Study on Chemical Investigation of Flowers and Leaves of *Catharanthus Roseus* L.

Ei Ei Sann[1], San San Aye[2], Ni Ni Than[3]

Abstract

Phytochemical investigation of *C. roseus* (Thin - baw - mahnyo - ahni) was carried out in this study. The preliminary phytochemical test revealed the presence of alkaloid, terpenoid, steroid, flavonoid, phenolic compounds, tannin, carbohydrate and glycosides compounds in leaves of *C. roseus*. The coloured pigments, anthocyanin, present in the pinkish-red flower of *C. roseus* was analysed in terms of anthocyanidin. The mineral contents such as K, Mg, Ca, Fe, Na, Zn and Cu were determined by atomic absorption spectroscopy (AAS) method from the leaves powder. Three compounds namely, petunidin, malvidin and kaempferol were isolated from flowers. Moreover, Ursolic acid was also isolated from leaves. The isolated compounds were identified by TLC and spectroscopic method.

Keywords: Phytochemical test, mineral contents, anthocyanidin

Introduction

Plants, mainly used for variety of disease related to cancer treatment. Plants produce several secondary metabolities including alkaloids, flavonoids, saponins, steroids, glycosides and terpenoids to protect themselves from the attack of naturally occurring pathogen, insects, pests and environmental stresses (Cragg *et al*., 2005). *Catharanthus roseus* L. (Thin - baw - mahnyo - ahni), commonly known as bright eyes, Cape periwinkle, graveyard plant, Madagascar periwinkle, rose periwinkle is a species of flowering plant in the dogbane family Apocynaceae (Marcone C., *et al*, 1997). *Catharanthus roseus* L. is an evergreen sub herb plant growing to 1 m tall. The leaves are oval of oblong, 2.5-9.5 cm long. The flowers are white to dark pink with a dark red center, with a basal tube about 2.5-3 cm long and a corolla about 2-5 cm diameter with 5 petals like lobes (Monika *et al*., 2013). There have been a great number of research papers on this plant from the viewpoints of Chemistry, Pharmacology and Botany in the foreign Literature. Especially, Chemical constituents and medicinal values reported on the plant are particular interest. In Myanmar, *C.roseus* is widely cultivated throughout the country and easily available. Scientific investigation on the chemical constituents present in the locally grown *C.roseus* is still lacking.

Materials and Methods

Plant material

Fresh leaves and flowers of *C. roseus* L. (Thin - baw - mahnyo - ahni) were collected from local garden, Yangon Region. The collected sample was confirmed as *Catharanthus roseus* L. as at Botany Department, Yangon University.

[1] Assistant Lecturer, Dr., Department of Chemistry, University of Yangon
[2] Professor, Dr., Department of Chemistry, Sittway University
[3] Professor and Head, Dr., Department of Chemistry, University of Yangon

Preliminary Phytochemical test

A few grams of dried leaves powder were subjected to the tests of alkaloids, α-amino acids, carbohydrates, flavonoids, glycosides, phenolic compounds, tannins, steroids and terpenoids according to the standard procedures, (Marini Bettolo, 1981 and Harborne, 1984).

Determination of elements

For determination by AAS, about 0.1 g of ash sample was accurately weighed and dissolved in 2 cm^3 of concentrated hydrochloric acid. The resulting solution of ash sample was evaporated to dryness and dissolved in 6 cm^3 of 25 % HCl solution (volume by volume) followed by centrifugation. The centrifuged solution was decanted and the clear solution was made up to 100 cm^3 with deionized water. The resultant solution was ready for analysis of mineral elements by AAS.

Preparation of anthocyanidin

The fresh petals (20 g) were dissolved in 2M HCl acid solution for 45 minutes on a water bath and filtered. The filtrate was partitioned with ethyl acetate. Then, the ethyl acetate insoluble layer was partitioned with amyl alcohol. When the amyl alcohol layer was evaporated to dryness, anthocyanidins crude extract was obtained (Harborne, 1984).

Isolation of compounds from leaves

The dried powdered leaves of *C. roseus* (100 g) was extracted with ethanol (300 mL). The green alcoholic extract deposited an amorphous mass (A). The concentrated extract when poured into water deposited a further amount of solid material (B). The light yellow amorphous fraction (A) and (B) were combined and dissolved in ethanol, and the alcoholic solution was treated with activated charcoal and filtered. From the clear filtrate, colourless crystals of ursolic acid was obtained.

Results and Discussion

The phytochemical constituents of *C.roseus* (leaves) were investigated by test tube method. Alkaloids, carbohydrates, glycosides, tannins, flavonoids, phenolic compounds and steroids were found to be present in leaves and α-amino acid was not detected.

Crude anthocyanidin extract from *C.roseus* (flowers) was separated by ascending preparative paper chromatography using (conc. HCl: $HCOOH:H_2O$) solvent (Figure 1). On preparative paper chromatography, two pink colour and pale yellow colour were observed. Thus, two pink coloured having R_f values of 0.3 and 0.25 and a pale yellow (R_f = 0.2) were obtained. Each constituent was further analysed by UV and FT IR spectral data. It has been reported that flowers of *C.roseus* contained malvidin, petunidin and kaempferol.

The wavelength of maximum absorption were found at 542 nm for pink fractions, malvidin and petunidin (Figure 2 (a,b)). The maximum absorption at 276 nm for pale yellow fraction, kaempferol) (Figure 2 (c)). The isolated compounds were also compared with reported UV and FT IR spectral data. It appears as dark brown under UV_{254} nm light when chromatographed on TLC plate using PE: EtoAc (1:1, v/v) as solvent system (R_f = 0.54) (Figure 3). In FT IR spectrum (Figure 4) of isolated

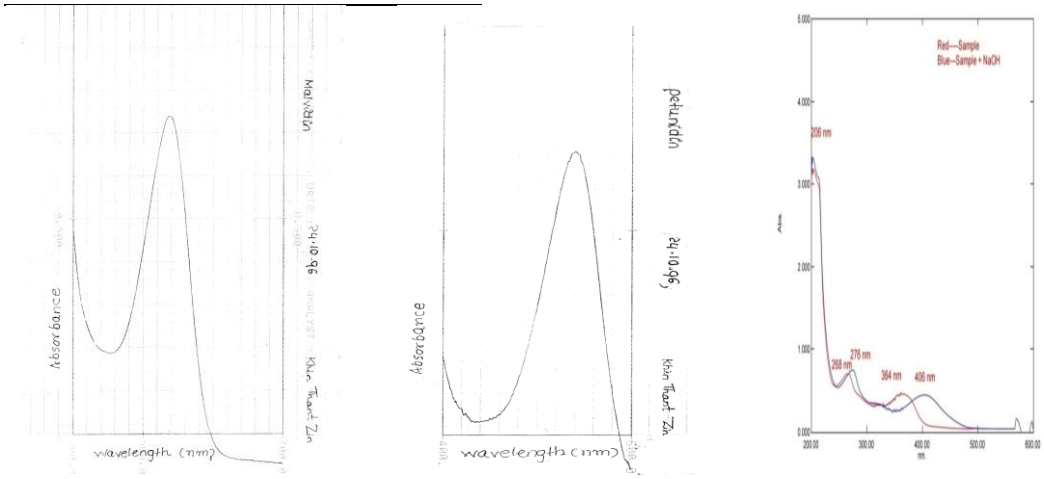
kaempferol at 3425, 3371cm^{-1} (υ_{O-H} of aromatic OH), 1654 cm^{-1} ($\upsilon_{C=O}$), 1612, 1519, 1458 cm^{-1} ($\upsilon_{C=C}$) and 1018 cm^{-1} (υ_{C-O-C} of aromatic CO group).

Ursolic acid (R_f = 0.57, PE: EtOAc, 1:1 v/v, 0.01 % yield) was obtained. It is UV inactive and its melting point (280-281 °C) is similar to that of authentic ursolic acid (m.pt = 285-287 °C) (Merck index, 2001) one of the constituents of *C.roseus* (leaves). It is soluble in pet-ether, ethyl acetate and ethanol but insoluble in water. In addition, authentic ursolic acid and both were observed as pink spots on TLC after spraying with 5% H_2SO_4 by heating (Figure 5). In FT-IR spectrum of ursolic acid (Figure 6) showed absorption bands at 3425 cm^{-1} (υ_{O-H} of COOH), 2923, 2862 cm^{-1} (υ_{asym} & υ_{sym} CH), 1689 cm^{-1} ($\upsilon_{C=O}$ of COOH), 1643 cm^{-1} ($\upsilon_{C=C}$) and 1458 cm^{-1} (δ_{C-O-H} in plane). All the results such as melting point, R_f value, chemical properties and FT IR spectral data of isolated compound were found to be coincident with that of authentic ursolic acid. Therefore, the isolated compound was assigned as ursolic acid.

Table 1. Elemental Contents of *C.roseus* Leaves

Sr.No	Elements	Percentage (%)
1.	Ca	0.078
2.	Zn	0.064
3.	Mg	0.031
4.	Fe	0.015
5.	K	0.151
6.	Na	0.068
7	Cu	0.002

Figure 1. Paper chromatogram of flowers of *C. roseus* (Thin-baw-mahnyo-ahni)



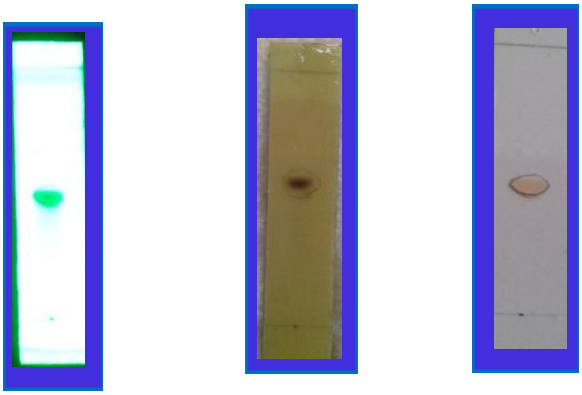
(a) (b) (c)

Figure 2. UV spectra of isolated compound from flowers of *C. roseus*

(a) malvidin

(b) petunidin

(c) kaempferol



(a) **(b)** **(c)**

(Under UV-254 nm) **(10 % $FeCl_3$)** **(5 % H_2SO_4)**

Solvent system = PE :EtOAc (1:1)

Spraying reagent = 10 % $FeCl_3$ and 5 % H_2SO_4

R_f = 0.54 (UV active)

Figure 3. TLC chromatograms of an isolated kaempferol

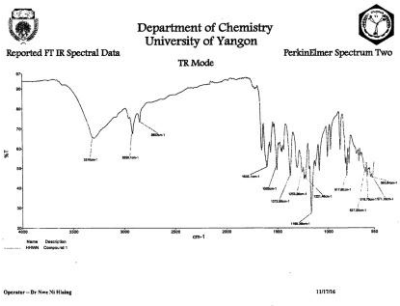
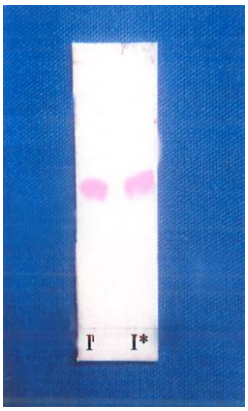


Figure 4. FT IR spectrum of isolated kaempferol



I = ursolic acid

I* = authentic ursolic acid

Solvent system : PE : EtOAc

1 : 1 v/v

Spraying reagent : 5 % H_2SO_4, Δ

R_f value : 0.57

Figure 5. TLC chromatogram of an isolated ursolic acid

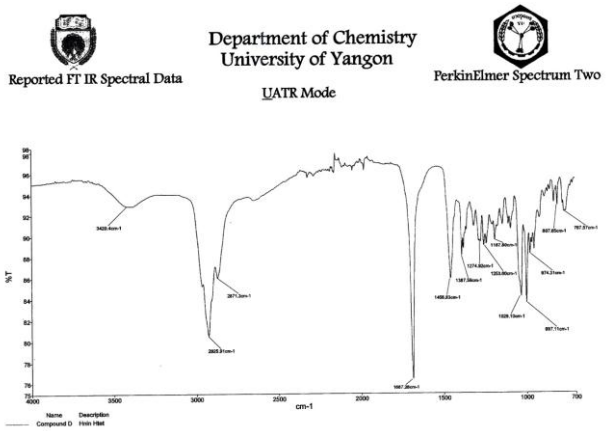


Figure 4. FT IR spectrum of isolated ursolic acid

Conclusion

From the present research work on "Study on Chemical Investigation of Flowers and Leaves of *Catharanthus roseus* L.", the following conclusions can be drawn. The phytochemical investigation of the selected *C. roseus* (leaves) revealed the presence of alkaloids, flavonoids, glycosides, carbohydrates, tannins, phenolic compounds, steroids but α-amino acid was not detected. Elemental analysis of plant sample by AAS method, Ca, K, Mg, Fe, Na, Cu and Zn were present as essential trace elements in selected leaves sample. By paper chromatographic separation technique, cyanidin was isolated and confirmed by spectroscopic method and also by comparing with its reported data. The colourful flowers are distinct characteristics of the plant. The flower indicates the presence of anthocyanidin compounds, namely petunidin and malvidin and flavonoid aglycone compound, namely kaempferol. In the present work, one triterpenoid compound: ursolic acid (0.01 %, m.pt = 280-281 °C) from deposition of 95 % EtOH extract of leaves. The isolated compounds were characterized by some physical and chemical properties and identified by UV and FT IR spectroscopic methods.

Acknowledgements

The authors would like to express their profound gratitude to the Department of Higher Education (Yangon Office), Ministry of Education, Yangon, Myanmar, for provision of opportunity to do this research. We also wish to express our grateful thanks to Dr Pho Kaung, Rector of University of Yangon.

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