ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Preliminary Phytochemical screening and HPTLC finger printing of leaf extracts of Cathranthus roseous

Anubha Arora
Department of Botany, V.M.K (P.G) College, Mangalore

Manuscript Info

Manuscript History:

Received: 17 August 2015
Final Accepted: 26 September 2015
Published Online: October 2015

Key words:

Catharanthus roseus , Phytochemical , Screening, HPTLC finger printing

Corresponding Author

Anubha Arora

Abstract

HPTLC is an analytical technique used for the qualitative and quantitative evalution of polyherbal formulations. *Catharanthus roseus* an important medicinal plant wide medicinal value is frequently used in a large no of traditional herbal preparations. For HPTLC statinory phase was silica gel 60 F254 plate. The mobile phase consisted of Toluene: Ethylacetate : Formic acd (7:2:0.5). In the present study the Preliminary Phytochemical screening of *Catharanthus roseus* leaf extraction has been done to dentify the chemical constituents and HPTLC fingerprinting of *Catharanthus roseus* leaf tracts has been performed which may be used as marks for quality evaluation and standardization of the drug.

INTRODUCTION

Periwinkle or *Catharanthus roseus* (L.) G. Don (Family Apocyanaceae), commonly known as "Nayantara" is an erect bushy perennial herb and evergreen shrub. The species was formerly known as *Vinca rosea*. The native of "Periwinkle" is mainly Madagaskar. This plant is grown commercially for its medicinal uses in Australia, Africa, India and Southern Europe. Except the highly alkaline or water logged soil"Periwinkle" does not require any special conditions of soil. It favorably grows in light sandy soil, rich in humus (Kokate 2009). The rainfall of about 100 cm is most suitable for it. The leaf is simple, opposite, estipulate, petiolate and PP Joy (1998) has enlisted phytochemical compositions of *Catharanthus roseus* (L.) G. Don resulting more than 100 alkaloids and related compounds have so far been isolated and characterized from this plant. It is native of West Indies, now commonly grown in gardens throughout India Drug consists of aerial parts of *Catharanthus roseus* (G.don) Apocynaceae . Plants plays an essential role in the health care needs for the treatment of diseases and to improve the immunological response against much pathology. (Borchers *et.al* 2000). *Catharanthus roseus* is an evergreen herbaceous plant growing to 1 m. tall. The leaves are oval to oblong, 2.5- 9.0 cm. long and 1- 3.5 cm. broad glossy green hairless with a pale midrib and a short petiole about 1- 1.8 cm. long and they are arranged in the opposite pairs. 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 five petal like lobes. The fruit is a pair of follicles about 2-4 cm. long and 3 mm broad.

Vernacular name:- **Sans:** Nitykaylani**; Hind**: Sadabahar; **Beng:** Nayantara; **Tam:** Sudukattu mallikai; **Eng:** Madagascar periwinkle

Systematc Position:-

Plantae
Magnoliophyta
Magnoliopsida
Gentianales
Apocynaceae

Catharanthus
Catharanthus roseus

Chemical constituents

The root contain d-yohimbine ajmalicine and alstonine, urosolic and olenolic acid and bornesitol.The root bark contains Vincaline I & II and serpentine and alstonine. The leaves contain leurosin, vindoline , Cartharanthine , lochnerine, tetrhydrolastonine, coronaridine, Vincarodine, catharanthamine, 21- oxo- leurosine,leurosidine - Nb-oxide, pericylivine, Vimblastine and Vincristine (Cordell 2001). The flowers yield alkaloid and non-alkaloid constituents and leurozine. The seeds contain Vinsedine, Vinsedicine and loganic acid. Besides, the plant has been reported to contain vincarodine, vincoline, leurocolombine, vinamidine, vincathicine, vincubine, 2 secoiridoid glycosides viz; secologanic acid and secologanoside , monoterpenoids glucosides, loganin, deoxy & dehydraloganin , sweroside with iridoid –like structure(Ghani 2003). Asolkar *et . al.* (1992) also reported vincristine and vinblostine from this plant help in controlling leukemic in children.

(

Agarwal, 1986) and bitter, acidic and stomachic (Warrier *et.al* 1994) and are used as tonic. The leaves in the form of an infusion are administered in menorrhagia and their juice is good for wasp- stings (Chopra *et.al* 1958). The whole plant is hypotensive, sedative and tranquiliser (Warrier *et.al* 1994) and is used as a safe remedy for diabetes. An extracts from the dried or wet flowers and leaves of plants are applied as a paste on wounds in some rural communities. The fresh juice from the flowers of *C. roseus* made into a tea has been used by Ayurvedic physicians in India for external use to treat skin problems, dermatitis, eczema and acne.

Method and materials: -

The plant material was collected from Roorkee district of Haridwar, Uttarkahand The samples were washed thoroughly to remove dirt particles present on the surface. The samples were then sun dried and cut into small pieces and plant identified with the help of Duthie flora (1903-1929) and Maheshwari (1962).

Qualitative phytochemical analysis

The crude powder of different flowers was subjected to qualitative phytochemical analysis describe by Gibbs, 1974; Johansen, 1955; Harborne, 1973; Paris, 1963; and Peach and Tracey , 1955.

- **Raphides :-** Hand sections of young shoot and petiole were cut treated with a saturated aqueous solution of cupric acetate. The crystals of calcium oxalate dissolved and oxalic acid so released diffused in to the intercellular spaces and yellow crystals of cupric oxalate appeared (Johansen, 1940).
- **Flavonoids:-**Alkaline reagent test was performed for checking the presence of flavonoids. The crude powder of flower was treated with a few drops of diluted sodium hydroxide (NaOH) separately.Formation of intense yellow colour which turned colourless on addition of a few drops of diluted HCl indicated the presence of flavonoids.
- **Tannins:-** The crude powder of flower was treated with alcoholic ferric chloride ($FeCl_3$) reagent. Blue colour indicated the presence of tannins.
- **Saponins:-** The presence of saponins was determined by Frothing test. The crude powder of flower was vigorously shaken with distilled water and was allowed to stand for 10 minutes and classified for saponin content as follows: no froth indicates absence of saponins and stable froth of more than 1.5 cm indicated the presence of saponins.
- **Steroids:-** Liebermann-Burchard reaction was performed for checking the presence of steroids. A chloroformic solution of the crude powder of flower was treated with acetic anhydride and a few drops of concentrated H_2SO_4 were added down the sides of the test tube. A blue green ring indicated the presence of steroids.
- **Cardiac glycosides:-** Keller-kiliani test was performed for checking the presence of cardiac glycosides. The crude powder of flower was treated with 1.0 ml mixture of 5% $FeCl_3$ and glacial acetic acid. To this solution, a few drops of concentrated H_2SO_4 were added. Appearance of greenish blue colour within few minutes indicated the presence of cardiac glycosides.
- **Alkaloids:-** The crude powder of flower was dissolved in 2 N HCl. The mixture was filtered and the filtrate was divided into 3 equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent and the other portion was treated with equal amount of Wagner's reagent. The creamish precipitate, orange precipitate and brown precipitate indicate the presence of respective alkaloids.

The preliminary qualitative phytochemical investigation of *Catharanthus roseus* leaf was performed which showed the presence of Flavonoids, Tannins, Steroids, Alkaloids by Dragondroff's reagent, Wagner's reagent and Mayer's reagent (Table 1).

HPTLC PROFILE:- Preparation of extract 1 gm of powdered plant material was extracted in soxhlet apparatus with methanol, individually on a water bath, filtered and made up to 10 ml in a standard flask. The sample were spotted in the form of width 8.00 mm with a CAMAG 100 µl sample (Hamilton , Bonaduz, Switzerland) syringe on silica gel per coated Aluminum plate 60 F-254 plates, (20cm × 10 cm with thickness , E Merk ,Darmstadt , Germany) using CAMAG Linomat V (Switzerland) sample applicator. The plates were prewashed with methanol and activated at 110°C for 5 min prior to chromatography. A constant application rate was used and the space between two bands was 8 mm. The slit dimension was kept at 6.00 mm×0.30 mm and the scanning speed was 20 mm/s. The mobile phase consisted of Toluene: Ethylacetate: Formic acid (7:2:0.5) and 10 ml of mobile phase was used per chromatography run. Linear ascending development was carried out in a 20 cm × 10 cm twin through glass chamber (CAMAG, Muttenz, Switzerland) saturated with the mobile phase. The optimized chamber saturation time for the mobile was 20 min at room temperature 25°C. Following the development the TLC plates were dried in a current of ar with the help of an air dryer in a wooden chamber with adequate ventilation . The flow rate in laboratory was maintained unidirectional (Laminor flow , towards the exhaust). Densitometric scanning was performed using a CAMAG TLC scanner III in the reflectance absorbance mode at 254 nm and operated by CATS Software (V 3.15, CAMAG).Concentrations of the compound chromatographed were determined from intensity of the diffused light. Evalution was by peak areas with linear regression.

Step involved in HPTLC

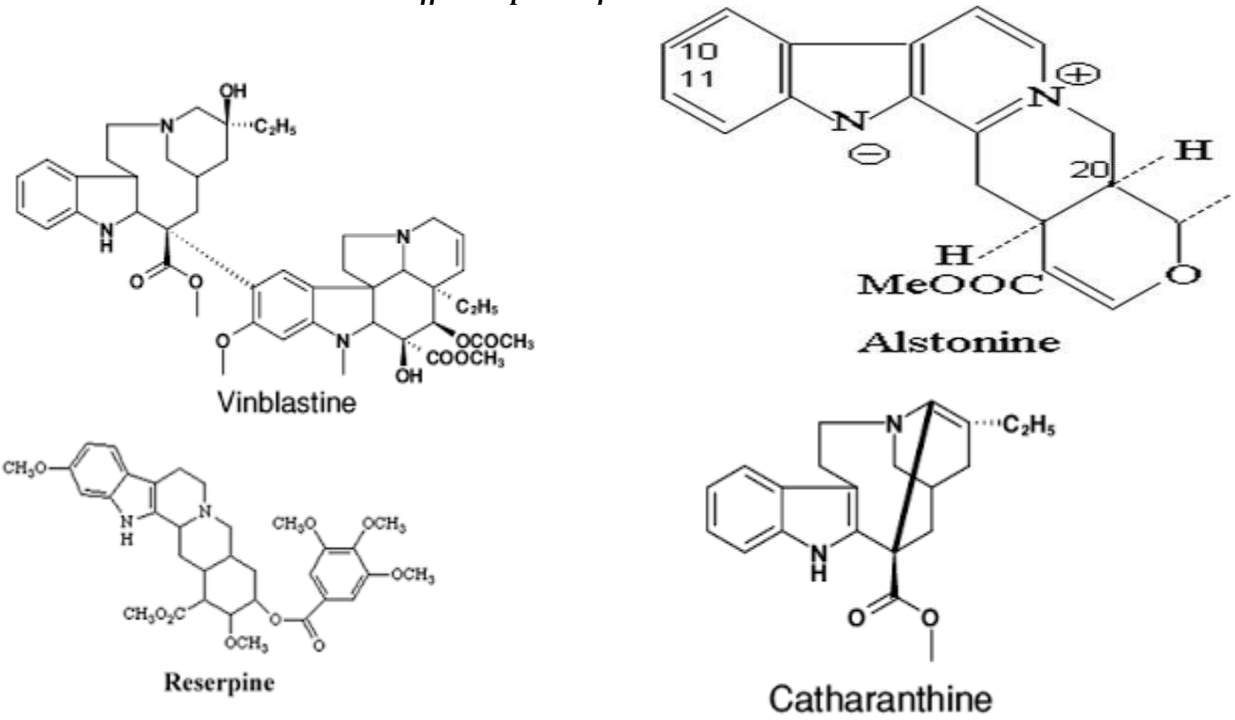
Section of chromatographic layer
↓
Sample collect and standard preparation
↓
Pre- washing Layer
↓
Layer pre- conditioning
↓
Application of sample and standard
↓
Development of Chromatography
↓
Spots dection
↓
Scanning
↓
Documentation of Chromatic layer

The results from HPTLC finger print scanned at wavelength 275nm for methanolic extract of *Catharanthus roseus* leaf showed 8 polyvaent phytoconstituents and corresponding ascending order Rf values start fom 0.25 to 0.99 in which highest concentration of the phytoconstituents was found to be 21.22 % and its corresponding Rf value was found to be 0.38 respectively and was recorded in Track .1 . The corresponding HPTLC chromatogram was present in Track.1

Conclusion :- The initial study was carried out HPTLC and result show that there are many compound are present *in Catharanthus roseus* From the HPTLC has been found that methanolic extracts contain not a single compound but mixtures of compound are present. This method is especially sutiable for the finger printing and analysis of botanical samples and herbal formulations.

I wish to express my sincere gratitude to principal and management V.M.K.P.G.College , Manglore , Haridwar, for providing me an opportunity to do my work. I am short of words to thanks Arbo pharmaceuticals for helping me in the analytical results.

Different species of Catharathus roseus

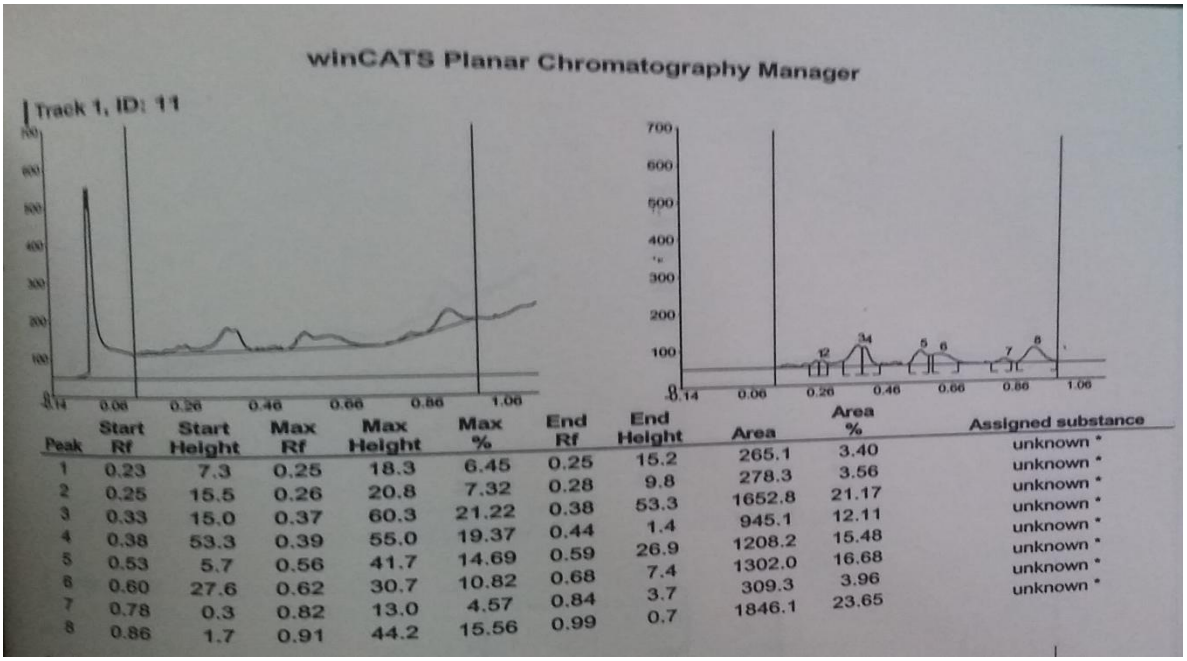


Folkloric use

The roots are sedative and tranquiliser

Table:-1 preliminary phytochemical screeing of Methanoloic extracts of C. roseus

constituents	Test	Methanolic extracts
Alkaoids	Mayer reagent	+
	Dragendorff reagent	+
	Hager reagent	+
	Wagner reagent	+
Glycosides	Keller-killiani test	+
Flavonids	Shinoda test B	+
Saponin	Foam test	+
Tannin	Ferric chloride test	+
Raphids	Calcium carbonate	+
Steriods	Liebermann-Burchard	+



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.23	7.3	0.25	18.3	6.45	0.25	15.2	265.1	3.40	unknown *
2	0.25	15.5	0.26	20.8	7.32	0.28	9.8	278.3	3.56	unknown *
3	0.33	15.0	0.37	60.3	21.22	0.38	53.3	1652.8	21.17	unknown *
4	0.38	53.3	0.39	55.0	19.37	0.44	1.4	945.1	12.11	unknown *
5	0.53	5.7	0.56	41.7	14.69	0.59	26.9	1208.2	15.48	unknown *
6	0.60	27.6	0.62	30.7	10.82	0.68	7.4	1302.0	16.68	unknown *
7	0.78	0.3	0.82	13.0	4.57	0.84	3.7	309.3	3.96	unknown *
8	0.86	1.7	0.91	44.2	15.56	0.99	0.7	1846.1	23.65	unknown *

Track.1- Chromatogram and Rf Values of Methanolic Extract of *Catharanthus roseus*

REFERENCE

- Agarwal V.S (1986): Economics Plants of India, Kailash Prakashan, Calcutta.
- Asolkar L. V., K.K. Kakkar and O.J. Charke (1992) : Second supplement to Glossary of Indian Medicinal Plants with Active Principles Part I (A-k) Publication & Information Directorate , C.S.I.R., New Delh. 1-414.
- Chopra R.N., I.. Chopra. K.L. Hada and L.D. Kapur (1958): Indigenous drugs of India , U.N. Dur and Sons Pvt.Ltd. Calcutta.
- Cordell GA, Quinn-Beattie ML, Farnsworth NR.(2001). The potencial of alkaloids in drug discovery. Phytother Res 15:183–205.
- Driver, J. E. (1960). Bentley and Driver's Text book of Pharmaceutical Chemistry, 7th Ed. Oxford Univ. Press, London.
- Duthie , J.F. (1903-1929). Flora of Upper Gangetic Plain and of the Adjacent Siwalik and Sub- Himalyan tracts. Calcutta. 3 vols.
- Ghani, A. (2003). Medicinal Plants of Bangladesh with chemical constituents and uses. 2nd edition, Asiatic Society of Bangladesh, 5 old Secretariate road, Nimtali, Dhaka, Bangladesh
- Gibbs, R.D. (1974): Chemotaxonomy of flowering plants Vol I-IV . Mc. Gill. Queens University Press, Montreal, London.

- Harborne ,J.B.(1973). Phytochemical Methods Edn 2, London: Chapman & Hall.
- Johansen, D. A. (1940). Plant Micro technique. Mc Graw Hill Pub. Co. Ltd., New York
- Kokate C.K, Purohit A.P, Gokhale S.B.(2009). A Textbook of Pharmacognosy, Nirali Prakashan, 1(3):27-28.
- Maheshwari ,J.K.(1962). Studies on the Naturalized Flora of India. Proc. Summer School of Botany, Darjeeling, 156-170
- PP Joy, J Thomas, Samuel Mathew, Baby P Skaria. Medicinal Plants, Kerala Agricultural University, Aromatic and Medicinal Plants Research Station Odakkali, Kerala, India, 1998.
- Peach, K. and Tracey, M.Y. (1955). Modern method of plant analysis, Vol. 1-4. Springer-Verlagm Berlin.
- Paris, R. (1963). The distribution of plant glycosides. In Chemical Plant Taxonomy T. Swain (Ed.) Pergaman Press, London. 333- 357.
- Warrier, P.K., V.P.K. Nambiar and E. Ramankutty (1994): Indian Medicinal plant Vol. II Orient Long man. Madras.