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Research Article

Screening of pollen of *Catharanthus roseus* L. – An anticancer plant

Jayshree S. Thaware*[1]
1. **Department of Botany, Seth Kesarimal Porwal College, Kamptee Dist –Nagpur, India**

Abstract

The role of cancer medicines is undoubtedly remarkable owing to the increasing scope and complexity of cytotoxic drugs. In this connection, a large number of phytomedicines tried and quite a good number of them have been used with success, *Catharanthus roseus* is one of them. *Catharanthus roseus*, which provided to the world the most potent weapon in the form of Vincristine and Vinblastine, crystalline alkaloids for the treatment of childhood leukaemia, is also used for the treatment of diabetics by primitive healers.

Pollen is known to have a higher energy investment per gram of organic tissue than other plant parts. For the breeders the pollen is a discrete mobile male partner of the fertilization process in higher plants. It carries the genotype of one partner of the pollination process, which in fact the process which breeder manipulates. In the present investigation, the different phytochemical investigations were carried out of *Catharanthus roseus* pollen grains. Pollen grain was screened for their phytochemical ingredients, total proteins, free amino acids and alkaloids. Pollen grain revealed the presence of steroids, alkaloids, totals pollen proteins, which were extracted spectrophotometrically and was found to be 33.56%. Analysis of free amino acids was carried out and total 17 different amino acids was separated and identified by using thin layer chromatographic method.

Most interesting point is that when pollen was screened for alkaloid detection Vincristine was detected from pollen. As the active alkaloid content of the plant is exceptionally low i.e. approximately 2 tons of crushed leaves yield 1 gram of active alkaloid. Detection of alkaloid for the first time from pollen of *Catharanthus* can prove to be a landmark for pharmaceutical industries.

Key Words: *Catharanthus roseus*, anticancer plant, pollen, phytochemical investigations, Vincristine

***Corresponding Author**

Introduction

Cancer - a dreadful disease

Cancer should be called a symptom rather than disease. It indicates the **abnormal uncontrolled and rapid growth of cells.** Because of this, cancer cells are malignant in nature. Cancer cells differ from normal biological cells mainly in two ways. as long as the host alive, cancer cells are also alive and rapidly multiply themselves to form new cancer cells. They **do not die until host dies.** But in case of ordinary cells, after a cycle they decay automatically.Cancer cell can undergo '**metastasis'**. The term metastasis means transfer of cancer cells from one organ of the body to another not directly connected with it. All **malignant cells are capable of metastasizing,** which means forming new loci in distant part of the body from the original position

The role of cancer medicines is undoubtedly remarkable owing to the increasing scope and complexity of cytotoxic drugs. In this connection, a large number of plants have been tried and quite a good number of them have been used with success[1].

In the recent years, greater emphasis of the plant scientists has been towards the search for plants with antitumour and anti cancerous activities. Er. J. Hartwell (1967-71) of National Cancer Institute, Bethesda, U.S., has assembled data on 3000 plants which possess anticancerous properties. Many of these plants were used by the primitive folk healers against various diseases. ***Catharanthus roseus*** is one of the most important anticancer plants which are the most potent remedy against a particular type of cancer even today.

So far, the only higher plant which is used as a source of patented anticancerous drug is ***Catharanthus roseus.*** The anticancer activity of the alkaloids of this plant was a chance discovery by Nobel et.al. [2] during their investigations against diabetes in Jamaica.

1. The two oncolytic alkaloids isolated from leaves are Vinblastine sulphate (marketed as VENBAN) which is used for treatment of Hodgkin's disease and other lymphomas solid tumours.
2. Vincristine sulphate (marketed as ONCOVIN) which is used in the treatment of acute lymphocytic leukemia which arrest the mitosis in metaphase and other tumours of childhood [3-6].

Aim and object

India has been the major exporter of the drug and the requirement are being met from large scale cultivation of this crop in South India. *Catharanthus roseus* being a hardy crop posed fewer problems in its cultivation. *Catharanthus roseus* has lent itself as a choice candidate for commercial cultivation

Pollen is known to have a higher energy investment per gram of organic tissue than other plant parts. For the breeders the pollen is a discrete mobile male partner of the fertilization process in higher plants. In the present investigation, the different phytochemical investigations were carried out of *Catharanthus roseus* pollen grains.

Pollen grains were screened for their phytochemical ingredients, total proteins, free amino acids and alkaloids.

Observations

Preliminary phytochemical screening of pollen grains

For the phytochemical screening, sufficient pollen material was collected from the *Catharanthus roseus* plant. It was dried, weighed and used for extraction of constituents

Extraction of constituents

The weighed quantity of pollen material was successively extracted with different organic solvents in a Soxalate apparatus and their extractive values are as follows in different solvents

Table 1: Extractive values and Physical Characteristics of *Catharanthus roseus* pollen

Solvent	% of extractive	Colour and appearance of the extractives
Petroleum ether	26.58	Yellowish brown, oily mass
Solvent ether	2.53	Not distinct
Chloroform	11.39	Brown dry mass
Ethanol	32.91	Dark brown mass
Water	1.33	Not distinct

Table 2: Preliminary Phytochemical screening of *Catharanthus roseus* pollen

Tests	Petroleum Ether extract	Solvent Ether extract	Chloroform extract	Alcohol extract	Water Extract
1)TEST FOR STEROIDS					
a) Salkowski reaction	+	+	-	-	-
b) Liebermann's-Burchard reaction	+	+	-	-	-
c) Liebermann's reaction	+	+	-	-	-
2)TEST FOR ALKALOIDS					
a) Dragendroff reagent	-	-	+	+	-
b) Mayer's reagent	-	-	+	+	-
c) Wagner's reagent	-	-	+	+	-
3)TEST FOR SAPONINS					
a) Foam test	-	-	-	-	-
4) TEST FOR GLYCOSIDES					
a) Keller Killiani test	-	-	-	-	-
b) Guignard test	-	-	-	-	-
c) Borntrager's test	-	-	-	-	-
5)TEST FOR COUMARINS	-	-	-	-	-
6)TEST FOR TANNINS					
a) Ferric chloride test	-	-	-	-	-
7)TEST FOR PROTEINS					
a) Biuret test	-	-	-	-	+
b) Millon's test	-	-	-	-	+
8) TEST FOR AMINO ACIDS					
a) Ninhydrin test	-	-	+	+	-
9) TEST FOR CARBOHYDRATES					
a) Molisch test					
b) Fehling's solution test	-	-	-	-	-
	-	-	-	-	-

Extraction of free amino acids

The paper chromatogram of test extract of pollen of plants under study indicated the presence of different amino acids in pollen.

The following amino acids were commonly found in ***Catharanthus*** pollen. They were Crysteine, valine, leucine, methionine, asparatic acid, asparagines, glutamic acid, glutamine, Arginine, lysine, proline, tyrosine, tryptophan and Histidine, Glycine, Alanine and serine

Estimation of total pollen proteins

The total pollen proteins was estimated by Lowry's method in which Ultra violet 118 spectrophotometer was used at 410 nm and the total proteins was found to be 33.56% from Vinca pollen.

Extraction of alkaloids

The weighed pollen sample was basified and was extracted for constituents with chloroform in Soxalate apparatus. Alkaloid detection was done with the Dragendorff's reagent.

The separation and identification of alkaloid by paper chromatography and with thin layer chromatography was done.

Good separation of alkaloid with well defined, compacrt spot was observed in the solvent system namely 1) n-butanol: hydrochloric acid (98:2) saturated with water and 2) n-butanol: hydrochloric acid (94:6) saturated with water .Both the system indicated the presence of only one alkaloid in Vinca pollen. When this alkaloid was compared with the authentic sample of Vincristine it indicated the presence of Vincristine in Vinca pollen.

In Vinca, good separation of alkaloid was obtained in the solvent systems 1) Ethyl acetate: absolute ethanol (3:1) and 2) n-butanol: glacial acetic acid: water (4:1:1). Both the systems showed only one spot of alkaloid. This spot was compact and well defined. When this spot was compared with the authentic Vincristine it showed exactly identical of value. So its presence in ***Vinca*** pollen is confirmed.

Vincristine

The material gave a single spot and the same Rf value (in different solvent system) corresponding to the authentic sample of Vincristine. Vincristine sulphate showed U.V. Maxima in ethanol at 220 nm, 225 named 296 nm. I.R. Spectra was identical to authentic Vincristine. In chloroform characteristic peak was obtained at 5.94 microns.

Conclusions

It is noteworthy that the only higher plant which is used as a source of patented anticancerous drug is ***Catharanthus roseus,*** Vincristine, the most important alkaloid was detected for the first time from the pollen.

The pollen is used as a convenient experimental system in genetic investigations, directed towards plant improvement.

The direct and indirect roles played by pollen in various spheres of applied biological research will be found useful in view of the fact that pollen is a material to work which seems to be providing an easier and even better means for experimentally controlling the genetic behaviors of the plants.

Pollen grains are rich source of phytochemicals. Phytochemical screening was carried out using different standardized tests. Pollen grain revealed the presence of steroids, alkaloids, amino acids, total pollen proteins. Total pollen proteins were extracted spectrophotometrically and were found to be 33.56%. Analysis of free amino acids was carried out and total 17 different amino acids was separated and identified by using paper chromatographic method.

Pollen were screened for alkaloid detection by paper and thin layer chromatography most interesting point is that Vincristine was detected from pollen. Vincristine is used in the treatment of acute leukemia, lymphosarcoma.

As the active alkaloid content of the plant is exceptionally low i.e. approximately 2 tons of crushed leaves yield 1 gram of active alkaloid. Detection of alkaloid for the first time from pollen of ***Catharanthus*** can prove to be a landmark for pharmaceutical industries.

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Table No. 3: Paper chromatography of alkaloid from the pollen of Vinca

Name	Solvent system	Rf value
Vincristine	n-butanol: HCl (94:6) Saturated with water.	0.88
Vincristine	n-butanol: HCl (98:2) Saturated with water	0.90

Table No. 4: Thin layer Chromatography of alkaloids from the pollens of Vinca

Name	Solvent system	Rf value
Vincristine	Ethyl acetate: absolute alcohol (3:1)	0.62
Vincristine	n-butanol: glacial acetic acid:water (4:1:1)	0.60