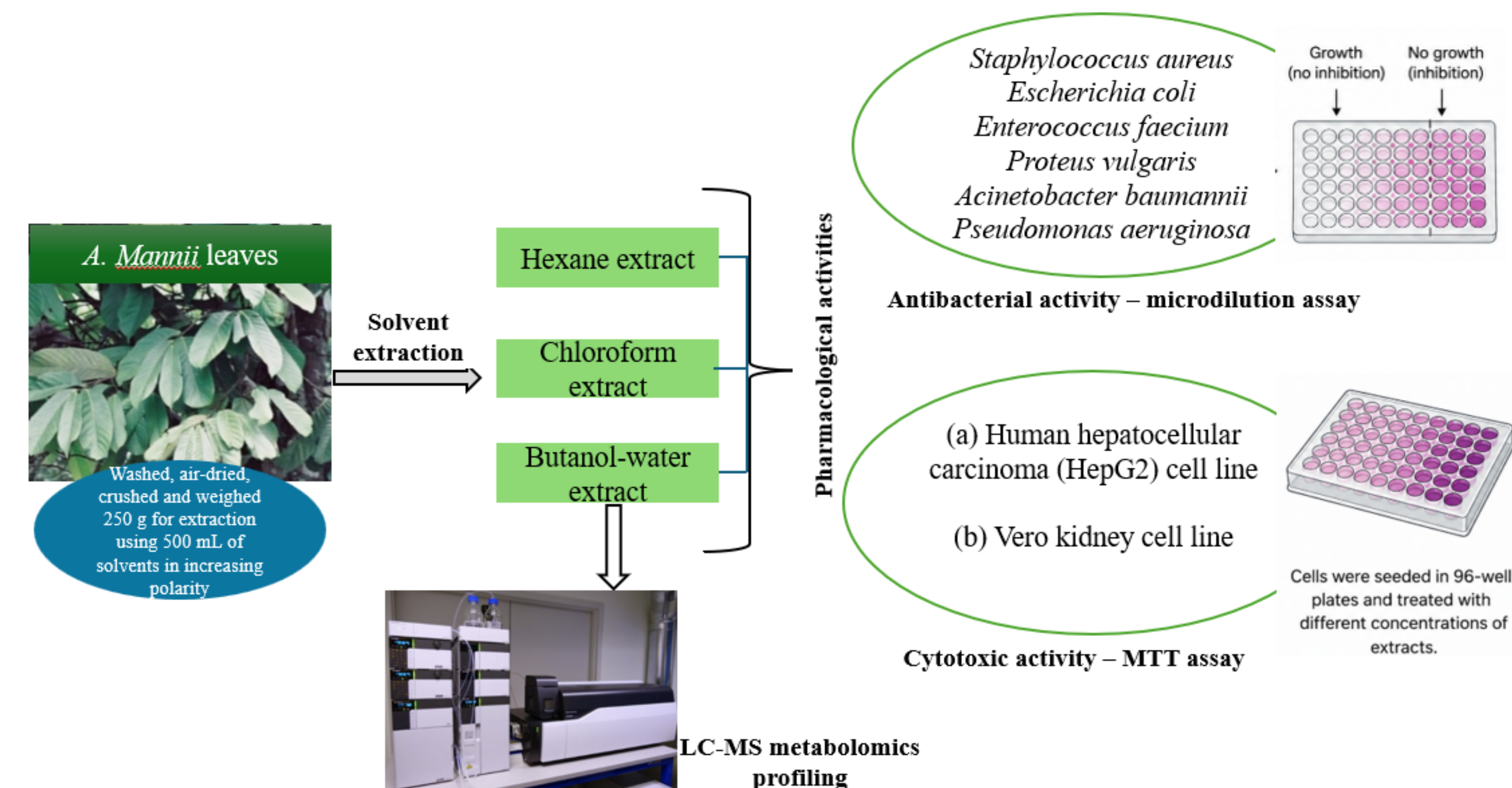


# 1. Introduction

- ❖ Antimicrobial resistance (AMR) is a growing global public health threat that compromises the effectiveness of existing antibiotics and urgently necessitates the discovery of lead antimicrobial drug candidates from natural sources. *Anonidium mannii* (Oliv.) Engl. & Diels is an under-utilised medicinal plant traditionally used for the management of infectious diseases, fever, and gastrointestinal disorders.
- ❖ With renewed global interest in plant-based medicine, phytochemical characterisation is essential to validate traditional uses and identify new bioactive compounds. Advances in metabolomics and analytical platforms, particularly LC-MS, now enable comprehensive profiling and rapid identification of antimicrobial constituents. Here, we investigated the chemical constituents of *A. mannii* through LC-MS analysis and evaluated the antimicrobial and cytotoxic activities of its extracts.

## 2. Methodology



### 3. Results and discussion

The findings are shown and discussed as follows in **Tables 1** and **2** as well as **Figures 1-4**.

**Table 1:** Antibacterial activity of the tested extracts of *A. mannii* against bacterial pathogens

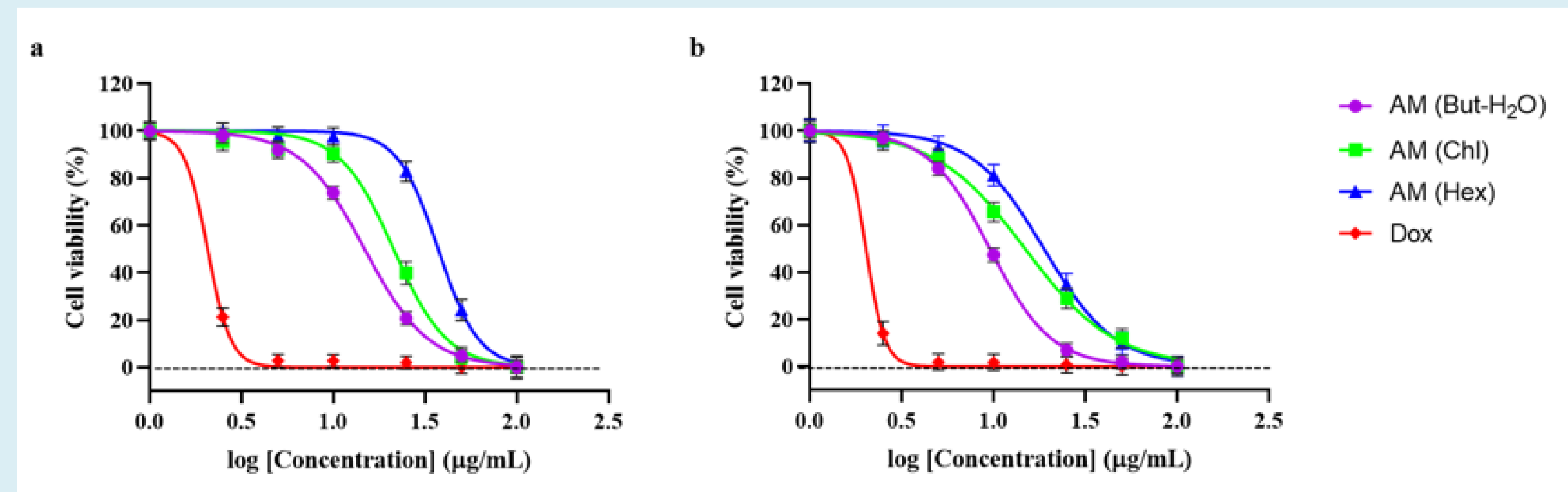
| Extracts                  | MIC (mg/mL)         |                |                   |                    |                      |                  |
|---------------------------|---------------------|----------------|-------------------|--------------------|----------------------|------------------|
|                           | <i>A. baumannii</i> | <i>E. coli</i> | <i>E. faecium</i> | <i>P. Vulgaris</i> | <i>P. aeruginosa</i> | <i>S. aureus</i> |
| AM (But-H <sub>2</sub> O) | 5.00                | 1.25           | 1.25              | 1.25               | 0.62                 | 0.62             |
| AM (Chl)                  | -                   | 2.50           | 5.00              | 2.50               | 2.50                 | 5.00             |
| AM (Hex)                  | -                   | -              | -                 | -                  | -                    | -                |
| Cip.                      | 0.0045              | 0.039          | 0.0024            | <0.0024            | 0.0045               | <0.0024          |
| Strep.                    | 0.078               | 0.078          | 0.078             | 0.0097             | 0.039                | 0.039            |

AM (But-H<sub>2</sub>O) – *A. mannii* butanol-water extract; AM(Chl) – *A. mannii* chloroform extract; AM (Hex) – *A. mannii* hexane extract; Cip. – Ciprofloxacin; Strep. – Streptomycin

**Table 2:** Cytotoxic activity, showing IC50 values of *A. mannii* extracts against Vero and HepG2 cell lines along with the selectivity index (SI)

| Extracts                  | IC <sub>50</sub> (μg/mL ± SD) |               |      |
|---------------------------|-------------------------------|---------------|------|
|                           | Vero                          | HepG2         | SI   |
| AM (But-H <sub>2</sub> O) | 14.82 ± 0.15*                 | 9.55 ± 1.00*  | 1.55 |
| AM ( <u>C</u> hl)         | 21.33 ± 1.50*                 | 15.00 ± 1.13* | 1.42 |
| AM (Hex)                  | 37.32 ± 1.52*                 | 18.80 ± 1.24* | 1.99 |
| Doxorubicin               | 0.78 ± 0.06                   | 0.68 ± 0.06   | 1.15 |

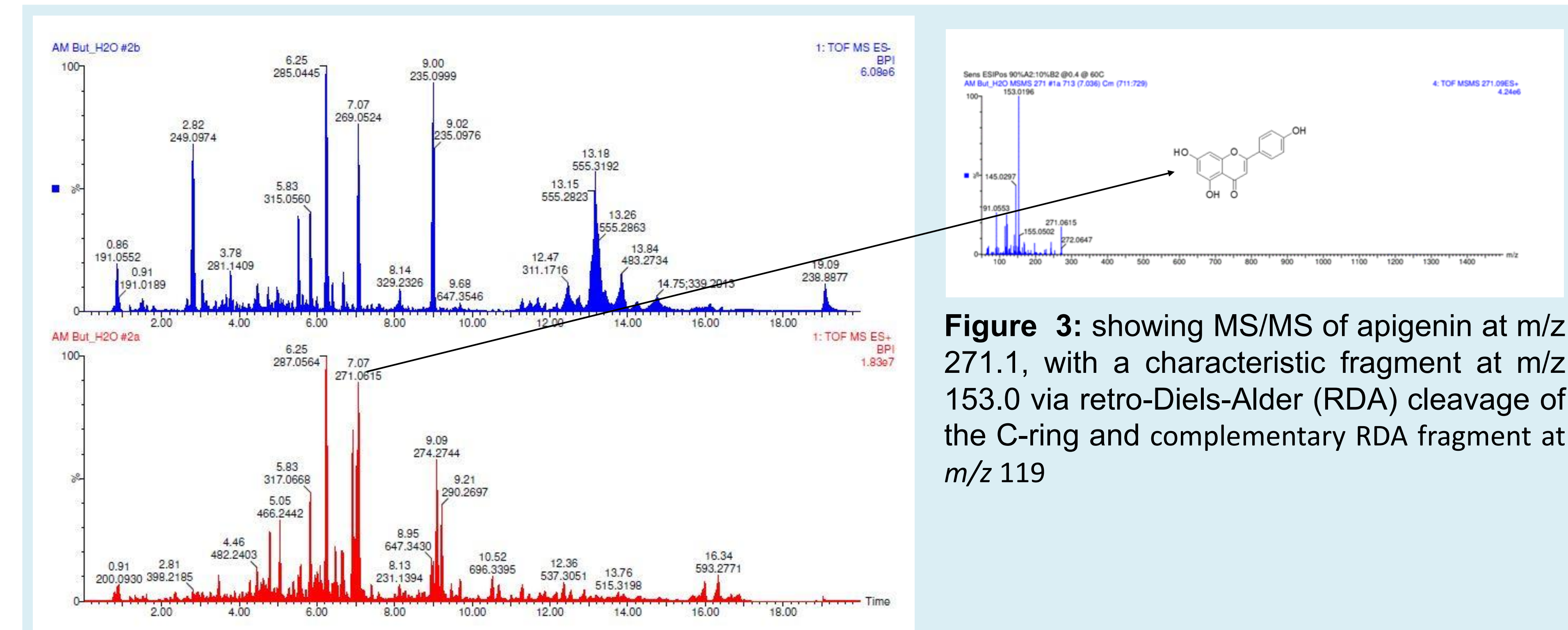
Data are presented as mean  $\pm$  SD. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. Where \* $p < 0.05$ , compared to doxorubicin (positive control).



**Figure 1:** The dose-response curves illustrating the cytotoxic effects (%) of *A. mannii* extracts and doxorubicin on: (a) Vero and (b) Hep2G cell lines.

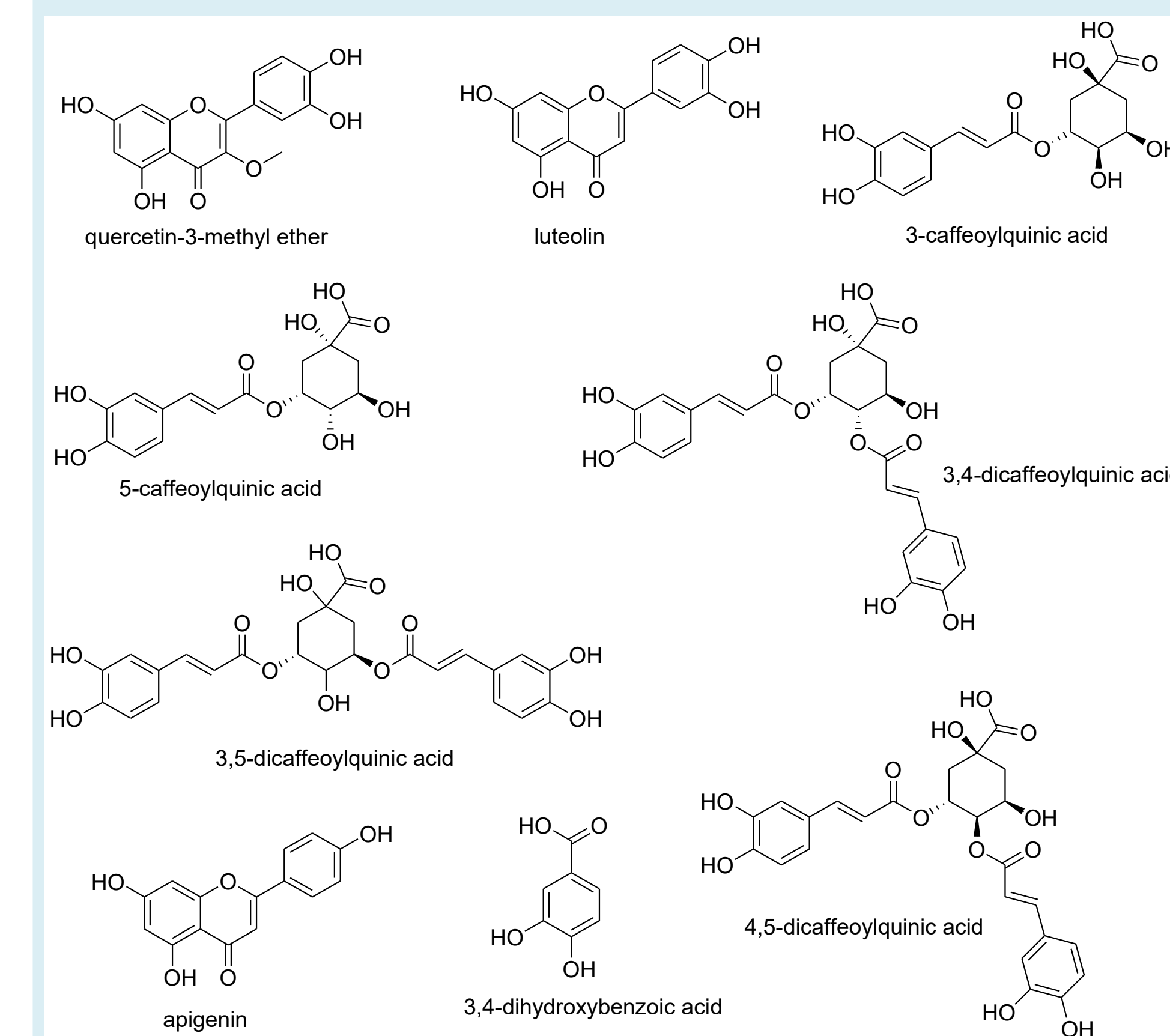
## 5. References

1. N. Chaachouay and L. Zidane, *Drugs and Drug Candidates* **3**, (2024):184–207
2. T. Manso, M. Lores, and T. de Miguel, *Antibiotics* **11**, (2022):46



**Figure 3:** showing MS/MS of apigenin at  $m/z$  271.1, with a characteristic fragment at  $m/z$  153.0 via retro-Diels-Alder (RDA) cleavage of the C-ring and complementary RDA fragment at  $m/z$  119

**Figure 2:** LC-MS chromatogram of *A. mannii* butanol-water extract, showing positive and negative ionization modes



**Figure 4:** Putatively identified compounds in *A. mannii* butanol-water crude extract via LC-MS/MS annotation

LC-MS profiling detected 14 compounds in the butanol-water extract, of which nine compounds were putatively annotated via LC-MS/MS analysis (**Figure 4**). These metabolites are known for their pharmacological activities, including antibacterial. Notably, apart from 3,4-dihydroxybenzoic acid, all the identified compounds are reported in *A. mannii* for the first time.

## 4. Conclusion

This study evaluated the bioactivities, and metabolites of *A. mannii* leaf extracts using LC–MS/MS. The butanol-water fraction showed the strongest antibacterial and anticancer activities. The findings support further drug discovery studies.