International Journal of Phytopharmacy **Research Article**
ISSN: 2277-2928 (Online)
Journal DOI:https://doi.org/10.7439/ijpp

Evaluation of fungitoxicity of the extractives of *Catharanthus roseus* (L.) G.Don

Eugene Sebastian J. Nidiry* and **A. N. Lokesha**

ICAR- Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore- 560 089, India

Abstract

The fungitoxic property of the hexane, ethyl acetate and methanol extractives of leaves and roots of *Catharanthus roseus* (L.) G. Don to the mycelial growth of *Colletotrichum gloeosporioides* by poisoned food technique and spore germination of *Cladosporium cucumerinum* by TLC bioautography was evaluated. Among all the extractives, the hexane extractive of the roots exhibited highest activity. The chromatographed fractions of hexane extractive were subjected to spectral studies (UV, IR, NMR and Mass), which indicated maandrosine, ammocalline, mitraphylline and pericalline as probable fungitoxic compounds present in the plant.

Keywords: *Catharanthus roseus* roots, hexane extractive, fungitoxicity, *Colletotrichum gloeosporioides*, *Cladosporium cucumerinum*.

***Correspondence Info:**
Dr. Eugene Sebastian J. Nidiry,
ICAR- Indian Institute of Horticultural Research,
Hessaraghatta Lake,
P.O., Bangalore- 560 089, India

***Article History:**
Received: 04/04/2020
Revised: 28/04/2020
Accepted: 30/04/2020
DOI: https://doi.org/10.7439/ijpp.v10i2.5465

QR Code

How to cite: Nidiry ESJ, and Lokesha AN. Evaluation of fungitoxicity of the extractives of *Catharanthus roseus* (L.) G.Don. *International Journal of Phytopharmacy* 2020; 10(2): e5465. Doi: 10.7439/ijpp.v10i2.5465
Available from:https://ssjournals.com/index.php/ijpp/article/view/5465

1. Introduction

Catharanthus roseus (L.)G. Don belongs to the family of *Apocyanaceae* and is found in many tropical and subtropical regions around the world [1]. This plant produces several pharmaceutically important secondary metabolites which are used as chemotherapeutic agents [2]. Antifungal properties of the plant extractives [3] and ursolic acid [4], 4-hydroxy flavones [5] and pericalline [6] present in the plant have been reported. However, a comparison of fungitoxic efficacy of different extractives of roots and leaves obtained by solvents of varying polarities has not yet been reported. In this paper, comparison of the fungitoxic properties of the extractives of roots and leaves obtained with hexane, ethyl acetate and methanol to the mycelial growth of *Colletotrichum gloeosporioides* by poisoned food technique [7,8] and spore germination of *Cladosporium cucumerinum* by TLC bioautography [9,10] are being reported. Chromatographic separations and spectral studies were attempted to chemically characterize the fungitoxic compounds present in the active fraction.

2. Materials and methods

2.1 Preparation of the extractive

Dried powdered roots and leaves of the plant (100 g each) were separately Soxhlet extracted first with hexane, then with ethyl acetate and finally with methanol. The respective extractives were obtained by completely distilling out the solvents on a water bath.

2.2 Poisoned food technique

Fungitoxic property of the extractives on *Colletotrichum gloeosporioides* was evaluated by the poisoned food technique [7] by the procedure previously reported from this institute [8]. Per cent mycelial growth inhibition was calculated using the formula P = (C-T/C) x 100, where C is the mycelial diameter of the control and T that of treated ones, after giving due adjustment of the mycelial diameter of the inoculum.

2.3 TLC bioautography

The conidial suspension of the fungus *Cladosporium cucumerinum* IMI 249540 in the required medium [9] was sprayed on the TLC plate and observations were taken after an incubation of 4 days [10].

2.4 HPLC

HPLC separation was done in two stages. In the first stage it was done using a Waters HPLC system (515 pump, 7725 Rheodyne injector, Waters 2487 Dual λ absorbance detector) under conditions as follows: Column: Prep Nova Pak HR Silica 7.8 x 300 mm, flow rate: 0.8 ml/min, UV detection at 254 nm, eluent: hexane. In the second stage of HPLC separation (after initial spectral studies and bioautography with *C. cucumerinum*), eluent was changed from hexane to dichloromethane and flow rate was changed from 0.8 ml/min to 1.0 ml/min maintaining other conditions same as those of the first stage.

2.5 Spectral studies

UV spectrum was obtained with Spectronic UV-Visible spectrophotometer. IR spectrum was obtained with a Perkin-Elmer spectrophotometer. ^{1}H NMR spectrum was recorded on a Bruker spectrometer operating at 300 MHz. The HRESIMS was obtained on a Micromass Q-TOF apparatus.

3. Results and Discussion

3.1 Evaluation of fungitoxicity

The results on the fungitoxicity of the extractives are presented in Table 1. The values are the averages of two replications of per cent mycelial growth inhibitions of *Colletotrichum gloeosporioides* on potato-dextrose-agar medium obtained by poisoned food technique [7,8]. The results of the antifungal activity of the extractives for spore germination inhibition of *Cladosporium cucumerinum* done by TLC bio-autography [9,10] are presented in Table 2. The results clearly show that the hexane extractive of the roots showed higher activity than ethyl acetate and methanol extractive indicating that the antifungal compounds present in the plant are non-polar in nature. The results also show that the root extractives show higher activity than leaf extractives.

Table 1: Antifungal activity of the extractives of the leaves and roots of *Catharanthus roseus* for the mycelial growth inhibition of *Colletotrichum gloeosporioides*

Plant part	Solvent used for preparation of the extractive	Conc. of the extractive in the medium (%)	Per cent mycelial growth inhibition
Leaves	Hexane	0.1	2.0
Leaves	Hexane	0.5	9.1
Leaves	Ethyl acetate	0.1	5.4
Leaves	Ethyl acetate	0.5	13.0
Leaves	Methanol	0.1	1.6
Leaves	Methanol	0.5	7.5
Roots	Hexane	0.1	21.2
Roots	Hexane	0.5	31.5
Roots	Ethyl acetate	0.1	19.2
Roots	Ethyl acetate	0.5	30.6
Roots	Methanol	0.1	8.7
Roots	Methanol	0.5	24.3

Table 2: Antifungal activity of the extractives of the leaves and roots of *Catharanthus roseus* for the spore germination inhibition of *Cladosporium cucumerinum*

Plant part	Solvent used for preparation of the extractive	Doses of the extractive spotted and inhibition			Rf value of inhibition spot after elution with EtOAc
		1.00 mg	0.50 mg	0.25 mg	
Leaves	Hexane	+	NI	NI	0.61
Leaves	Ethyl acetate	+	NI	NI	0.72
Leaves	Methanol	NI	NI	NI	NI
Roots	Hexane	+++	++	+	0.62
Roots	Ethyl acetate	++	+	NI	0.78
Roots	Methanol	NI	NI	NI	NI

Observation on *Cladosporium cucumerinum* inhibition taken after an incubation of 4days; Thickness of the plate = 0.5 mm; Diameter of the spot 1.2 cm; + indicates inhibition; ++ indicates conspicuous inhibition; +++ indicates very conspicuous inhibition; NI = No inhibition.

3.2 Chromatographic purification of the hexane extractive

Since the hexane extractive of the roots showed highest activity, it was chromatographed over alumina. The fraction eluted with hexane showed conspicuous inhibition of *Cladosporium cucumerinum* by TLC bioautography. This fraction was further subjected to the first stage of HPLC fractionation.

Four peaks were observed. The fraction corresponding to the 1st peak starting at retention time of 3.4 min and ending at 4.5 min was collected, concentrated and bioassay with *C. Cucumerinum* was performed. It was found to be active. Other fractions did not show activity. The chromatographed fraction of hexane extractive which showed activity was subjected to spectral studies (UV, IR, NMR and MS)

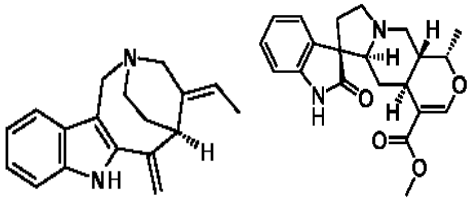
3.3 Spectral data

The hexane extractive gave UV peaks at 202 and 227 nm (shoulder). EIMS spectrum of the fraction showed peaks at m/z 365, 351, 337,323, 309, 295, 281, 267, 253, 239, 225, 211, 197, 183, 169, 155, 141, 127, 111, 97, 85, 71, 57 (base peak), and 43 (all accounted for hydrocarbons) in addition to peaks at m/z 352, 302, 288, 274 and 245 which are unaccounted. It also showed a peak at m/z 264. Its IR spectrum showed peaks at 2923.1 and 2851.6 cm^{-1} (alkyl CH_2 stretching), 1458.5cm^{-1} (aromatic C=C stretching), 1264.4 cm^{-1} (C-O stretching) and 741.5 cm^{-1} (C-H bending). Its ^1H NMR gave signals at 7.95 (d, 2H), 7.70 (d, 1H) and 7.55 (dd, 1H).

From the spectral data it was clear that the fraction is impure with the presence of hydrocarbons. It was treated with 0.1 N HCl, and shaken with hexane to separate the hydrocarbons from the aqueous fraction. The aqueous fraction was neutralized using aq. ammonia and extracted with chloroform. (At this stage, bioassay with *C. Cucumerinum* showed that the hexane fraction containing hydrocarbons did not show activity, but the chloroform fraction was active). The chloroform extract was concentrated and purified by column chromatography over silica gel. Bioassay showed that most of the active fractions were eluted with hexane. The fractions eluted with hexane were concentrated and preparative thin layer chromatography was performed using hexane as the mobile phase. The portion with R_f value 0.9 was found to be active by bioassay with *C. Cucumerinum*. This portion was extracted with hexane and subjected to a second stage of HPLC separation with the following column conditions. Flow rate 1 mL/min., eluent- dichloromethane, wavelength 254 nm, Column- Prep Nova Pak HR Silica 7.8 x 300 mm. The fraction corresponding to the first peak which came at a retention time of 9.1 minutes showed activity against *C. Cucumerinum*. It was concentrated and analysed by HRESIMS. It gave peaks of sodiated ions at 301.1260, 391.1714 and 685.4400.

The spectral data obtained were compared with those compounds from *C. roseus* reported in literature [11-12]. The first UV peak of 202 nm of the fraction is very near to the first UV peak of 204 nm of maandrosine [12] and to 207 nm of pericalline [11] published in the literature. The EIMS spectrum of the active fraction before treatment with 0.1N HCl had shown that it is a mixture of several compounds including hydrocarbons. But a survey of literature on compounds present in *Catharanthus roseus* indicated that the peak at 264 is attributable to pericalline [11]. IR spectrum of the fraction showed a peak at 1458.5 cm^{-1} (aromatic C=C stretching) (reported in literature [12] for pericalline as 1458 cm^{-1}). Its ^1H NMR gave signals at δ = 7.95 (d, 2H), 7.70 (d, 1H) and 7.55 (dd, 1H) attributable to the aromatic protons of pericalline or mitraphylline. HRESIMS of the fraction obtained after the second stage of HPLC showed a peak at m/z 301.1260 which is attributable to sodiated ion of ammocalline [12] having a formula of $C_{19}H_{22}N_2$ and molecular mass of 278 and the peak at 391.1714 is attributable to sodiated ion of mitraphylline [2] having a molecular mass of 368. It may be noted that in the HRESIMS taken after the second stage of HPLC purification, the peak corresponding to sodiated ion of pericalline (expected at 287) was missing. The peak at 685.4400 in the HRESIMS remains unaccounted.

Thus the spectral data showed that the fraction is a mixture of several compounds. The data did not give any conclusive evidence on the structure of fungitoxic compounds present in the plant. Probably more extensive studies commencing with larger amount of plant material and several stages of HPLC purification are required to accurately pinpoint chemical nature of fungitoxic compounds present in *C. rosues.* But the present studies indicate the presence of earlier reported maandrosine, ammocalline, pericalline and mitraphyllinein the active fraction. It may be noted that among these four compounds maandrosine and ammocalline have not yet been fully characterized. Fungitoxicity of pericalline has already been reported [6]. The structures of pericalline and mitraphylline are given in Figure 1.



(i) (II)

Figure 1: Pericalline (I) and Mitraphylline (II), probable antifungal compounds present in *Catharanthusroseus*roots

4. Conclusions

It may be noted that among the different extractives of roots and leaves of *Catharanthus roseus* investigated, the hexane extractive of the roots showed maximum fungitoxicity to the mycelial of *Colletotrichum gloeosporioides* and spore germination of *Cladosporium cucumerinum.* Chromatographic separations and spectral studies indicated the probable presence of earlier reported alkaloids maandrosine, ammocalline, pericalline and mitraphylline in the active fraction.

Acknowledgement

The authors thank The Director, ICAR-Indian Institute of Horticultural Research, Hessaraghatta Lake P.O., Bangalore for his encouragement in the studies.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Abbreviations

UV: Ultra Violet; **IR**: Infra Red; **NMR:** Nuclear Magnetic Resonance; **HRESIMS**: High Resolution Electron Spray Ionization Mass Spectrometry; **TLC:** Thin Layer Chromatography; **HPLC:** High Performance Liquid Chromatography.

References

[1]. Heijden R, Jacobs DI, Snoeijer W, Hallard D, Verpoorte R. The *Catharanthus* Alkaloids: Pharmacognosy and Biotechnology. *Current Medicinal Chemistry* 2004; 11(5): 607-628.

[2]. Nisar A, Mamat AS., Hatim MI, Aslam MS, Ahmat MS. An updated review of *Catharanthus roseus*: phytochemical and pharamacological analyses. *Indian Res J Pharm and Sci* 2016; 3(2): 631-653.

[3]. Kumari K, Gupta S. Antifungal properties of leaf extract of *Catharanthus roseus* L (G.) Don. *American Journal of Phytomedicine and Clinical Therapeutics* 2013; 9: 698-713.

[4]. Zahari R, Halimoon N, Ahmed MF, Ling SK. Antifungal compound isolated from *Catharanthus roseus* L. (Pink) for Biological Control of Root Rot Rubber diseases. *International Journal of Analytical Chemistry* 2018; Article ID 8150610, 1-6.

[5]. Roy S, Chatterjee PA. Non-toxic antifungal compound from the leaves of *Catharanthus roseus* characterized as 5-hydroxy flavone by UV spectroscopic analysis and evaluation of its antifungal property by agar-cup method. *Industrial Crops and Products*2010; 32(3): 375-380.

[6]. Clark AM, Hufford CD. Antifungal alkaloids in 'The Alkaloids: Chemistry and Pharamacology (Ed, G. A. Cordell) Academic Press. 1992; 137.

[7]. Nene YL, Thapliyal PN. Fungicides in plant disease control. Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi. 2002; 531.

[8]. Nidiry ESJ, Ganeshan G, Lokesha AN. Antifungal activity of *Mucuna pruriens* Seed Extractives and L-DOPA., *Journal of Herbs, Spices and Medicinal Plants,* 2011; 17:139-143.

[9]. Homans AL, Fuchs A. Direct bioautography on thin layer chromatograms as a method for detecting fungitoxic substances. *Journal of Chromatography* 1970; 51: 327-329.

[10]. Nidiry ESJ, Ganeshan G, Lokesha AN. Antifungal activity and isomerization of octadecyl *p*-coumarates from *Ipomoea carnea* subsp. *fistulosa*, *Natural Product Communications*, 2011; 6 (12): 1889-1892.

[11]. Svoboda GH. Alkaloids of Vincarosea Linn (*Catharanthus roseus* G.Don) XVIII. Root alkaloids. *Journal of Pharmaceutical Sciences* 1963; 52(4): 407-408.

[12]. Taylor WI. The Vinca alkaloids in The Alkaloids: Chemistry and Physiology (Ed, Manske RHF), Elsevier 2014; 100.