mixture was evaporated and after dilution with  $\text{H}_2\text{O}$  (10 ml), the hydrolysate was extracted with  $\text{CHCl}_3$  (3 × 20 ml, 10 min). The  $\text{CHCl}_3$  extract was then evaporated to afford the aglycone. The aqueous layer was neutralized with 2 N KOH solution and concentrated to 1 ml under reduced pressure (50°), which was then compared with standard sugars by TLC in a solvent system consisting of  $\text{CHCl}_3$ :MeOH: $\text{H}_2\text{O}$  (30:12:4 v/v/v).

#### Antimicrobial assay:

Antimicrobial activity of the *n*-hexane, ethyl acetate and *n*-butanol fractions of *Digitaria sanguinalis* and the isolated compounds was investigated *in vitro* using the diffusion agar technique<sup>[18]</sup>. The following bacterial and fungal strains were used: Gram-positive bacteria, *Staphylococcus aureus* (RCMB 010028) and *Bacillus subtilis* (RCMB 010067), Gram-negative bacteria, *Escherichia coli* (RCMB 010052) and *Pseudomonas aeruginosa* (RCMB 010043); fungal strains, *Aspergillus fumigates* (RCMB 02568) and *Candida albicans* (RCMB 05031). Ampicillin, gentamycin and amphotericin B were used as standard reference antimicrobial drugs. The microbial species are environmental and clinically pathogenic

microorganisms obtained from Regional Center for Mycology and Biotechnology antimicrobial unit (RCMB) at Al-Azhar University, Cairo, Egypt. The tested samples were prepared in a concentration 1 mg/ml for fractions and 250 µg/ml for isolated compounds dissolved in DMSO. The results are presented in Table 1.

#### Determination of MRSA isolates and antibiotic susceptibility:

For MRSA differentiation from other *S. aureus* isolates, Muller-Hinton agar medium (Oxoid Oxoid Ltd., UK) was used. The strains in a liquid medium of 0.5 McFarland standard concentrations were grown in Muller-Hinton agar medium, and antibiotic discs with ampicillin (10 µg), erythromycin (15 µg), penicillin (10 U), ciprofloxacin (5 µg), clindamycin (2 µg), vancomycin (30 µg), methicillin (5 µg) and gentamycin (10 µg) (Mast Group Ltd, UK) were then placed on the medium and incubated for 24 h at 37°<sup>[19]</sup>. The diameter of the clear zone around the discs was measured following Clinical Laboratory Standard Institute<sup>[20]</sup> standards. The results are presented in Table 1.

#### Antiviral assay:

Screening of antiviral activity of *n*-hexane, ethyl acetate and *n*-butanol fractions of alcohol extract was conducted with the aerial parts of *Digitaria sanguinalis*, and the isolated compounds were then evaluated using cytopathic effect inhibition assay<sup>[21]</sup>. The assay was performed using hepatitis A virus (HAV), oral herpes simplex virus (HSV-1) and genital herpes simplex virus (HSV-2). The results are presented in Table 2.

#### Cytotoxicity assay:

The cytotoxicity of *n*-hexane, ethyl acetate and *n*-butanol fractions from the alcohol extract of the aerial parts of *Digitaria sanguinalis* as well as the isolated compounds were tested against three human tumour cell lines, hepatocellular carcinoma cells (HepG-2), colon carcinoma cells (HCT-116) and breast carcinoma cells (MCF-7), in addition to normal fibroblast cells (L929). The cells were obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were grown on Roswell Park Memorial Institute 1640 medium (Nissui Pharm. Co., Ltd., Tokyo, Japan) and supplemented with 10 % inactivated foetal calf serum with 50 µg/ml gentamycin. The cells were maintained at 37° in a humidified atmosphere with 5 %  $\text{CO}_2$  and were sub cultured 2 to 3 times a week. The cytotoxic activity was determined using cell viability

assay method<sup>[22]</sup>. The experiments were performed in triplicate and percent cell viability was calculated as the mean absorbance of control cells/mean absorbance of treated cells. The concentration-response curves were prepared, and the IC<sub>50</sub> values were calculated and presented in Table 3.

### Statistical analysis:

All experiments were replicated three times. The results are presented as the means±standard deviation. The statistical comparisons of data were performed using an ANOVA and t-test using SPSS 18.0 (IBM Corporation, US). Differences were considered significant at the  $p < 0.001$  level.

## RESULTS AND DISCUSSION

Structure elucidation data of isolated compounds is as follows, *p*-coumaric acid (1) colourless needles, UV  $\lambda_{\max}$  (MeOH) nm: 207, 340; IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3420 (OH), 3100-2700 (OH), 1675 (CO), 1620, 1600, 1520 (C=C); <sup>1</sup>H NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$  7.50 (1H, *d*,  $J=16.0$  Hz, H- $\beta$ ), 7.36 (2H, *d*,  $J=8.4$  Hz, H-2, H-6), 6.73 (2H, *d*,  $J=8.4$  Hz, H-3, H-5), 6.25 (1H, *d*,  $J=15.8$  Hz, H- $\alpha$ ); <sup>13</sup>C NMR ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$  167.66 (CO), 159.90 (C-4), 145.59 (C- $\beta$ ), 129.88 (C-2, 6), 125.73 (C-1), 115.47 (C-3, 5), 113.43 (C- $\alpha$ ); EIMS  $m/z$  164 [M]<sup>+</sup>, 242 (100 %), 178 (43 %), 120 (12.2 %), 40 (19 %).

Tricin (2)- yellow needles, UV  $\lambda_{\max}$  (MeOH) nm: 245, 269, 349, (NaOMe) nm: 264, 275 sh, 330, 416, ( $\text{AlCl}_3$ ) nm: 258 sh, 278, 305, 372 sh, 396 ( $\text{AlCl}_3/\text{HCl}$ ) nm: 259 sh, 279, 305, 364, 388, (NaOAc) nm: 264, 275 sh, 322, 362, (NaOAc/boric acid) nm: 260, 304 sh, 343; IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3340 (OH), 1655 (CO), 1614, 1505 (C=C aromatic); <sup>1</sup>H NMR ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$ : 7.38 (2H, *s*, H-2', 6'), 6.73 (1H, *s*, H-3), 6.54-6.56 (1H, *d*,  $J=1.6$  Hz, H-8), 6.19-6.21 (1H, *d*,  $J=1.7$  Hz, H-6), 3.88 (6H, *s*, 3', 5'-OMe), 12.94 (1H, *s*, 5-OH); <sup>13</sup>C NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$  182.26 (C-4), 164.19 (C-2), 164.58 (C-7), 161.79 (C-5), 157.81 (C-9), 148.62 (C-3', 5'), 140.24 (C-4'), 120.86 (C-1'), 104.71 (C-2', 6'), 104.00 (C-3), 104.16 (C-10), 99.33 (C-6), 94.71 (C-8), 56.80 (11, 12-OMe); EIMS  $m/z$  330 [M]<sup>+</sup>, 331 [M+H]<sup>+</sup>.

*p*-Hydroxy benzoic acid (3)- white solid, IR (KBr)  $\nu_{\max}$  3450, 3000, 2850, 2650, 2550, 1660, 1590, 1510, 1450; <sup>1</sup>H NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.84 (1H, *d*,  $J=8.7$ , H2-H8), 6.78 (1H, *d*,  $J=8.7$ , H3-H5); <sup>13</sup>C NMR (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  170.14 (-COOH), 127.7 (C-1),

116.8 (C-3, 5), 163.32 (C-4), 130.95 (C-2, 6); ESIMS  $m/z$  137 [M-H]<sup>-</sup>.

Stigmasterol (4)- white crystalline needles, IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3380 (OH), 2942 and 2870 (aliphatic CH), 1643 (C=C); <sup>1</sup>H NMR ( $\text{CDCl}_3$ , 400 MHz)  $\delta$  5.28 (1H, *d*,  $J=4.6$  Hz, H-6), 5.14 (1H, *dd*,  $J=15.1, 8.6$  Hz, H-22), 4.95 (1H, *dd*,  $J=15.1, 8.6$  Hz, H-23), 3.48 (1H, *m*, H-3), 0.95 (3H, *d*,  $J=7.4$  Hz, Me-21), 0.91 (3H, *s*, Me-19), 0.80 (3H, *t*,  $J=6.4$  Hz, Me-29), 0.77 (3H, *d*,  $J=7.4$  Hz, Me-26), 0.75 (3H, *d*,  $J=7.4$  Hz, Me-27), 0.60 (3H, *s*, Me-18); <sup>13</sup>C NMR ( $\text{CDCl}_3$ , 100.0 MHz)  $\delta$  140.53 (C-5), 138.52 (C-20), 129.58 (C-21), 121.72 (C-6), 71.94 (C-3), 56.78 (C-14), 56.22 (C-17), 50.25 (C-9), 46.13 (C-22), 42.35 (C-4), 42.31 (C-13), 40.62 (C-18), 39.83 (C-12), 37.56 (C-1), 36.53 (C-10), 32.95 (C-2), 31.83 (C-7), 31.81 (C-8), 30.62 (C-25), 28.95 (C-16), 25.49 (C-23), 24.43 (C-15), 21.70 (C-19), 21.39 (C-11), 20.22 (C-26), 19.82 (C-27), 19.33 (C-19), 12.25 (C-29), 11.96 (C-24); EIMS  $m/z$  412 [M]<sup>+</sup>.

$\beta$ -Sitosterol 3-*O*- $\beta$ -D-glucoside (5)- white amorphous powder, IR (KBr)  $\nu_{\max}$  3432, 1634  $\text{cm}^{-1}$ ; <sup>1</sup>H NMR ( $\text{DMSO}-d_6$ , 400 MHz)  $\delta$  0.68 (*s*, Me-18), 0.86 (*d*,  $J=6.5$  Hz, Me-27), 0.89 (*t*,  $J=7.0$  Hz, Me-29), 0.92 (*d*,  $J=6.5$  Hz, Me-26), 0.94 (*d*,  $J=6.5$  Hz, Me-21), 0.97 (*s*, Me-19), 3.12-3.39 (*m*, H-2'-H-5'), 3.43 (*m*, H-6'b), 3.46 (*m*, H-3), 3.62 (*dd*,  $J=10.7, 5.8$  Hz, H-6'a), 4.25 (*m*, H-1'), 5.34 (*m*, H-6); <sup>13</sup>C NMR ( $\text{DMSO}-d_6$ , 100 MHz)  $\delta$  aglycon: 141.22 (C-5), 120.23 (C-6), 75.65 (C-3), 56.37 (C-14), 55.47 (C-17), 49.69 (C-9), 45.24 (C-24), 41.67 (C-13), 39.37 (C-4), 37.26 (C-12), 36.73 (C-1), 36.04 (C-10), 35.25 (C-20), 33.09 (C-22), 31.28 (C-7), 31.06 (C-8), 29.21 (C-2), 28.31 (C-25), 27.44 (C-16), 25.38 (C-23), 23.62 (C-15), 22.46 (C-28), 20.13 (C-11), 19.74 (C-26), 19.03 (C-19), 18.72 (C-27), 18.45 (C-21), 11.22 (C-29) and 11.05 (C-18) ppm. Sugar: 100.87 (C-1'), 76.46 (C-3'), 76.33 (C-5'), 73.22 (C-2'), 70.17 (C-4'), 62.43 (C-6') ppm. ESI-MS  $m/z$  577 [M+H]<sup>+</sup>

Tricin-7-*O*- $\beta$ -D-glucopyranoside (6)- yellow amorphous powder, UV  $\lambda_{\max}$  (MeOH) nm: 254, 270, 344, (NaOMe) nm: 261, 300 sh, 430, ( $\text{AlCl}_3$ ) nm: 270, 297 sh, 344, 385, ( $\text{AlCl}_3/\text{HCl}$ ) nm: 272, 297 sh, 335, 344, (NaOAc) nm: 255, 266 sh, 350, 410, (NaOAc/boric acid) nm: 254, 338; IR  $\nu_{\max}$  (KBr)  $\text{cm}^{-1}$ : 3420 (OH), 1653 (CO), 1618, 1499 (C=C aromatic); <sup>1</sup>H NMR ( $\text{DMSO}-d_6$ , 400 MHz) aglycon  $\delta$  12.95 (1H, *s*, 5-OH), 7.38 (2H, *s*, H-2', 6'), 6.93-6.98 (1H, *s*, H-3), 6.54- 6.50 (1H, *brs*, H-8), 6.48 (1H, *brs*, H-6), 3.89 (6H, *s*, 3', 5'-OMe), sugar  $\delta$  5.26 (1H, *d*,  $J=7.1$  Hz,

H-1''), 3.72 (1H, m, H-6''a), 3.42 (1H, m, H-6''b), 3.32 (1H, t,  $J=9.6$  Hz, H-3''), 3.29 (1H, m, H-5''), 3.27 (1H, t,  $J=8.6$  Hz, H-2''), 3.15 (1H, d,  $J=8.7$  Hz, H-2'');  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz) aglycon  $\delta$  182.49 (C-4), 164.65 (C-2), 163.46 (C-7), 161.55 (C-5), 157.35 (C-9), 148.75 (C-3', 5'), 141.50 (C-4'), 121.0 (C-1'), 105.83 (C-10), 105.00 (C-2', 6'), 104.10 (C-3), 99.97 (C-6), 95.75 (C-8), 56.83 133(3', 5'-OMe), sugar  $\delta$  98.5 (C-1''), 77.82 (C-5''), 76.95 (C-3''), 73.62 (C-2''), 70.12 (C-4''), 61.11 (C-6''); ESIMS (positive mode)  $m/z$  493 [M+H]<sup>+</sup>, [M+Na]<sup>+</sup>, 331 [aglycon+H]<sup>+</sup>, 353 [aglycon+Na]<sup>+</sup>; ESIMS (negative mode)  $m/z$  491 [M-H]<sup>-</sup>, 329 [aglycon-H]<sup>-</sup>. TLC analysis of the aqueous portion of the hydrolysate of compound (6) revealed the presence of glucose as sugar moiety.

Isoorientin (7)- yellow amorphous powder, UV  $\lambda_{\text{max}}$  (MeOH) nm: 240 sh, 255, 270, 348, (NaOMe) nm: 265, 275, 338, 402, (AlCl<sub>3</sub>) nm: 277, 298 sh, 331, 425, (AlCl<sub>3</sub>/HCl) nm: 264 sh, 272, 290, 362, 384, (NaOAc) nm: 275, 322, 393, (NaOAc/boric acid) nm: 265, 373, 432; IR  $\nu_{\text{max}}$  (KBr) cm<sup>-1</sup>: 3400 (OH), 1658 (CO), 1585, 1510 (C=C aromatic);  $^1\text{H}$  NMR (DMSO- $d_6$ , 400 MHz) aglycon  $\delta$  13.54 (1H, brs, 5-OH), 7.42 (1H, brs, H-2'), 7.40 (1H, brs, H-6'), 6.91 (1H, d,  $J=8.5$  Hz, H-5'), 6.65 (1H, s, H-3), 6.58 (1H, brs, H-8), sugar  $\delta$  4.55 (1H, d,  $J=9.5$  Hz, H-1''), 4.07 (1H, t,  $J=9.0$  Hz, H-1''), 3.70 (1H, d,  $J=11.4$  Hz, H-6''a), 3.45 (1H, m, H-6''b), 3.25 (1H, t,  $J=8.9$  Hz, H-3''), 3.22 (1H, t,  $J=8.5$  Hz, H-4''),

3.14 (1H, m, H-5''),  $^{13}\text{C}$  NMR (DMSO- $d_6$ , 100 MHz) aglycon  $\delta$  182.45 (C-4), 163.2 (C-2, C-7), 161.13 (C-5), 156.63 (C-9), 152.425 (C-4'), 143.28 (C-3'), 122.35 (C-1'), 120.35 (C-6'), 116.33 (C-5'), 114.62 (C-2'), 104.73 (C-10), 103.56 (C-3), 102.89 (C-6), 94.13 (C-8), sugar  $\delta$  82.22 (C-5''), 80.32 (C-3''), 73.46 (C-1''), 71.66 (C-2''), 70.44 (C-4''), 60.67 (C-6''); ESIMS (positive mode)  $m/z$  471 [M+Na]<sup>+</sup>; ESIMS (negative mode)  $m/z$  447 [M-H]<sup>-</sup>, 285 [aglycon-H]<sup>-</sup>.

All the tested fractions showed antimicrobial activity against all the tested microorganisms and exhibited no MRSA activity (Table 1). Ethyl acetate fraction showed the highest activity against Gram-positive, Gram-negative and fungi, followed by *n*-hexane then *n*-butanol fractions. *p*-Coumaric acid, triclin,  $\beta$ -sitosterol-3-*O*-glucoside and triclin-7-*O*-glucopyranoside have shown to possess antiMRSA activity. *p*-Coumaric acid exhibited the highest activity followed by triclin-7-*O*-glucopyranoside then  $\beta$ -sitosterol-3-*O*-glucoside and triclin.

Isoorientin produced no antimicrobial activity against all the tested microorganisms. *p*-Coumaric acid showed the highest activity against Gram-positive bacteria, followed by triclin-7-*O*-glucopyranoside then *p*-hydroxybenzoic acid and  $\beta$ -sitosterol-3-*O*-glucoside.

Triclin exhibited no activity against *B. subtilis*, but showed significant activity against *S. aureus*

**TABLE 1: ANTIMICROBIAL AND ANTIMRSA ACTIVITY OF DIFFERENT FRACTIONS AND ISOLATED COMPOUNDS FROM DIGITARIA SANGUINALIS**

Organism	Diameter of inhibition zone $\pm$ SD (mm)										Standard
	Fractions			Isolated compounds							
	<i>n</i> -hexane	Ethyl acetate	<i>n</i> -butanol	1	2	3	4	5	6	7	
<b>Gram-positive</b>											Ampicillin
<i>S. aureus</i>	15.4 $\pm$ 0.25*	16.9 $\pm$ 0.58*	11.8 $\pm$ 0.27*	28.3 $\pm$ 0.62*	19.5 $\pm$ 0.41*	16.2 $\pm$ 0.32*	13.5 $\pm$ 0.65*	18.3 $\pm$ 0.63*	26.6 $\pm$ 0.47*	NA	28.9 $\pm$ 0.14
<i>B. subtilis</i>	17.9 $\pm$ 0.37	20.9 $\pm$ 0.25	12.66 $\pm$ 0.64	22.6 $\pm$ 0.35	NA	17.4 $\pm$ 0.42	14.2 $\pm$ 0.27	16.7 $\pm$ 0.28	22.5 $\pm$ 0.38	NA	28.3 $\pm$ 0.37
<b>Gram-negative</b>											Gentamycin
<i>P. aeruginosa</i>	12.3 $\pm$ 0.38*	15.2 $\pm$ 0.58*	11.1 $\pm$ 0.37*	30.6 $\pm$ 0.36*	NA	15.6 $\pm$ 0.64*	12.4 $\pm$ 0.48*	17.2 $\pm$ 0.35*	16.4 $\pm$ 0.54*	NA	20.3 $\pm$ 0.37
<i>E. coli</i>	13.7 $\pm$ 0.26*	17.1 $\pm$ 0.25*	12.6 $\pm$ 0.63*	32.7 $\pm$ 0.28*	15.6 $\pm$ 0.57*	13.3 $\pm$ 0.37*	13.1 $\pm$ 0.26*	16.2 $\pm$ 0.74*	18.5 $\pm$ 0.56*	NA	21.4 $\pm$ 0.25
<b>Fungi</b>											Amphotericin-B
<i>A. fumigates</i>	14.7 $\pm$ 0.54*	18.2 $\pm$ 0.44*	12.6 $\pm$ 0.36*	18.6 $\pm$ 0.73*	NA	18.6 $\pm$ 0.47*	12.6 $\pm$ 0.28*	17.4 $\pm$ 0.43*	12.3 $\pm$ 0.46*	NA	22.9 $\pm$ 0.44
<i>C. albicans</i>	13.8 $\pm$ 0.40*	20.9 $\pm$ 0.25*	12.3 $\pm$ 0.24*	17.5 $\pm$ 0.36*	NA	16.4 $\pm$ 0.31*	11.4 $\pm$ 0.44*	15.4 $\pm$ 0.48*	13.8 $\pm$ 0.27*	NA	21.4 $\pm$ 0.25
<b><i>S. aureus</i> (MRSA)</b>											
<i>S. aureus</i> (MRSA)	NA	NA	NA	34.4 $\pm$ 0.58*	17.2 $\pm$ 0.53*	NA	NA	20.3 $\pm$ 0.45*	25.5 $\pm$ 0.49*	NA	

The testing was done using agar diffusion method. \*Significant at  $p < 0.001$  NA: no activity

and *E. coli*. *p*-Coumaric acid showed highest activity against Gram-negative microorganisms, followed by  $\beta$ -sitosterol-3-*O*-glucoside then triclin-7-*O*-glucopyranoside, *p*-hydroxybenzoic acid and stigmasterol. *p*-Coumaric acid exhibited highest antifungal activity followed by *p*-hydroxybenzoic acid, followed by  $\beta$ -sitosterol-3-*O*-glucoside, stigmasterol and triclin-7-*O*-glucopyranoside.

All the tested fractions showed antiviral activity against HAV, and *n*-hexane showed the highest activity followed by ethyl acetate, and *n*-butanol. *n*-Hexane and ethyl acetate showed antiviral activity against HSV-1, while *n*-butanol was inactive. Only *n*-hexane showed antiviral activity against HSV-2 (Table 2). *p*-Coumaric acid and stigmasterol showed no antiviral activity against all the tested viruses, while triclin showed antiviral activity against all the tested viruses. Triclin and triclin-7-*O*-glucopyranoside showed high antiviral activity against HAV and HSV-1, and isoorientin was proven to have antiviral activity against HAV; *p*-hydroxybenzoic acid

and  $\beta$ -sitosterol 3-*O*-glucoside showed activity against HSV-1. Only triclin and *p*-hydroxybenzoic acid have exhibited antiviral activity against HSV-2.

*n*-Hexane and ethyl acetate fractions exhibited significant cytotoxic activity against HepG-2 and MCF-7 cell lines but had no significant effect against the HCT-116 cell line (Table 3). The ethyl acetate fraction produced a higher cytotoxic effect (IC<sub>50</sub>- 10.2 and 1.42  $\mu$ g against HepG-2 and MCF-7, respectively) than the *n*-hexane fraction (IC<sub>50</sub>- 11.8 and 5.85  $\mu$ g against HepG-2 and MCF-7, respectively).

Triclin-7-*O*- $\beta$ -D-glucopyranoside showed the highest cytotoxic activity against HepG-2 (IC<sub>50</sub>- 9.7  $\mu$ g), followed by *p*-hydroxy benzoic acid,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucoside, triclin and *p*-coumaric acid (IC<sub>50</sub>- 12.3, 13.2, 13.5 and 15.6  $\mu$ g, respectively). Compound  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucoside exhibited the highest cytotoxic activity against MCF-7 (IC<sub>50</sub>- 3.8  $\mu$ g), followed by compounds triclin-7-*O*- $\beta$ -D-glucopyranoside, *p*-coumaric acid, *p*-hydroxy benzoic acid and triclin (IC<sub>50</sub>- 4.8, 7.3, 8.6 and 14.6  $\mu$ g, respectively). Stigmasterol and isoorientin showed no significant activity against any of the tested cell lines, and none of the tested fractions and isolated compounds exhibited significant cytotoxic activity against the HCT-116 cell line. None of the tested extracts and isolated compounds showed cytotoxic effect against normal fibroblast cells (L929), indicating their safety towards normal cells.

Seven compounds (1-7) were isolated for the first time from the *n*-hexane, ethyl acetate and *n*-butanol fractions of the alcohol extract of the aerial parts of *D. sanguinalis* (fig. 1). The isolated compounds were identified by comparing their spectral data with the published literature<sup>[23-38]</sup>. They were identified as

**TABLE 2: ANTIVIRAL ACTIVITY OF DIGITARIA SANGUNALIS FRACTIONS AND ISOLATED COMPOUNDS**

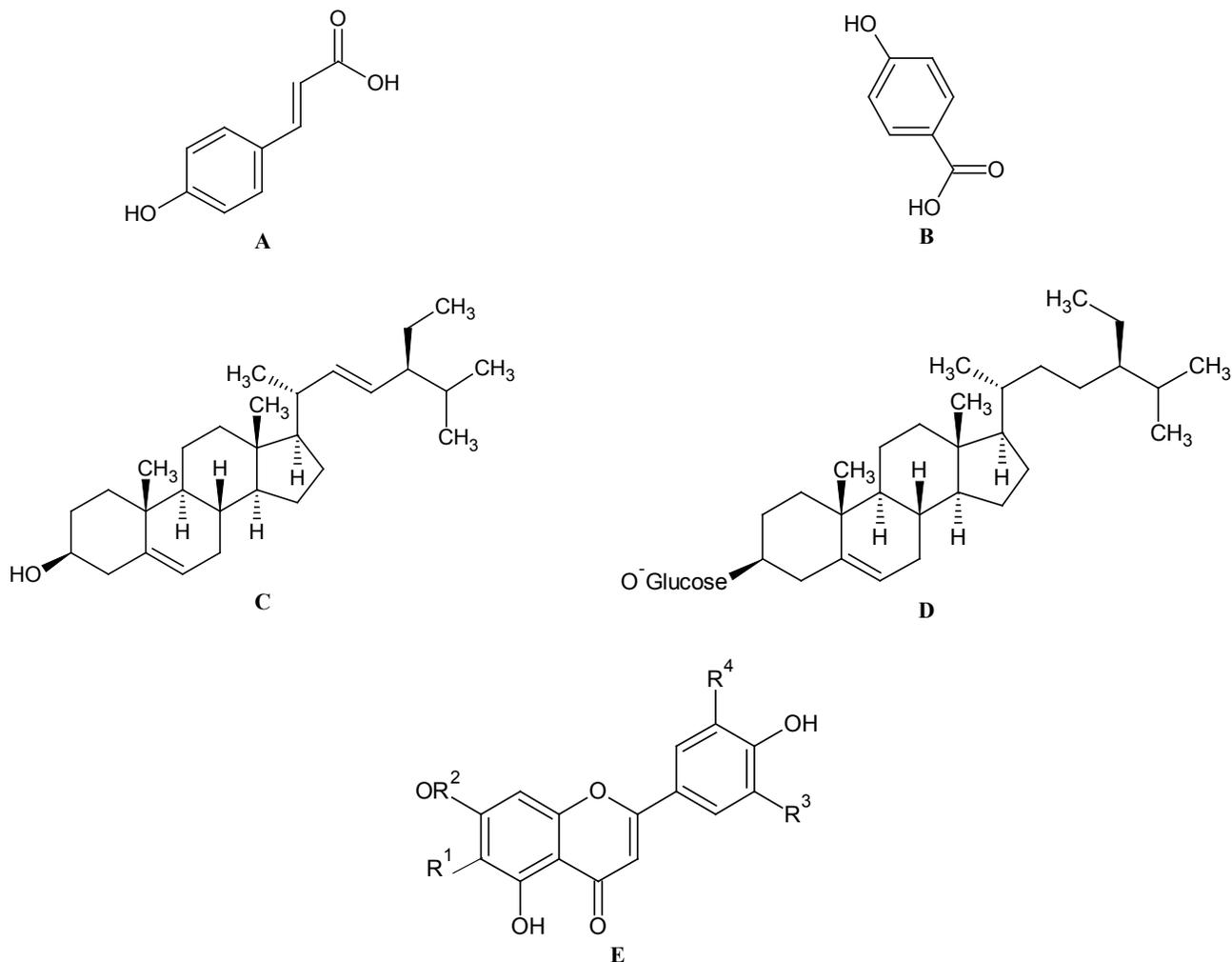
Sample	Tested Viruses		
	HAV	HSV-1	HSV-2
<i>n</i> -hexane fraction	+++ve	++ve	+ve
Ethyl acetate fraction	++ve	++ve	-ve
<i>n</i> -butanol fraction	+ve	-ve	-ve
<i>p</i> -coumaric acid	-ve	-ve	-ve
Triclin	++ve	++ve	++ve
<i>p</i> -hydroxybenzoic acid	-ve	+ve	+ve
Stigmasterol	-ve	-ve	-ve
$\beta$ -sitosterol-3- <i>O</i> -glucoside	-ve	+ve	-ve
Triclin-7- <i>O</i> -glucoside	++ve	++ve	-ve
Isoorientin	+ve	-ve	-ve

'+++' High activity, '++' moderate activity, '+' slight activity, '-' no activity

**TABLE 3: CYTOTOXICITY OF DIFFERENT FRACTIONS AND ISOLATED COMPOUNDS FROM DIGITARIA SANGUNALIS AGAINST HEPG-2, HCT-116 AND MCF-7 CELL LINES**

Cell line	IC <sub>50</sub> ±SD ( $\mu$ g)										
	Fractions			Isolated compounds							Standard
	<i>n</i> -hexane	Ethyl acetate	<i>n</i> -butanol	1	2	3	4	5	6	7	Vinblastine
HepG-2	11.8±0.53*	10.2±0.48*	69.5±1.20	15.6±0.42*	13.5±0.27*	12.3±0.3*	77.8±1.36	13.2±0.39*	9.7±0.16*	65.7±1.10	0.01±0.002
HCT-116	38.8±0.64	29.5±0.44	52.0±1.26	22.7±0.40	42.6±0.47	25.2±0.43	156.3±1.57	18.5±0.26	24.7±0.24	76.3±1.32	0.01±0.001
MCF-7	5.85±0.28*	1.42±0.26*	43.4±0.78	7.3±0.47*	14.6±0.34*	8.6±0.27*	95.2±0.87	3.8±0.12*	4.8±0.09*	52.8±0.88	0.01±0.002
L929	64.2±0.34	54.8±0.14	<80	46.3±0.47	48.6±0.46	45.5±0.62	<80	63.2±0.73	58.6±0.55	<80	ND

Cytotoxicity of different fractions and isolated compounds from *Digitaria sanguinalis* was evaluated using vinblastine as standard reference against HEPG-2, HCT-116 and MCF-7 cell lines. \*Significant at  $p < 0.001$  ND: not determined



**Fig. 1: Structure of the isolated compounds**

(A) Compound (1); (B) compound (3); (C) compound (4); (D) compound (5); (E) compound (2): R1- H, R2- H, R3- OCH<sub>3</sub>, R4- OCH<sub>3</sub>; compound (6): R1- H, R2- glucose, R3- OCH<sub>3</sub>, R4- OCH<sub>3</sub>; compound (7): R1- glucose, R2- H, R3- OH, R4- H

*p*-coumaric acid (1), tricin (2), *p*-hydroxybenzoic acid (3), stigmasterol (4), which were isolated from the *n*-hexane fraction,  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glucoside (5), tricin-7-*O*- $\beta$ -D-glucopyranoside (6), which were isolated from the ethyl acetate fraction and isoorientin (7), which was isolated from the *n*-butanol fraction.

The *n*-hexane and ethyl acetate fractions exhibited significant antimicrobial activity against the tested microorganisms, which may be attributed to the presence of *p*-coumaric acid and *p*-hydroxybenzoic acid in the *n*-hexane fraction and to the presence of  $\beta$ -sitosterol-3-*O*- $\beta$ -glucoside and tricin-7-*O*- $\beta$ -D-glucopyranoside in the ethyl acetate fraction, as these compounds were previously proved to have antimicrobial activity<sup>[39,40]</sup>. Isoorientin showed no antimicrobial activity supporting previously published findings<sup>[41]</sup>. *p*-Coumaric acid showed significant antiMRSA activity again following previously published data<sup>[42]</sup>. However, this study is the first to

find antiMRSA activity in tricin,  $\beta$ -sitosterol-3-*O*- $\beta$ -glucopyranose and tricin-7-*O*- $\beta$ -D-glucopyranoside.

The antimicrobial inhibitory mechanisms of phenolic compounds found to be active in this study may be due to iron deprivation or hydrogen bonding with vital proteins such as microbial enzymes<sup>[43]</sup>. Lipophilic flavonoids may disrupt microbial membranes whereas terpenes may have the ability to disrupt microbial membrane and this may explain their antimicrobial properties<sup>[44]</sup>.

A study that investigated the relationship between structure, activity and the mechanism of action of phenolic acids as antimicrobial and antiMRSA agents found that phenolic acids (benzoic and cinnamic acid derivatives) possess antimicrobial activity, highlighting the importance of the carboxylic group in the molecule structure (proton acceptor), in addition to presence of OH (proton donor) and either OCH<sub>3</sub> group (proton acceptor) or H in position 5 of the benzene ring. The

docking studies provided strong evidence that the molecular basis for antiMRSA activity is probably due to PBP2a inhibitors<sup>[42]</sup>.

The *n*-hexane and ethyl acetate fractions showed significant antiviral activity against HAV and HSV-1, which may be attributed to the presence of triclin and triclin 7-*O*- $\beta$ -D-glucopyranoside in the *n*-hexane and ethyl acetate fractions, respectively. Tricin presented a significant antiviral activity against HAV, HSV-1 and HSV-2; this result agrees with the published data, which proved the antiviral activity of triclin against influenza virus<sup>[45]</sup>. Tricin-7-*O*- $\beta$ -D-glucopyranoside presented a significant antiviral activity against HAV and HSV-1; the present study is the first study to demonstrate the antiviral activity of triclin-7-*O*- $\beta$ -D-glucopyranoside, as previous study found no antiviral activity against respiratory syncytial virus (RSV)<sup>[46]</sup>. This is also the first study to examine the antiviral properties of stigmasterol; however, it showed no antiviral activity. In addition, *p*-coumaric acid showed no antiviral activity, which agrees with previously published findings on the antiviral activity of *p*-coumaric acid<sup>[47]</sup>. *p*-Hydroxybenzoic acid was found to show weak antiviral activity against both HSV-1 and HSV-2,  $\beta$ -sitosterol-3-*O*-glucoside represented weak antiviral effect against HSV-1, and isoorientin was proven to have a weak effect against HAV; this study is the first antiviral study of *p*-hydroxybenzoic acid,  $\beta$ -sitosterol-3-*O*-glucoside and isoorientin.

The *n*-hexane and ethyl acetate fractions exhibited significant cytotoxic activity against HepG-2 and MCF-7 cell lines, which may be attributed to the presence of *p*-coumaric acid and triclin in the *n*-hexane fraction and the presence of  $\beta$ -sitosterol-3-*O*- $\beta$ -glucoside and triclin-7-*O*- $\beta$ -D-glucopyranoside in the ethyl acetate fraction. *p*-Coumaric acid exhibited significant cytotoxic activity against HepG-2 and MCF-7 cell lines, and this result agrees with previous published studies, which proved that *p*-coumaric acid exhibited cytotoxic effect on neuroblastoma and H9c2 cardiomyoblasts<sup>[48]</sup>. Tricin and triclin-7-*O*-glucoside presented significant cytotoxic activity against HepG-2 and MCF-7, extending previous studies findings on their potent cytotoxic activity *in vitro*<sup>[49]</sup>.  $\beta$ -sitosterol-3-*O*-glucoside exhibited significant cytotoxic activity once again confirming published data as  $\beta$ -sitosterol-3-*O*-glucoside is known to possess significant cytotoxic activity towards the Caco-2 cell line<sup>[50]</sup>. Stigmasterol, *p*-hydroxybenzoic acid and isoorientin

exhibited no significant cytotoxic effect; however, no previous studies have examined the cytotoxic effect of *p*-hydroxybenzoic acid and isoorientin, but a previous study on stigmasterol found that it had no significant cytotoxic effect towards KB, CasKi, HCT 116, MCF7 and A549 cell lines<sup>[51]</sup>.

In this study, triclin's mechanism of cytotoxic action was examined to find that it was docked onto the indomethacin-bound structure of cyclooxygenase COX-2. It is considered to be an important mechanism of colorectal cancer chemoprevention. Ligand docking suggested that triclin can occupy the active site of COX-2<sup>[52]</sup>. In prior work, Ovesna *et al.*<sup>[53]</sup> stated that  $\beta$ -sitosterol and its glycoside derivatives can affect different levels of tumour development, such as their inhibitory effects on creation, promotion and induction of cancerous cells, as well as the inhibition of cell invasion and metastasis.

This study isolated seven compounds (1-7) from *D. sanguinalis* for the first time. Compounds 1, 2, 5 and 6 showed significant antiMRSA and cytotoxic activities, while compounds 2 and 6 showed significant antiviral effects. The tested fractions and isolated compounds of *D. sanguinalis* demonstrated potential to be future therapeutic agents.

### Conflicts of interest:

The authors declare no conflict of interest.

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