

Proximate Content, In vitro Antioxidant and Antimicrobial activity of *Eulophia hereroensis*

Celine MUKAKALISA¹, Renate H. HANS², Kathithileni M. Kalili², Martha A. KANDAWA-SCHULZ¹, and Mervyn BEUKES³, Vinesh Maharaj⁴

¹Department of Biochemistry, Microbiology and Biotechnology, University of Namibia

²Department of Physics, Chemistry and Material Science, University of Namibia

³Department of Biochemistry, Stellenbosch University

⁴Department of Chemistry, University of Pretoria

Introduction and motivation

- Eulophia hereroensis*, a perennial plant¹, belongs to the Orchidaceae family, a family of beautiful orchids
- Found across Southern Africa², especially in Angola, Namibia, Botswana, Zimbabwe, Mozambique, and South Africa
- Identified by attractive bright yellow flowers and edible corms that are found underground (Fig.1)¹
- Orchidaceae species have been extensively studied worldwide, and contain a number of medicinal benefits^{3,4}, however, little is known about the nutritional and medicinal values of Southern African orchids.
- Whereas indigenous leafy vegetables have been extensively studied, edible tubers are relatively unexplored in terms of their nutritional and medicinal values.

Objectives

- To evaluate the macronutrient content
- To evaluate the antimicrobial and antibiofilm activity of extracts
- To evaluate the antioxidant and cytotoxic activity of extracts
- To identify the phenolic compounds



Figure 1. *Eulophia hereroensis* (A) flower and (B) corms

Methodology

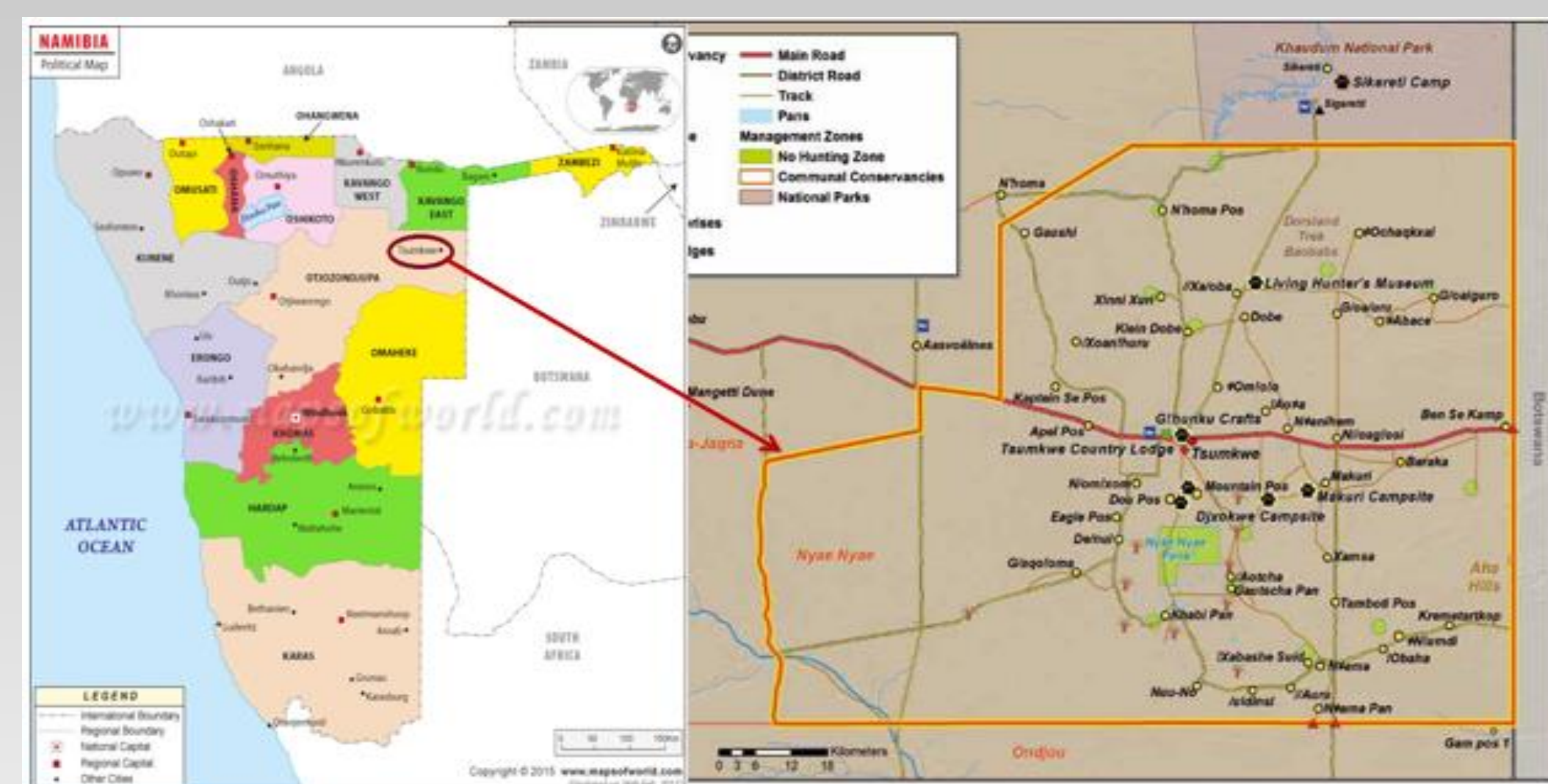


Figure 2. Study site

Antioxidant activity

- Extraction in 80% methanol
- DPPH assay with ascorbic acid as control measured at 517nm
- Reducing power with ascorbic acid as control measured at 700 nm
- Hydrogen peroxide scavenging assay with ascorbic acid as control measured at 230 nm

Cytotoxic activity

- 80% methanolic extracts tested
- Tested against a non-cancerous cell line (Vero) and one cancerous cell line (HT-29 cells)
- Cell viability evaluated using MTT assay

Total phenolic content

- Determined using the Folin-Ciocalteu method
- Gallic acid used as standard and absorbance measured at 750nm

Total flavonoid content

- Determined using the aluminium chloride colorimetric method
- Quercetin used as standard and absorbance measure at 510nm

UPLC-MS analysis

- TLC and prepartive Lcwere used to isolate pure compounds
- Compound ran in a UPLC BEH C18 column and both negative and positive ionization modes were used in the MS
- Data were acquired using Waters Masslynx V4.1 software
- Literature review gave tentative identification

Table 1. Phytochemical screening of the corms

Phytochemicals	EH
Anthraquinones	-
Anthranoids	-
Cardenolides	-
Saponins	+
Terpenes	-
Polyphenols	++
Flavonoids	+
Alkaloids	+
Tannins	++

Table 2. Proximate composition

	EH
Moisture (% fresh weight)	92±0.24
Dry matter (% fresh weight)	8±0.31
Ash (g/100g dry weight)	4±0.32
Crude protein (g/100g dry weight)	7.1±1.05
Crude fat (g/100g dry weight)	0.5±0.003
Total carbohydrate (g/100g dry weight)	88±0.85
Energy (kcal/100g dry weight)	384.9

- Presence of saponins give indication of antimicrobial action
- Presence of phenols and flavonoids indicate an number of activities, including antioxidant and anticancer activities
- Good and high protein content compared to domesticated tubers

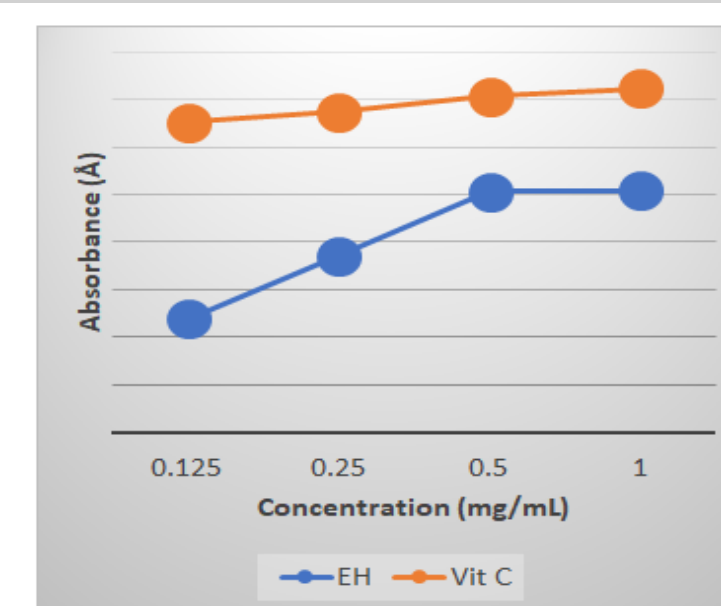


Figure 4. Reducing power

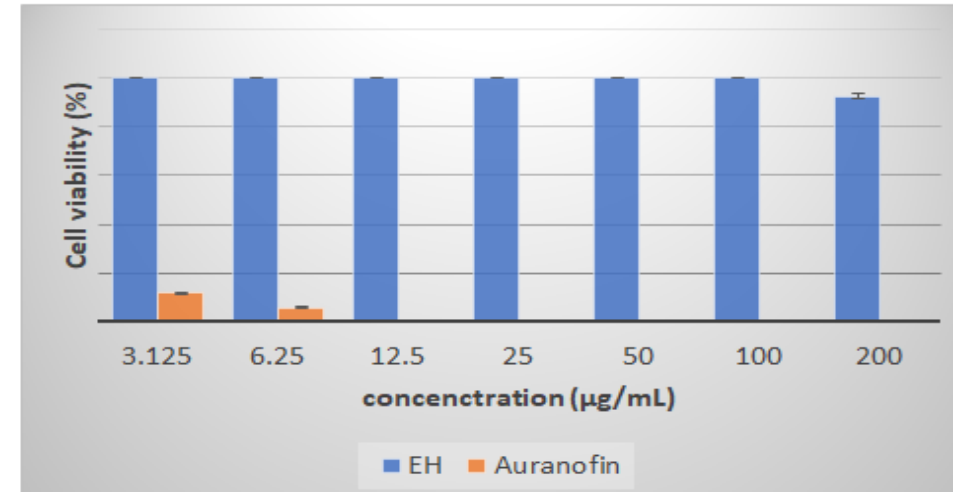


Figure 5. Cytotoxic activity against Vero cells

- The reducing power showed good antioxidant potential
- The corms are good antioxidant agents with IC₅₀s below 100µg/mL
- CC50 was above 200 ug/mL, showing the non-toxic aspect and reduction in HT-29 cell viability shows chemopreventative activity

Conclusion and way forward

- The corms exhibited good macronutrient content with protein standing out
- The DCM extract showed good antimicrobial and antibiofilm results
- The corms had good antioxidant activity and were found to non-toxic but the decline in cell viability of HT-29 – chemopreventative agents with Caffeic acid-O- hexoside tentatively identified as the active phenol
- Overall the study showed that the tubers of *E. hereroensis* has nutritional and medicinal value.
- Further studies use better purification and identification techniques. Extend cancer models for cytotoxic assays, and evaluate micronutrient content

Results and discussion

Table 3. Antimicrobial activity of six solvent extracts

	Inhibition zones (mm)			
Extract	<i>C. albicans</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
Hexane	—	—	—	—
DCM	9.0 (±0.71)	10.5 (±0.71)	10.0 (±0.00)	12.5 (±1.41)
Ethyl acetate	6.5 (±0.71)	8.5 (±2.12)	7.0 (±0.00)	10.5 (±1.41)
Acetone	5.5 (±0.71)	7.5 (±0.71)	5.5 (±0.71)	7.0 (±0.71)
Ethanol	—	—	—	—
Water	—	—	—	—

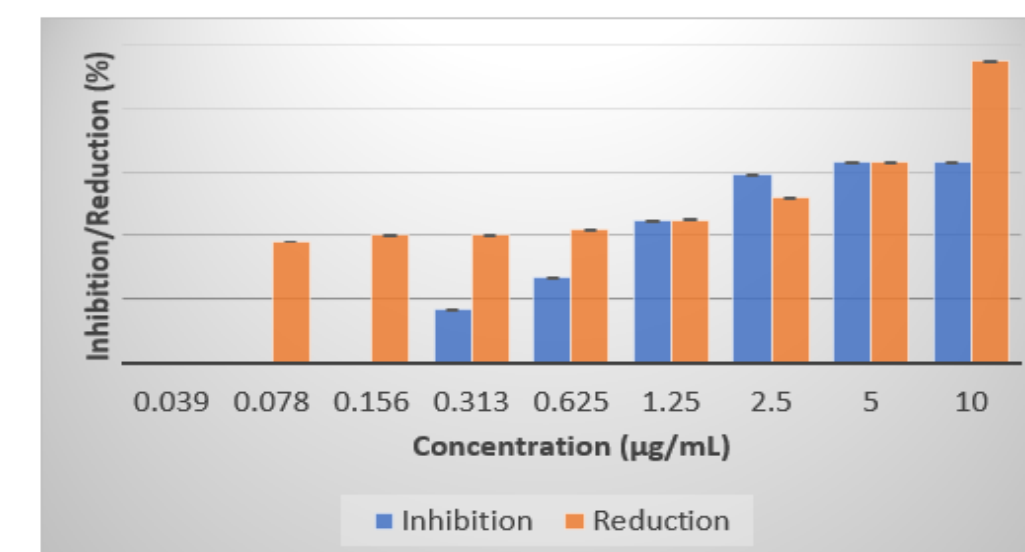


Figure 3. Biofilm inhibition and reduction by the corms' DCM extract

Table 4. MIC

	MIC (mg/mL)			
	<i>C. albicans</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>
DCM	10	2.5	5.0	10
Ethyl acetate	10	10	10	10
Acetone	10	10	10	10

Table 5. MBIC₅₀ and MBEC₅₀ of the DCM extract

Samples	MBIC ₅₀ (ug/ml)	MBEC ₅₀ (ug/ml)
<i>E. hereroensis</i>	1.960 (±0.040)	0.785 (±0.023)
Ampicillin	<0.078	<0.078

- The antimicrobial screening and MIC results showed that DCM extracts had the best activity
- High activity against *E. coli* validates traditional use in treating stomach ailments
- MIC values too high for good antimicrobial agents (0.1 – 1 mg/mL)
- Good antibiofilm activity

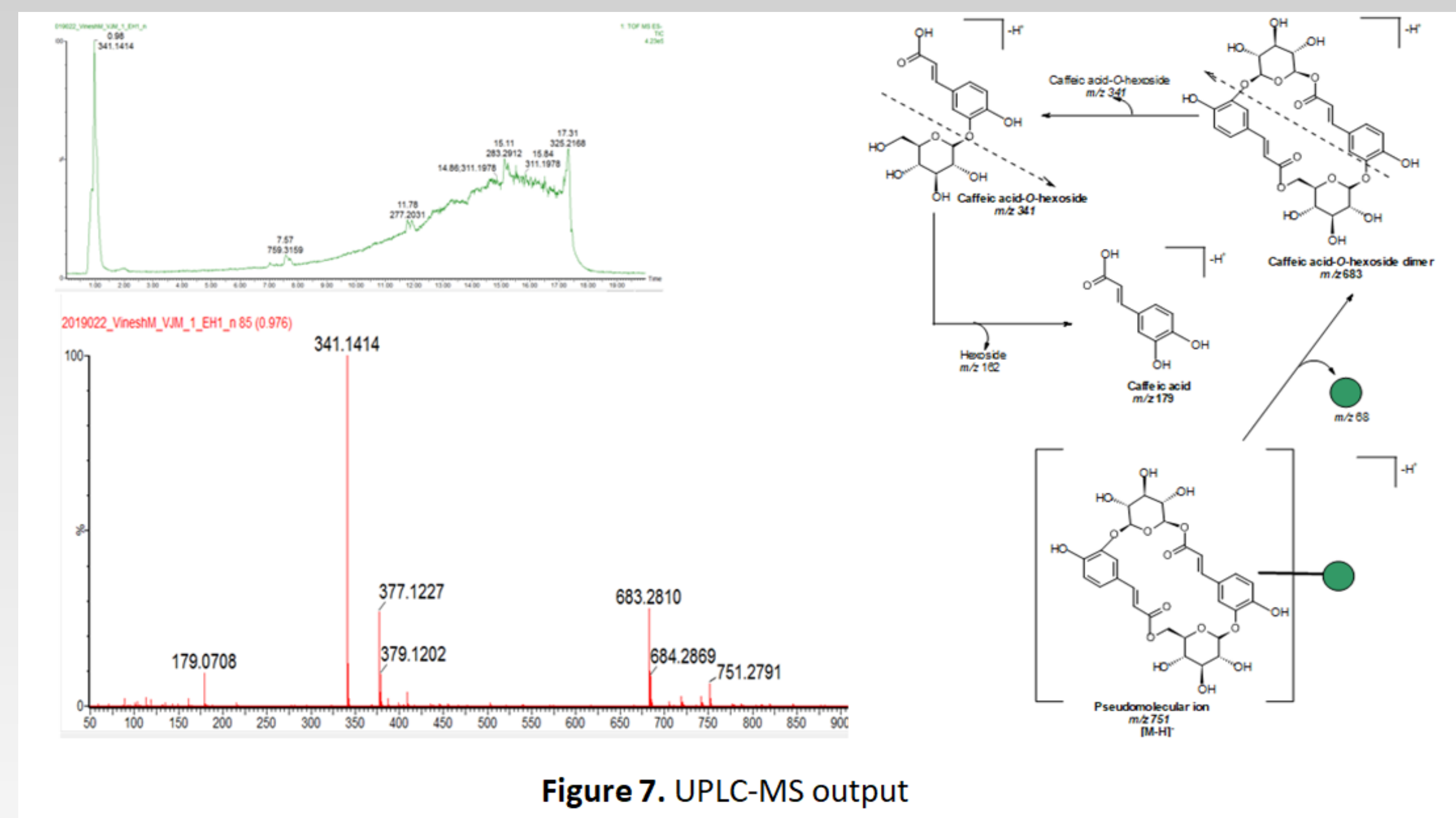


Figure 7. UPLC-MS output

- Identification through literature review looking at the compounds fragmentation patterns
- m/z 683 was assigned as a dimer of caffeic acid-O-hexoside (m/z 341)
- m/z 179 as [caffeic acid-H]⁻ after the loss of a sugar moiety

References

- Leffers, A. (2003). Gemsbok bean & Kalahari truffle: Traditional plant use by the Jul'hoansi in North-Eastern Namibia. Macmillan Education Namibia Publishers (Pty) Ltd
- Chinsamy M., Finnie J.F., and Van Staden, J. (2011). The ethnobotany of South African medicinal orchids. *South African Journal of Botany*, 77: 2-9
- Hossain M.M. (2011). Therapeutic orchids: traditional uses and recent-advances – An overview. *Fitoterapia* 82 (2011): 102 -140
- Keatinge J.D.H., Wang J.-F., Dinssa F.F., Ebert A.W., Hughes J.D.A., Stoilova T., Nenguwo N., Dhillon N.P.S., Easdown W.J., Mavlyanova R., Tenkouano A., Afari-Sefa V., Yang R.-Y., Srinivasan R., Holmer R.J., Luther G., Ho F.-L., Shahabuddin A., Schreinemachers P., Iramu E., Tikai P., Dakuidreketi-Hickes A., Ravishankar M. (2015). Indigenous vegetables worldwide: their importance and future development. *ISHS Acta Horticulturae*, 1102:1-20