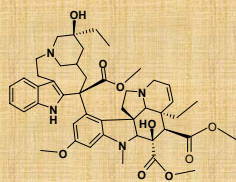


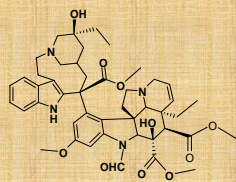
Introduction:

Catharanthus roseus is a well-known medicinal plant belonging to the family Apocynaceae. It is regarded as a rich source of pharmaceutically important indole alkaloids that are used as antitumor, hypotensive and antiarrhythmic agents. Among the indole alkaloids, vinblastine (1) and vincristine (2) are currently used to treat a wide variety of neoplasms and is recommended for treatment of Hodgkin's disease, acute leukemia and choriocarcinoma, which is resistant to other types of therapy. The unique antitumor activities of these indole alkaloids have resulted in a great demand for vinblastine (1) and vincristine (2) as anticancer agents.[1,2]



Vinblastine (1)

Formula C₄₆H₅₆N₄O₉



Vincristine (2)

Formula C₄₆H₅₆N₄O₁₀



Catharanthus roseus



Supercritical Fluid Extractor (SFE)
(SFT-100 XW)

Aim of the Study:

The aim of the present study is to assess the suitability of supercritical carbon dioxide for the extraction of *Catharanthus* alkaloids with optimized variables that affect the extraction, such as pressure, temperature, time of extraction and the amount of modifier, using factorial designed experiments.

Plant Material:

The leaves of *Catharanthus roseus* were planted and collected from the Department of Chemistry, Faculty of Science, University of Malaya.

SFE Instrumentation:

Supercritical fluid extractions were performed with an SFT-100 XW (New York, USA) instrument using 100mL extraction vessels. In each experiment, 25g of dried leaves of *C. roseus* was loaded into the extraction vessels. The temperature of the restrictor was maintained at 80°C and the static extraction time was 60 min. The oven temperature was 80°C. The carbon dioxide used for extraction was 99.8%; SFE grade and it was purchased from Malaysian Oxygen (Petaling Jaya, Selangor). The SFE instrument was equipped with a double 12mL syringe pump for the addition of carbon dioxide at a flow rate of 3mL/min at 5000 psi and a 20-80% gradient of EtOH polar modifier was used. The extractions were repeated using different sample batches.

Results:

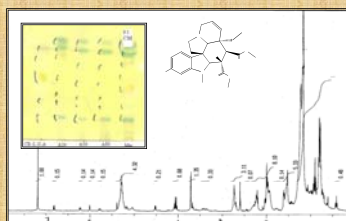
Table: 1 (Physical conditions of extracts)

Code	Solvent ratio	Pressure	Temperature	No. of soakings	Yield
CR-L-S-AN20 ₁₋₃ CR-L-S-AN20 ₄₋₁₀	CO ₂ : EtOH+ NH ₃ 80% : 20%	5000	80 °C	10	0.64 g 2.00 g
CR-L-S-1	CO ₂ : EtOH 80% : 20%	5000	80 °C	5	1.00 g

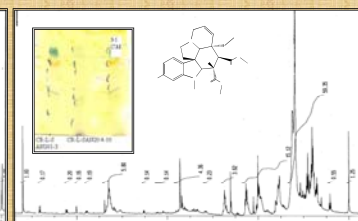
Table: 2 (Physical conditions of extracts for consequent series)

Code	Solvent ratio	Pressure	Temperature	No. of soaking	Yield
CR-L-S-A	CO ₂ : 100%	5000	80 °C	10	0.55 g
CR-L-S-A20	CO ₂ : EtOH 80% : 20%	5000	80 °C	5	0.47 g
CR-L-S-A50	CO ₂ : EtOH 50% : 50%	5000	80 °C	5	1.80 g
CR-L-S-A80	CO ₂ : EtOH 20% : 80%	5000	80 °C	6	1.60 g

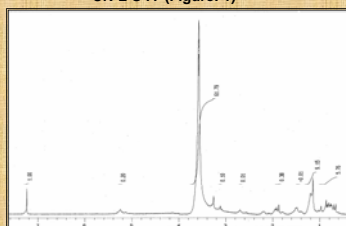
- In this study of the dry leaves of *Catharanthus roseus*, three parameters were applied (temperature, pressure and solvent ratio) in which the same temperature and pressure was used, but the solvent ratio was changed (Table 1 and Table 2).
- Table 1 lists the parameters used for 2 different extracts using 2 different samples batches.
- Table 2 lists the parameters used for 4 different extracts obtained from one sample which is extracted several times consequently using the parameters listed.



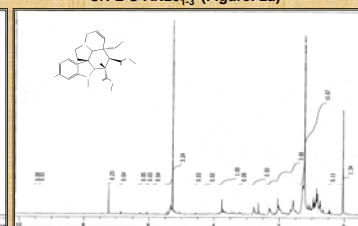
CR-L-S-A (Figure: 1)



CR-L-S-AN20₁₋₃ (Figure: 2a)



CR-L-S-AN20₄₋₁₀ (Figure: 2b)



CR-L-S-1 (Figure: 3)

Discussion:

- Comparison of the ¹H-NMR spectra (Fig.1, 2a & 3) of all the extracts obtained, we observed that vindoline is present, in a significant amount, in extracts CR-L-S-A (100% CO₂), CR-L-S-1 (80% CO₂ : 20% EtOH) and CR-L-S-AN20₁₋₃ (80% CO₂ : 20% EtOH+ NH₃).
- It was also observed that the extracts contain vindoline when the concentration of the CO₂ is high such as 100% and 80%. In all cases of 50% EtOH and 80% EtOH, vindoline's signals do not seem to appear in the ¹H-NMR spectrum.



Acknowledgement:

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