Supplementary information

2.4.2 LC-ESI-QTOF-MS/MS

UPLC-ESI-TOF-MS is an advanced sensitive analytical technique used for the qualitative and quantitative identification of secondary plant metabolites. To identify the different phytoconstituents of *Bauhinia purpurea* and *Bauhinia madagascariensis* extracts, both negative and positive electrospray ionization modes were used. The temperature of the LC system was set to room temperature, (50 mg) was dissolved in 1 mL of (DI-Water: Methanol: Acetonitrile – 50: 25: 25) using vortexing (for 2 min) and ultrasonication(for 10 min). Centrifuge for 10 min at 10000 rpm. 50 µl stock was diluted with 1000 µl reconstitution solvent. Finally, the injected concentration was 1µg/µl, inject 10 µl on positive mode and negative mode and inject 10 µl reconstitution solvent as a blank sample. Gradient elution was performed using mobile phase A (5 mM ammonium formate in 1% methanol; pH = 3) for positive mode, B (5 mM ammonium formate in 1% methanol; pH = 8) for negative mode, and C (100% acetonitrile) for both positive and negative modes. For the first 20 min, 10% (C) was utilized, followed by 90% (C) until 25 min, and finally 10% (C) from 25.01 to 28 min with a flow rate of 0. ml/min. UPLC-MS analysis was carried out on an AB SCIEX Triple TOF 5600+ system outfitted with an electrospray ionization mode using a Phenomenex in-line filter disks precolumn (0.5 µm × 3.0 mm), and Waters X Select HSS T3 column (2.5 µm, 2.1 × 150 mm). Targeted analytes were identified by comparing LC/MS data with previously reported compounds and reference databases (ReSpect positive or ReSpect negative databases). MasterView was used for feature (peaks) extraction from Total ion chromatogram (TIC) based on the following criteria; Features should have Signal-to-Noise greater than 5 (Non-targeted analysis). Features intensities of the sample-to-blank should be greater than 3.

2.5.1. DPPH Radical Scavenging Activity

Freshly prepared (0.004%w/v) methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10C in the dark. A methanol solution of the test compound was prepared. A 40 uL aliquot of the methanol solution was added to 3ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$PI = [\{(AC - AT)/ AC\} \times 100]$ (1)

Where *A*C = Absorbance of the control at t = 0 min and *A*T = absorbance of the sample+DPPH at t = 16 min**.**

The 50% inhibitory concentration (IC_{50}), the concentration required for 50% DPPH radical scavenging activity was estimated from graphic plots of the dose-response curve using Graphpad Prism software (San Diego, CA. USA).

2.5.2. Hydrogen Peroxide Radical (H_2O_2) Scavenging Activity

A solution of H_2O_2 (43 mM^{-1}) was prepared in phosphate buffer (pH 7.4). A volume 0.2mL of extract in distilled water (at different concentrations) was added to the H_2O_2 solution (600uL of 43mM^{-1}). The absorbance of H_2O_2 at 230nm was read after 10min for comparison with a blank solution containing phosphate buffer without H_2O_2. The H_2O_2 radical scavenging percentage of the extracts was calculated using the following equation:

H_2O_2 radical scavenging percentage = $[(A_{blank} - A_{sample})/ A_{blank}] \times 100$ g extract.

The 50% inhibitory concentration (IC_{50}), the concentration required to 50% radical scavenging activity was estimated from graphic plots of the dose-response curve using GraphPad Prism software (San Diego, CA. USA).

2.5.3. Ferric reducing antioxidant power (FRAP)

This method is based on the reduction of ferricyanide relative to extract sample. Samples (1mg/ml) in 1mL of methanol were mixed with 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v). After 20 min of incubation at 50 °C, the reaction mixture was acidified with 2.5 mL of trichloroacetic acid (10%, w/v). The reaction mixture was centrifuged at 1000 *xg* for 10 min. The supernatant solution (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of freshly prepared ferric chloride (0.1%, w/v). The absorbance of the resulting solution was measured at 700 nm versus a blank using a spectrophotometer (Milton Roy, Spectronic 1201). The reducing capability percentage (%) was calculated as follows

$$\text{Reducing capability (\%)} = 100 - \left[\frac{Ao - As}{Ao} x100\right]$$

Where, A_0: absorbance of the control solution. As: sample absorbance.

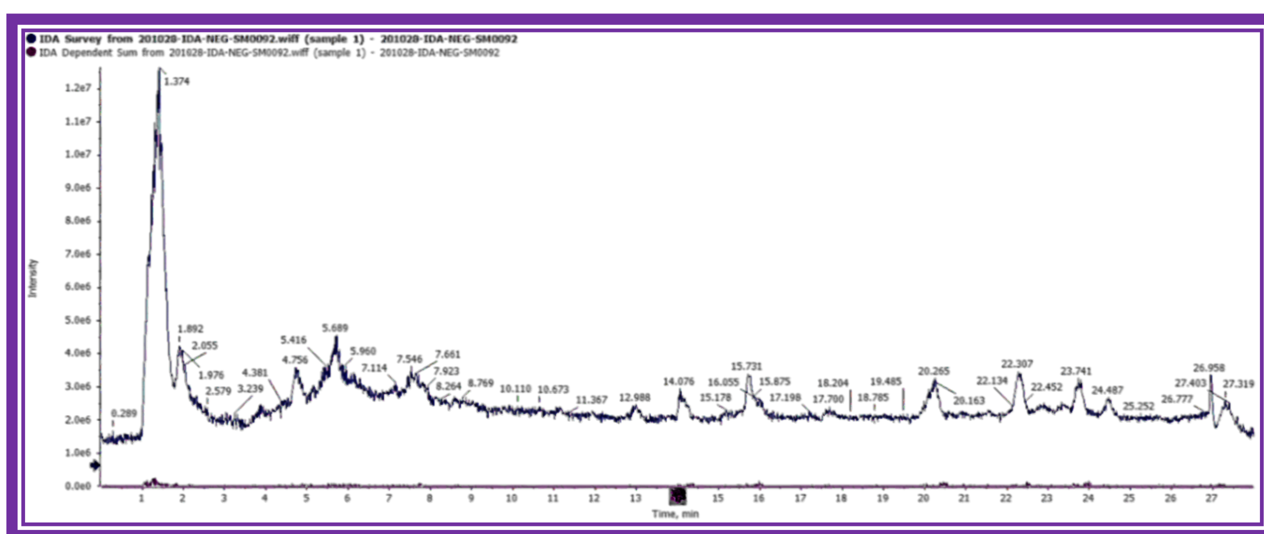

Figure 6. Negative -MODE – TIC of *B. madagascariensis.*

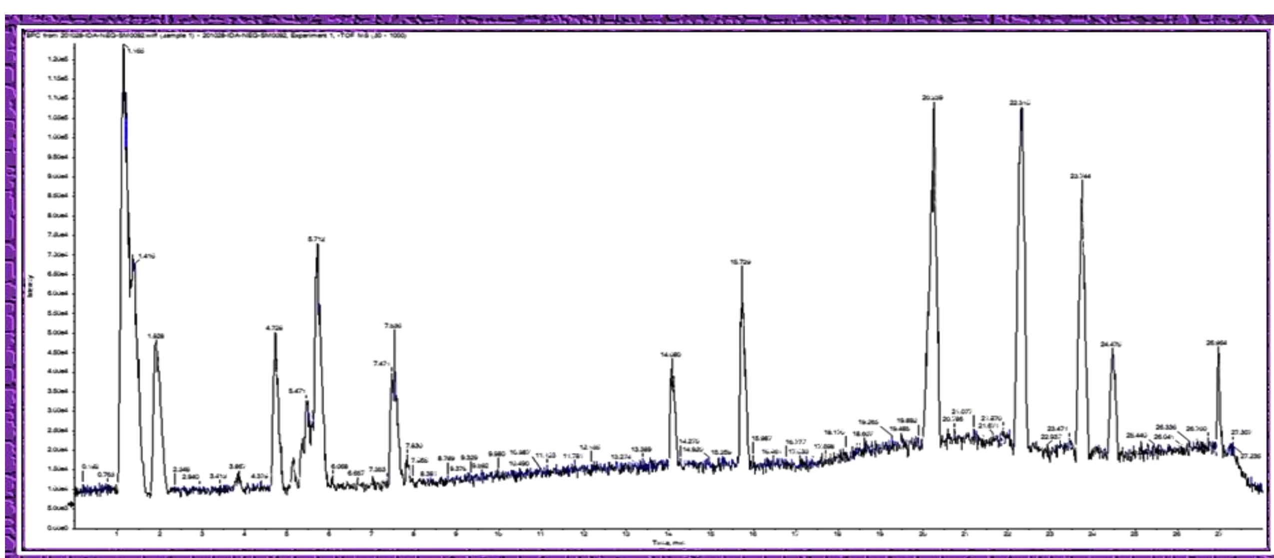

Figure 7. Negative -MODE – BPC of *B. madagascariensis*.

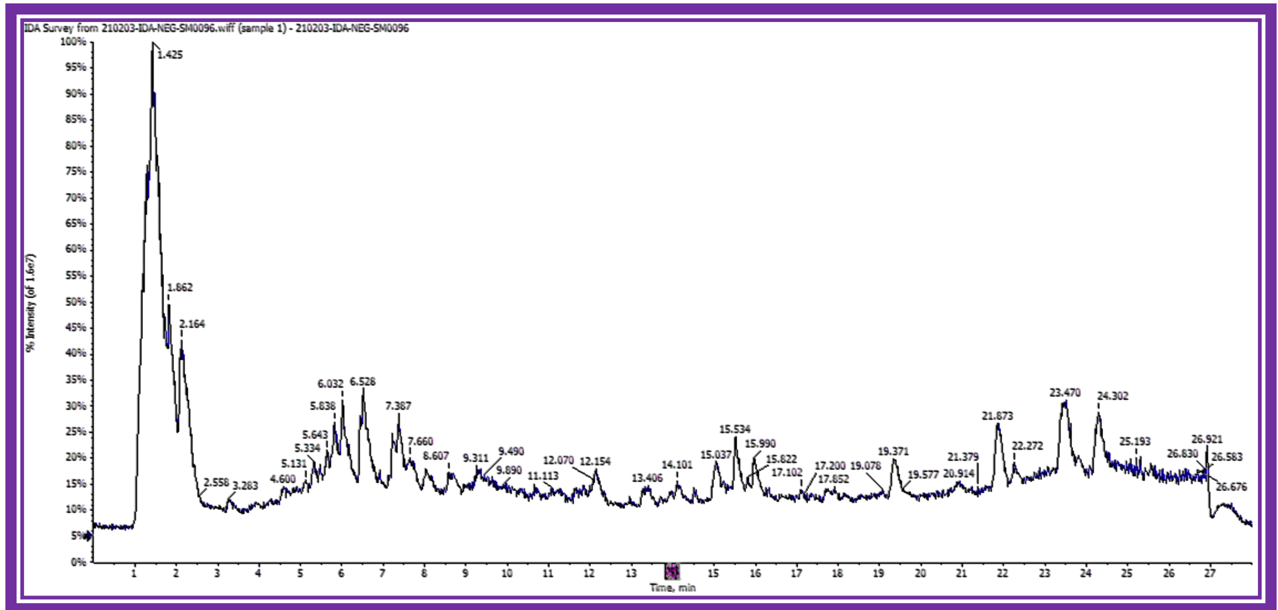

Figure 8. Negative -MODE – TIC of *B. purpurea*.

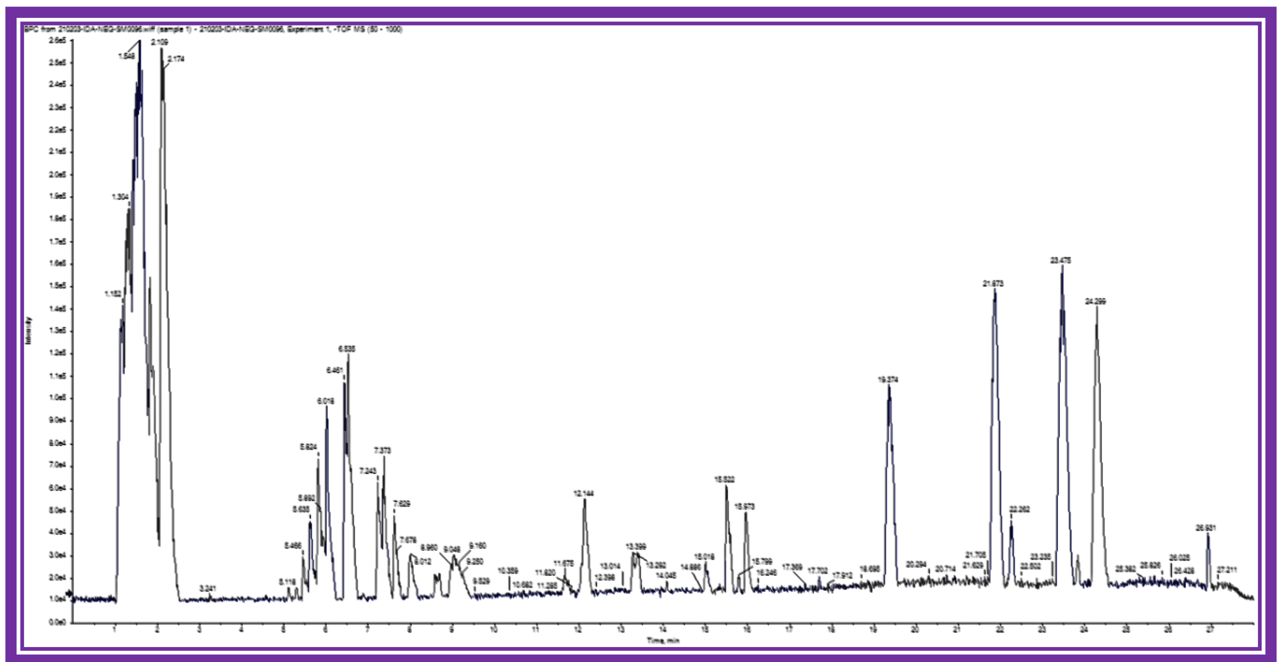

Figure 9. Negative -MODE - BPC of *B. purpurea.*