# The Identification and Isolation of Compounds from *Sutherlandia frutescens*

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## Introduction

Plants constitute the basic food chain and provide the most valuable source of natural nutrients; in addition, constituents of medicinal importance are also present in plants[1]. For drug discovery and other purposes, we must characterise the active plant compounds and study their chemical structures and basic properties. *Sutherlandia frutescens* is one of the best-known, multi-purpose medicinal plants[2]. Its therapeutic properties are based on its ability to help the human body to mobilise its own immunological and physiological resources to help combat diseases and physical and mental stress[2].

**Figure 1: Distribution of *Sutherlandia* in S.A.**

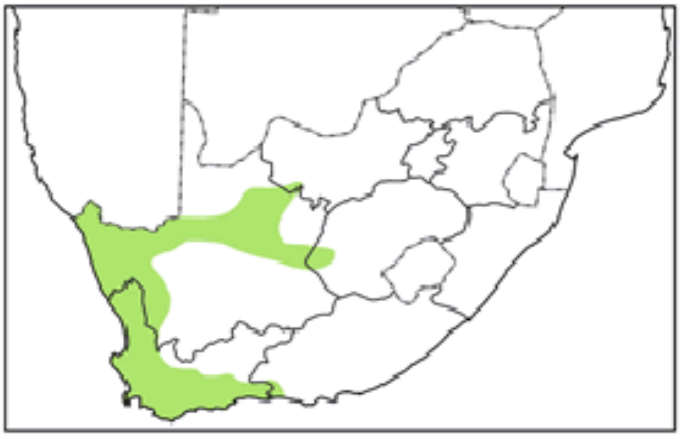


**Figure 2: *S. frutescens***

**Table 1: Historical uses of *Sutherlandia***

| Tribe | Historical uses |
|---|---|
| Khoi San & Nama people | Washing wounds, fever remedy[3] |
| Zulu people | Antidepressant, relaxant[3] |
| Early colonists & Rastafarians | Chicken pox, eye troubles, internal cancers, stomach problems[4,5] |
| Southern Sotho | 'Dropsy of the heart', fever remedy[6] |
| Nowadays | Flu, asthma, bronchitis, TB, dysentery, diabetes, inflammation, mental & emotional stress, anxiety, depression, aids in digestion and improves appetite[4] |

## Objective

To phytochemically investigate the spray-dried extract made from *Sutherlandia frutescens*, and to isolate compounds while evaluating their immune-stimulating/boosting properties.

**Methodology**

A spray-dried extract of the dried leaves of the plant was prepared in the Botanical Supplies Unit of Biosciences at the CSIR. This process is illustrated below:

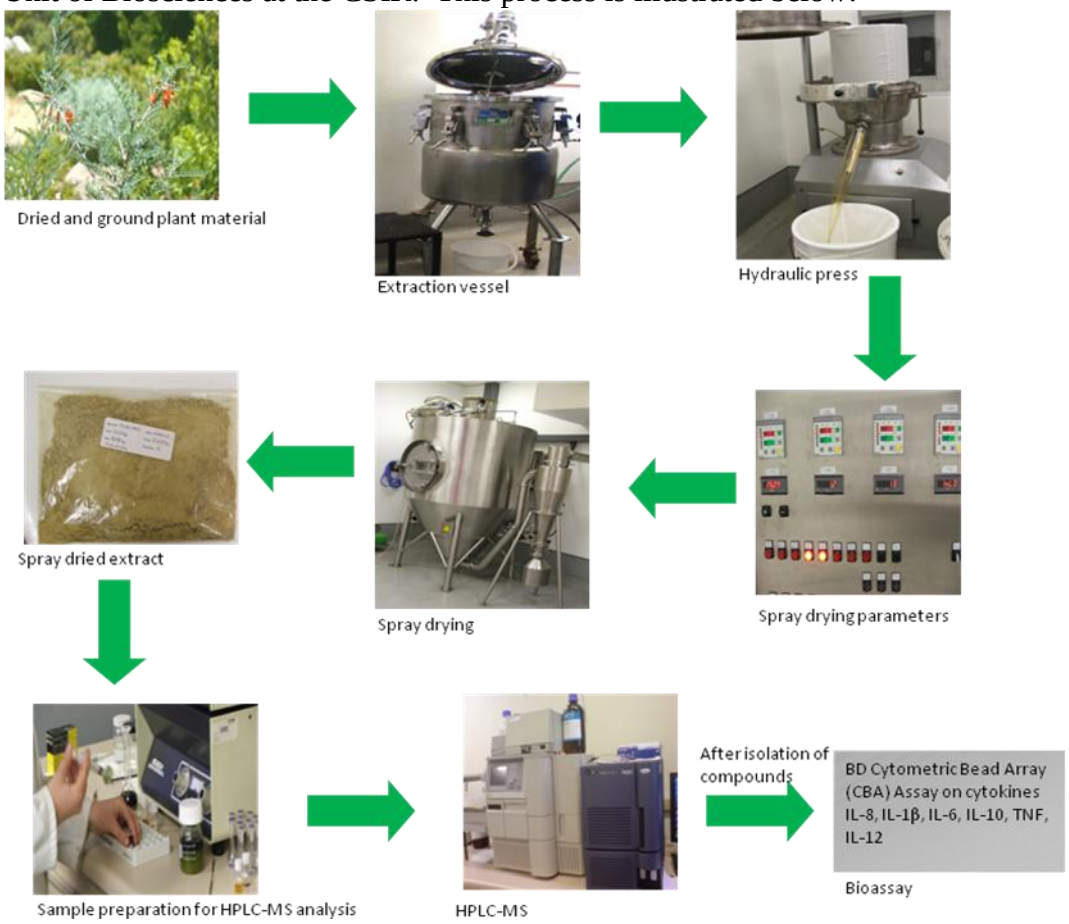



**Figure 3: Schematic diagram of methodology used**

**HPLC-MS analysis**

The spray-dried extract was analysed using a WATERS 2695 separation module. Two Atlantis T3 columns connected in series were used for the separation. UV detection was done on a WATERS 2996 PDA scanning from 200–600 nm. The mobile phase used was 0.1% formic acid in water (A) and acetonitrile (B).

| **Time (min)** | **Flow (ml/min)** | **% A** | **%B** |
|---|---|---|---|
| 0 | 0.3 | 100 | 0 |
| 10 | 0.3 | 100 | 0 |
| 20 | 0.3 | 95 | 5 |
| 30 | 0.3 | 75 | 25 |

| | | | |
|---|---|---|---|
| 90 | 0.3 | 0 | 100 |

**Mass spectrometry**

Mass spectrometry detection was performed using a WATERS SQD scanning from 100 – 1200 m/z with +/- switching.

**Results & Discussion**

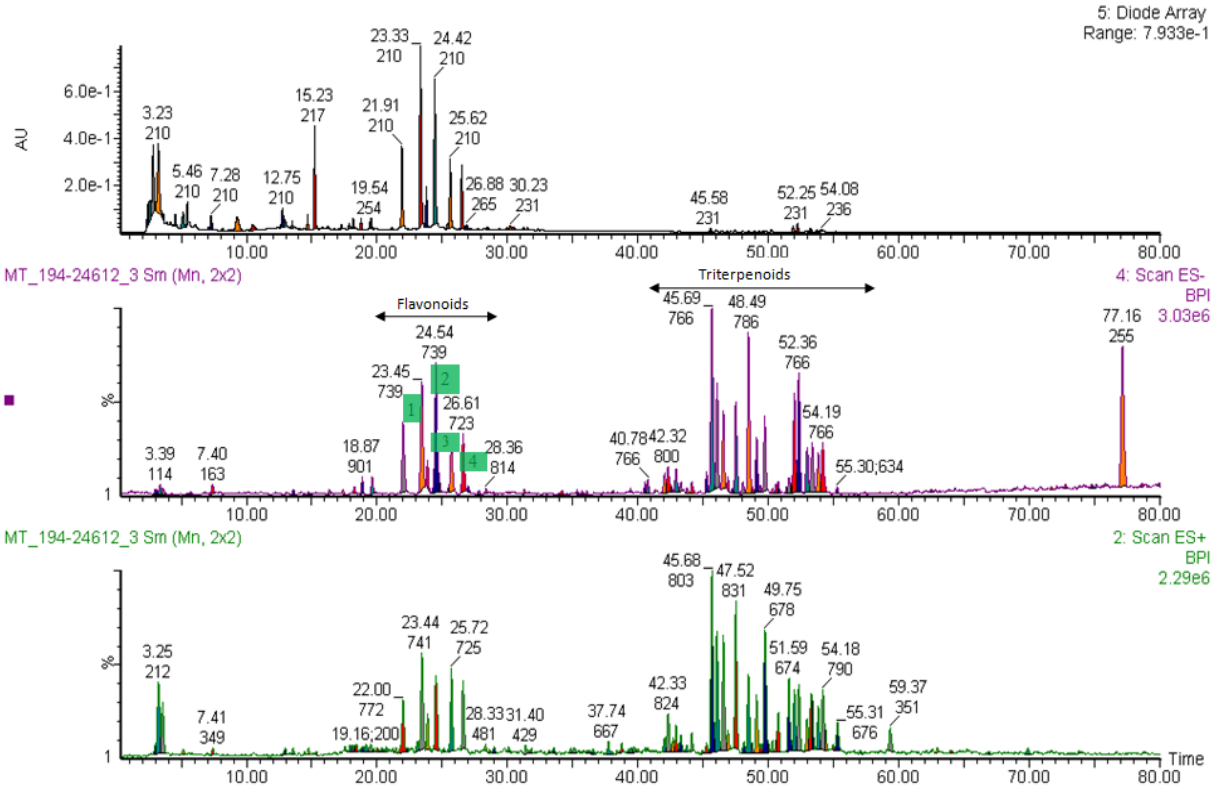
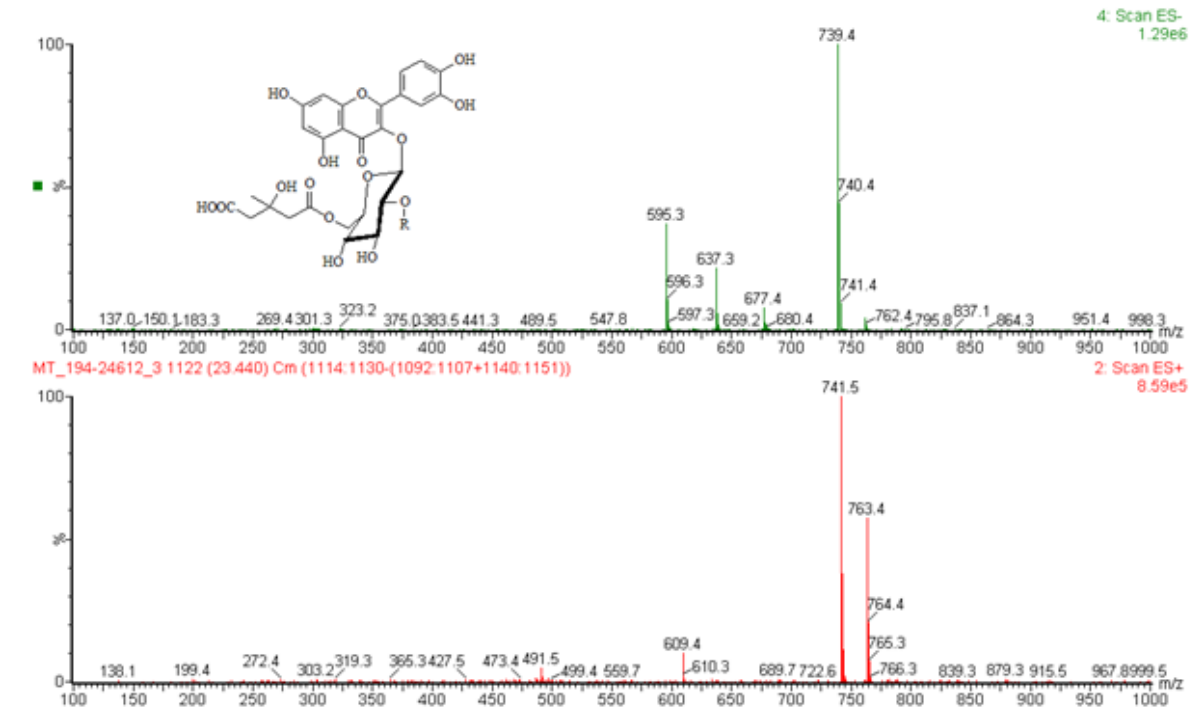



**Figure 4: UV and Total Ion Chromatogram (TIC) Analysis of spray-dried extract**

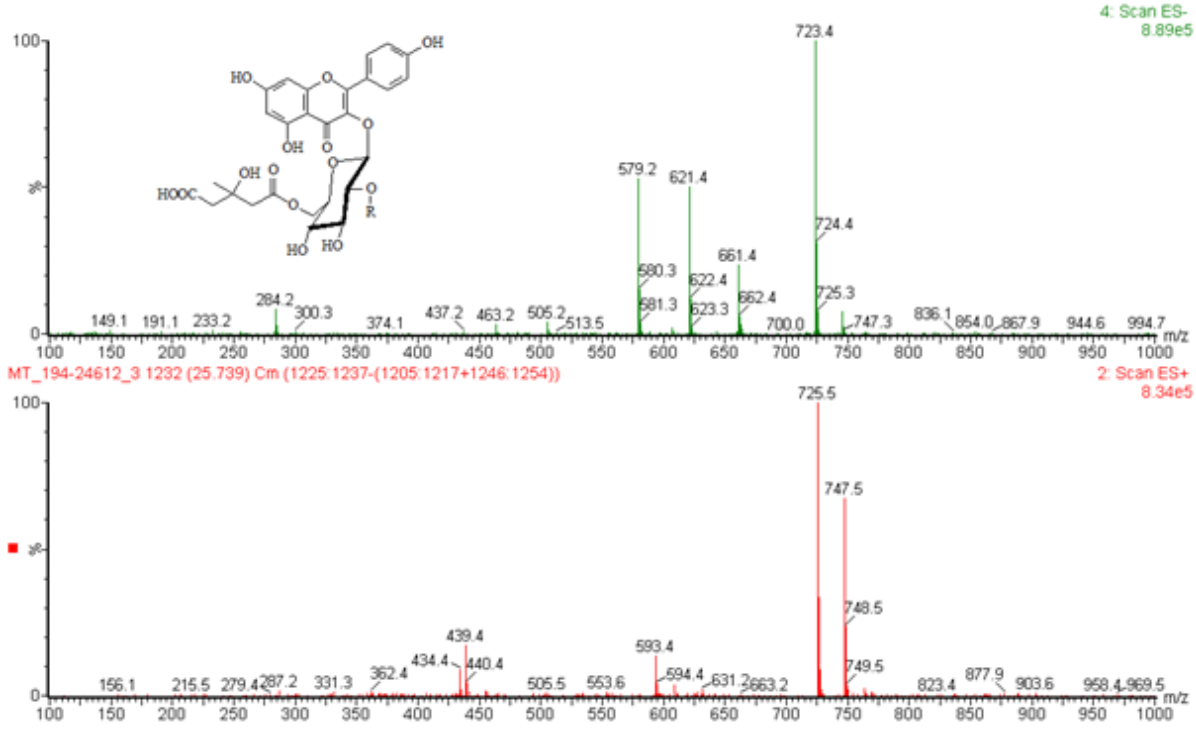
The spray-dried extract was resolved by HPLC-UV-MS to show the presence of a number of different compounds. Two different regions were identified in the chromatogram. The first region between 20–30 minutes was found to be a flavonoid-rich area, while the region between 40 –60 minutes was found to be a triterpenoid-rich area. These regions were identified based on the spectral data.

A database search of the triterpenoid molecular ions observed in our chromatogram did not identify any of the triterpenoid masses reported in literature. An attempt to isolate these compounds will be done as part of the next stage in the project. Figures 5 and 6 below show the mass spectral data of the peaks labelled 1 and 3 from the TIC chromatogram of Figure 4. These peaks were identified as being flavonol glycosides based on their mass fragmentation patterns. The presence of these flavonoids in *Sutherlandia* extracts has been reported by Avula (2010).




R = or R =

**Figure 5: Mass spectral data and structure of flavonoid 1**




R = or R =

**Figure 6: Mass spectral data and structure of flavonoid 3**

*Sutherlandia* consists of a complex variety of compounds with the main class of compounds found to be glycosidic cycloartane-type triterpenoids. This class has been reported to stimulate appetite and contribute to adaptogenic and immune-boosting effects[2]. The other class of compounds identified, namely flavonol glycosides, is said to have anti-oxidant activity and help to eliminate mutagen and carcinogens - which are of value in cancer prevention[8].

## Conclusion & Future work

Due to the complex nature of the plant extract, there are still many compounds to be isolated and identified. The isolation and identification is still in progress. At present two triterpenoid-type compounds have been isolated and structure elucidation is in progress. The compounds isolated and identified will be biologically evaluated for their immune-stimulating/boosting properties using the Cytometric Bead Array assay.

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