Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2019, 11(6):33-40

Research Article

ISSN: 0975-7384

CODEN(USA): JCPRC5

Screening of Available Components of Egyptian *Catharanthus Roseus* Cultivars using HPLC-MS

Amal M Zweil*, Ibrahim A Ibrahim and M Bekhit

Genetic Engineering and Biotechnology Research Institute, University of Sadat City, Egypt

---

ABSTRACT

The HPLC method has been successfully applied to detect some indole alkaloids in the three cultivars of Catharanthus roseus plants to facilitate further study of the metabolic pathway and its regulation in Catharanthus roseus plants; the Albus cultivar is the richest in content then Ocellatus and the poorest is Roseu.

While on intensity level of valuable components Ocellatus cultivar has more advantages than Albus and Roseus respectively. Albus cultivar had the highest content of vincristine (1.5 fold) and catharanthine (1.6 fold); while Ocellatus cultivar had the highest content of vinblastine (1.7 fold), vindoline (6.6 folds) and tryptophan (1.7 fold); Roseus cultivar had the third grade on all levels. From that we can suggest Ocellatus cultivar then Albus cultivar for tissue culture work as a starting material as they had the highest content.

Keywords: *Catharanthus roseus*; Albus; Ocellatus; Roseus; HPLC; MS; Vincristine; Vinblastine.

INTRODUCTION

Catharanthus Roseus

The Madagascar periwinkle or rosy periwinkle (*Catharanthus roseus* L. G. Don), a member of the family Apocynaceae, is one of the most intensively studied medicinal plants [1-3]. Aerial parts of the plant contain between0.2 and 1% of a mixture of more than alkaloids [3]. The most abundant 120 are the monomers such as catharanthine and vindoline [4].

Figure 1. *Catharanthus roseus* cultivars' (Albus, Ocellatus and Roseus respectively)

The dimeric alkaloids that result from the joining of two compounds can display interesting pharmaceutical activities. Thus, vinblastine and vincristine are used in the chemotherapy of leukemia and in the treatment of Hodgkin's disease [5,6].

Additionally, ajmalicine, a monomeric indole alkaloid presents in the root of *C. roseus*, is an antihypertensive alkaloid [7]. High performance liquid chromatography HPLC) and electron ionization mass spectrometry (EIMS) play a major role in their respective fields, solving a variety of analytical problems They are the extremes of two different technical approaches: The first one typically employs very high pressure for the efficient separation of analytes dis-solved in a sometimes complex liquid phase; the second one, a sophisticated detector for chromatography, measures the mass-to-charge ratio of the analytes and operates at a very high vacuum, a rarefied gas phase with no tolerance for extraneous substances .The two techniques, which in principle appear totally incompatible, may nevertheless show an impressive number of overlapping applications. Small to medium sized molecules, typically under 1000 u, that for several reasons are suitable for HPLC analysis, can generate highly informative, reproducible, library match able EI mass spectra for an easier identification and characterization. Thousands of these compounds can be found in many different areas of Interest. Therefore, a single analytical tool that combines the environmental, biological, or pharmaceutical techniques without limiting their potential is highly desirable. In the Direct-EI, each instrument becomes the natural completion of the other without limitation of the specific potential of each. In addition, liquid chromatography can take advantage of the added specificity offered by the electron ionization detection and mass spectrometry can benefit from a simple and reliable inlet system for liquid samples [2].

A variety of drugs such as Velsar®, Velban®, Velbe® (vinblastine sulfate), etc. con- tain vinblastine as a major component. Also, many commercially available drugs such as Oncovin®, Vincasar PFS®, Vincrex®, and Marqibo® (vincristine sulfate) contain vincristine as a major component. These drugs are recommended for the treatment of blood cancer and hypertension. These drugs are produced by international companies such as Eli Lilly and Company, Teva Pharmaceutical Industries, Bristol-Myers Squibb Pharmaceutical, Spectrum Pharmaceuticals, Adva Care Pharma, Talon Therapeutics, Fresenius Kabi, Adria Laboratories, and Hospira, Inc.The consumption of vinblastine and vincristine worldwide in 2005 was estimated with total value of US$ 150–300 million [8]. These important anticancer compounds, i.e., vinblastine and vincristine, were derived from the coupling of catharanthine with vindoline. Catharanthine is produced almost exclusively in the wax exudates on the leaf surface, whereas vindoline is accumulated in specialized internal leaf cells, suggesting a hypothesis of the involvement of transport processes for their coupling to take place [6]. Another major alkaloid is ajmalicine or raubasine which have a broad application in the treatment of circulatory diseases, especially in the relief of obstruction of normal cerebral blood low [5].

The aim of this study is screening of *Catharanthus roseus* cultivars' available components to detect indole alkaloids in the three cultivars (Albus, Ocellatus and Roseus) to facilitate further study of metabolic engineering and its regulation.

MATERIALS AND METHODS

This work had been done in Genetic Engineering and Biotechnology Research Institute's (GEBRI), University of Sadat city.

Catharanthus cultivars Albus, Ocellatus and Roseus (as shown in figure 1) were collected from GEBRI's greenhouse (obtained kindly from the farm of faculty of pharmacy Cairo university), plants identified and confirmed kindly by: prof. Dr. Kassem Fouad Kassem El-Sahhar, Department of Agricultural Botany, Faculty of Agriculture, Cairo University; and prof. Dr. M.Nabil El- Hadidi,Cairo Herbarium, Faculty of Science Cairo University, Egypt) samples were collected at the beginning of the flowering stage.

Fresh tissues (30 g) of each cultivar were homogenized with liquid nitrogen in a mortar then extracted by HPLC grade methyl alcohol 60 ml then stored in-20°C.

Then the filtrate was analyzed by HPLC as described previously [8,9].

HPLC-MS SYSTEM

The HPLC-MS system consisted of electrospray ionization (ESI)

Separation was carried by a Dionex bounded silica C18 column (4.6× 150 mm 3 um),100 µL of extract of each sample dissolved in 1 ml of methyl alcohol to carry out analysis, after that samples were filtrated by Teflon 0.22 membrane filter and used for analysis.

Method

Solvents: Methyl alcohol, acetonitril, formic acid, and high purity water (420:252:1:227 v/v/v/v), as mobile phase, Flow rate 1ml/min, Volume 10 µL, Run time 40 min, temperature 25°C, MS/MS ESI. Nitrogen was used as nebulizing gas at a pressure of 50 PSI and the flow was adjusted to 11 l/min. the heated capillary was maintained at 25°C.

RESULTS AND DISCUSSION

Identification of Compounds using HPLC-MS

Compounds were identified by comparing the retention indices of their peaks with literature values, and finally confirmed by comparison of mass spectra of the peaks with published data and computer-matching with Wiley and NIST libraries.

Phytochemical research typically involves isolation and Characterization of secondary metabolites from plant tissues, and since they are often non- volatile and polar substances, liquid chromatographic (LC) separation and analysis is employed [10].

Regular photometric detection methods in LC are usually satisfactory when the interest is focused on one substance at a time, which is isolated and characterized from starting material.

However, the interest has increasingly been directed to biochemical studies, where whole metabolic routes and consequently series of closely related compounds should ideally be covered simultaneously [11-15].

Table 1. representing major of *Catharanthus roseus* cultivar Albus content of compounds of interest

S	Name	Chemical formula	MW	Comp. no.	MZ	Rt	Abundance
1	Tryptophan	C11H12N2O3	220	4	218.9+1	4.3	148756
2	Catharanthine	C12H24N2O2	336	432	335+1	9.7	39809
3	Vincristine	C46H56N4O10	824	741	823.1+1	13	26394
4	Vinblastine	C46H58N4O9	810	1422	810.8+1	21	4240
5	Vindoline	C25H32N2O6	456	1733	456.8+1	24.4	4423

from table (1) we can see that tryptophan and catharanthine gave the highest abundance (inensity) in albus cultivar while vindoline and vinblastine gave the lowest ,but vincristine gave the moderate intensity.

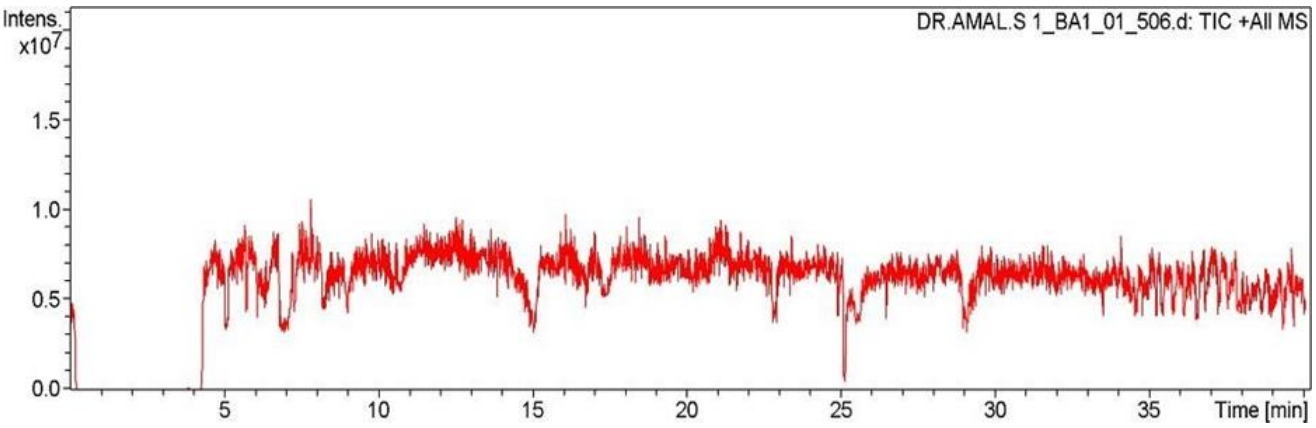

Figure 2. chromatogram of *Catharanthus roseus* cultivar Albus representing total 2962 compounds

Total compounds identified in Albus cultivar 2962 compounds

Table 2. representing major of *Catharanthus roseus* cultivar Ocellatus content of compounds of interest

S	Name	Chemical formula	MW	Comp. no.	MZ	Rt	Abundance
1	Vindoline	C25H32N2O6	456	260	455.1+1	4.1	29176
2	Vinblastine	C46H58N4O9	810	294	810.5+1	4.6	72591
3	Tryptophan	C11H12N2O3	220	380	220.9+1	5.9	253144
4	Catharanthine	C12H24N2O2	336	477	336.5+1	7.3	25562
5	Vincristine	C46H56N4O10	824	1397	824.7+1	20.6	17166

from table (2) we can see that Ocellatus cultivar contains the highest tryptophan abundance then all other compounds gave moderate content.

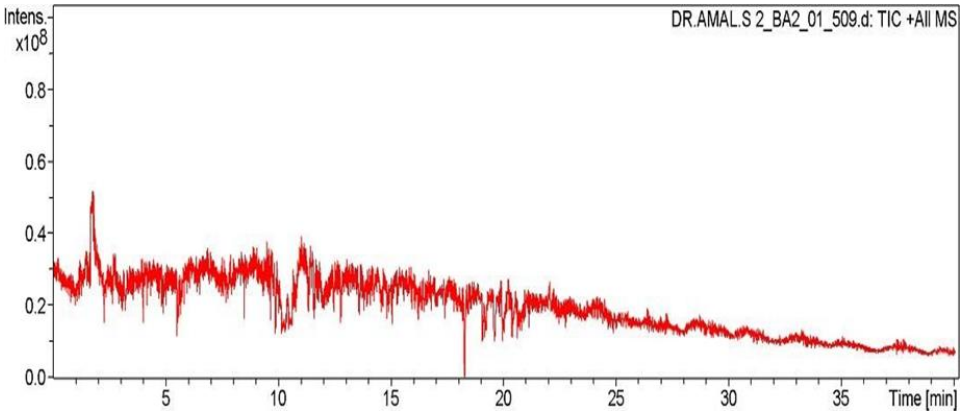

Figure 3. chromatogram of *Catharanthus roseus* cultivar Ocellatus representing total 2866 compounds

Total compounds identified in Ocellatus cultivar 2866 compounds.

Table 3. Representing major of *Catharanthus roseus* cultivar Roseus content of compounds of interest

S no	Name	Chemical formula	MW	Comp. no.	MZ	Rt	Abundance
1	Vincristine	C46H56N4O10	824	91	824.3+1	1.6	7423
2	Catharanthine	C12H24N2O2	336	181	336.3+1	3.5	7651
3	Vindoline	C25H32N2O6	456	748	457.4+1	14.1	946
4	Vinblastine	C46H58N4O9	810	762	810.8+1	14.7	1791
5	Tryptophan	C11H12N2O3	220	873	220.9+1	17.6	19793

from table (3) we can see that has high level, vincristine and catharanthine gave moderate amounts while vinblastine and vindoline gave lowest content in Roseus cultivar.

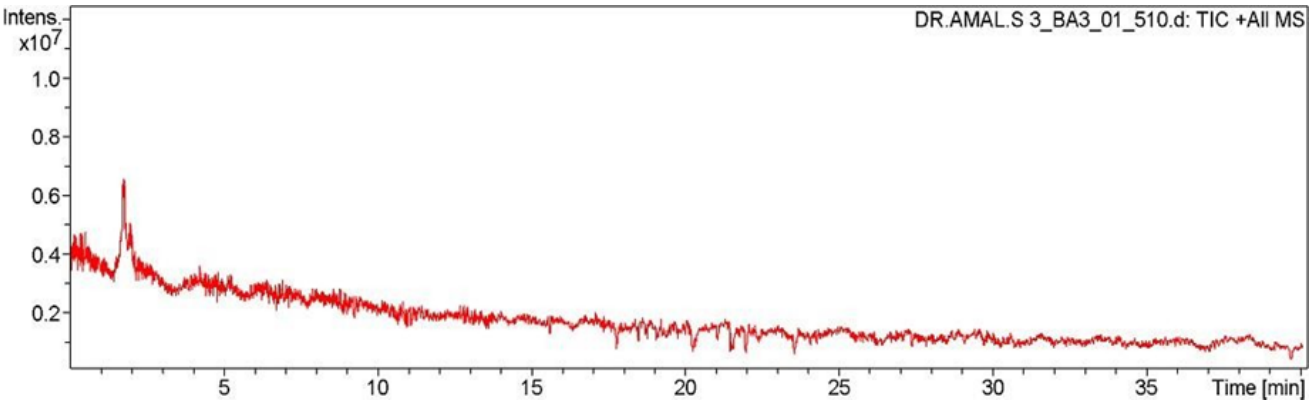

Figure 4. Chromatogram of *Catharanthus roseus* cultivar roseus representing total 1582 compounds.

Total compounds identified in Roseus cultivar 1582 compounds.

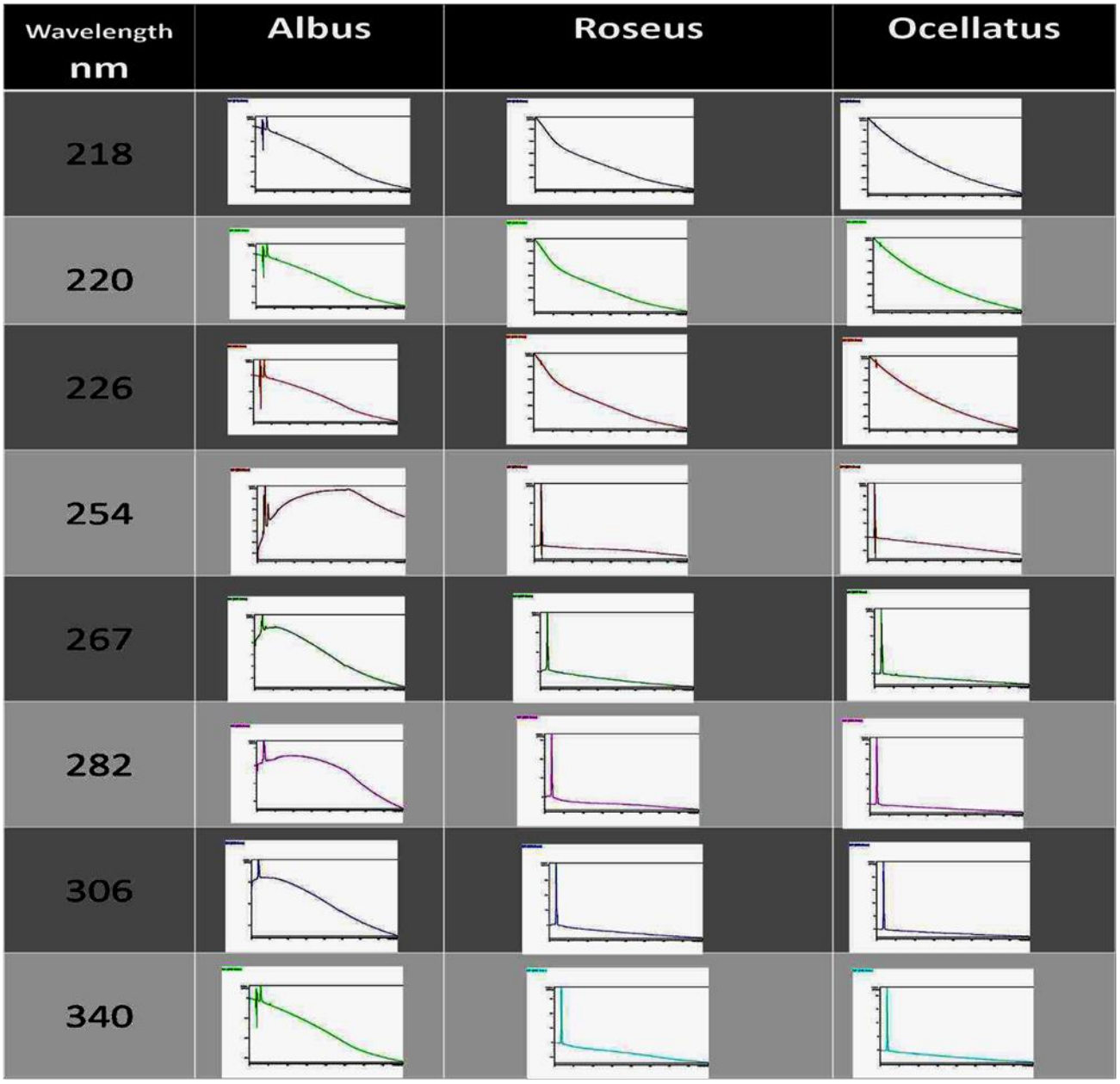

Figure 5. Representing components of the three cultivars under certain eight wavelengths (218,220,226,254,267,282,306 and 340 respectively):

From earlier represented data (tables 1-3, figures 4 and 5) it's very clear that Albus cultivar has more components than Ocellatus and Roseus cultivars

Table 4. Summarizing a comparison between cultivars according to compounds intensity

Cultivar	VLB	VCR	Vindoline	Catharanthine	Tryptophan
Albus	4240	26394	4423	39809	148756
Ocellatus	72591	17166	29176	25562	253144
Roseus	1791	7423	946	7651	19793

From table (4) above, it's so clear that Ocellatus cultivar has more advantages in high content of compounds of interest than albus and roseus respectively.

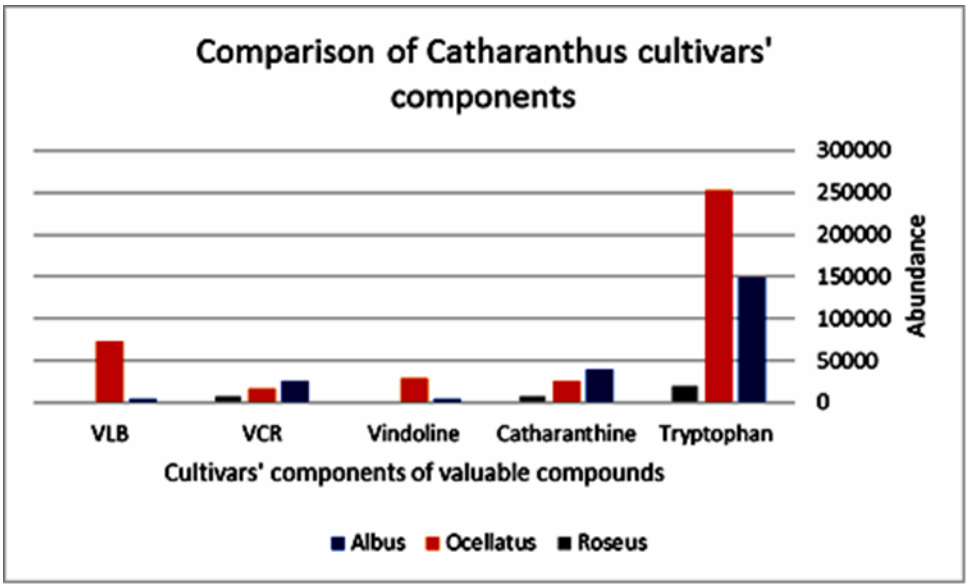

Figure 6. A chart representing a comparison Summary of components of *Catharanthus roseus* cultivars (Albus, Ocellatus and roseus) of valuable compounds

Albus cultivar had the highest content of vincristine (1.5 fold) and catharanthine (1.6 fold); while Ocellatus cultivar had the highest content of vinblastine (1.7 fold), vindoline (6.6 folds) and tryptophan (1.7 fold); Roseus cultivar had the third grade on all levels. From that we can suggest Ocellatus cultivar then Albus cultivar for tissue culture work as a starting material as they had the highest content (Figure 6).

CONCLUSION

From this study we can conclude that:

The albus cultivar is the richest in content then ocellatus and the poorest is roseus as total compounds identified in albus cultivar 2962 compounds while in ocellatus cultivar 2866 compounds and in roseus cultivar 1582 compounds. While on intensity level of valuable components ocellatus cultivar has more advantages than albus and roseus respectively. These results are aligning with [13] findings.

REFERENCES

[1] M Sottomayor; M Lopez-Serrano; F DiCosmo; A Ros-Barcelo. *FEBS Lett*. **1998**, 428, 299-230.

[2] R Van der Heijden; DJ Jabos; W Snoeijer; D Hallard; R Verpoorte. *Curr Med Chem*. **2004,** 11, 1241-1253.

[3] JH Renault; JM Nuzillard; G Le Croue´rour; M Ze`ches-Hanrot; L Le Men-Olivier. *J Chromatogr A*. **1999,** 849, 431-421.

[4] A Cappiello; G Famiglini; F Mangani; P Palma. *J Am Soc Mass Spectrom.* **2002**, 13, 265-273.

[5] MS Hanafy; MA Matter; MR Rady. Production of Indole Alkaloids in *Catharanthus roseus* L. Hairy Root Cultures. Production of Plant Derived Natural Compounds through Hairy Root Culture, **2017**.

[6] F Ferreres; DM Pereira; P Valentao; JMA Oliveira; J Faria; L Gaspar; M Sottomayor; PB Andrade. J Pharm Biomed Anal. **2010,** 51, 65-69.

[7] AI Ibrahim; AAl Hemeida, AM Hussain; M Bekhit; AA Zweil. J Product Dev. **2013**, 18(1), 77-84.

[8] E Ellis. *Canadian J Botany*. **1984,** 62(12), 2912-2917

[9] RL Noble. *Biochem Cell Biol*. **1990**, 68, 1344-1351.

[10] R Verpoorte; AB Svendsen. Chromatography of Alkaloids, **1964**: Part 6: Gas-Liquid Chromatography and High-Performance Liquid Chromatography, pp. 331. Elsevier Amsterdam.

[11] H Zhou; Y Tai; C Sun; Y Pan. *Phytochem Anal*. **2005,** 16, 328-333.

[12] FM El-Domyati; AZ Abdel Azeiz; A Atef; A Elhalwagi; JSM Sabir; AM Ramadan; NO Gadalla; S Edris; MN Baeshen; NH Hajrah; MA Al-Kordy; ASM Al-Hajar; CC Akoh; A Bahieldin. *Life Sci*. **2014**, 11(10), 1213-1220.

[13] C Tikhomiroff; M Jolicoeur. *J Chromatogr A*. **2002**, 955, 87-93.

[14] J Murata; V De Luca. *The Plant Journal.* **2005**, 44: 581-594.

[15] M Magnotta; J Murata; J Chen; V De Luca. Metabolic, Biochemical and Molecular Profiling of Catharanthus roseus Flower Cultivars and Transformed Hairy Roots for Monoterpenoid Indole Alkaloid Accumulation .MSc. thesis Brock University St. Catharines, Ontario. **2007**.