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Sandip Kale
 College of Agricultural
 Biotechnology, Ahmednagar,
 Maharashtra, India

Tejaswini B Pathare
 College of Agricultural
 Biotechnology, Ahmednagar,
 Maharashtra, India

Shivaji A Lavale
 College of Agricultural
 Biotechnology, Ahmednagar,
 Maharashtra, India

Correspondence
Tejaswini B Pathare
 College of Agricultural
 Biotechnology, Ahmednagar,
 Maharashtra, India

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Identification of phytochemicals and alkaloids in *Catharanthus roseus* and study of their antimicrobial activity

Sandip Kale, Tejaswini B Pathare and Shivaji A Lavale

Abstract

Phytochemical analysis of two variants of *Catharanthus roseus* (Rosea and Alba) was done to know the presence of different secondary metabolites. Phytochemicals like Alkaloids, Saponins, Phenol and Tannins, Flavonoids, Terpenoids, Quinines, and Phytosterols were present in the plant extract. Presence of alkaloids like Vinblastine, Vincristine and Vindoline was confirmed by separating them on Thin Layer Chromatography (TLC). Antimicrobial and antifungal nature of this plant extract was also tested. Bacterium like *Bacillus subtilis*, *Salmonella typhi* and *Pseudomonas aeruginosa* were found to be inhibited by the plant extract effectively.

Keywords: Secondary metabolites, Alkaloids, TLC, *Catharanthus roseus*, Phytochemicals

1. Introduction

Catharanthus roseus (L.) is an important medicinal plant of the family Apocynaceae which contains useful alkaloids and secondary metabolites used in treatments of diabetes, blood pressure, asthma, constipation, cancer problem. Phytochemical compounds are products of plants normal metabolic processes. Most of these constituents are potent bioactive compounds found in medicinal plant parts which are precursors for the synthesis of useful drugs. Alkaloids rank among the most efficient and therapeutically significant plant substances. The anti-cancer drugs namely Vincristine and Vinblastine are alkaloids produced from *C. roseus* (Chandrasekaran *et al*, 2014) [2]. Flavonoid are widely distributed in plants performing various functions including producing yellow or red pigmentation in flowers and protection from attack by microbes and insects. They are synthesized by plants in response to microbial infection and have been found *in vitro* to be effective against a wide array of micro-organisms (Esther *et al*, 2016).

The different parts of *C. roseus* are used for medicinal uses. Mainly leaves and flowering tips are used for extraction of oil. This oil has been found to have anti-bacterial, anti-yeast action. Due to medicinal virtues to this plant, it is used in Ayurvedic preparations for treating various diseases (Giri *et al*, 2012). The current experiment was designed to know the presence of different secondary metabolites and different alkaloids. We also studied efficacy of plant extract of *C. roseus* against different bacteria and fungi.

Material and methods

Collection of plant sample

The seeds of two variants of *Catharanthus roseus* viz. Rosea and Alba were collected from Dhanwantari Botanical garden, Mahatma Phule Krishi Vidyapeeth, Rahuri, India. These seeds were sown in pots and leaf samples were collected from healthy plants, one month after sowing.

Preparation of plant extract

Fresh leaves were washed with distilled water and dried completely in shed. The powder is prepared from dried leaves with the help of mixer grinder and obtained powder was then kept in air-tight container. The powder was added to Chloroform and Methanol separately at 5g/50ml of solvent, and kept on rotating shaker for 72 hours. After that the mixture was filtered and extract was used for further analysis.

Qualitative analysis of Phytochemicals

The plant extract with both, chloroform and Methanol was subjected to qualitative analysis for different phytochemicals like, Alkaloids, saponins, Phenol and Tannins, Flavonoids, Terpenoids, Quinines, Anthocyanin and Betacynin, Protein and, Phytosterols (Nandkarni, 2004; Khare, 2007; Bakshi, 1999) [6, 1].

Thin Layer Chromatography

The chloroform and methanol extracts were added as spot on TLC plates using capillary tubes on the one end of the thin layer plate. Plate was allowed to air dry and then it was placed in a beaker containing solvent Chloroform: Methanol in the ratio of 9:1. The samples were allowed to separate towards the other end of the plate. The sheet was removed, allowed to air dry and Dragendroff's reagent was sprayed. The plate was then visualized for presence of colour spot on the plate.

Antibacterial activity

The antibacterial activity of the extract was estimated by using Agar disc diffusion method. It was tested against five bacterial species viz. *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella typhi*. Sterile disk of Whatmen filter paper of 6 mm diameter was soaked into the plant extract and kept onto nutrient agar plates pre-inoculated with the bacterium and incubated at 35°C for 24 hrs. After completion of incubation, the diameter of zone of inhibition was measured.

Antifungal activity

The antifungal activity of the extract was estimated by using Agar disc diffusion method. It was tested against *Aspergillus Niger*. Sterile disk of Whatmen filter paper of 6 mm diameter was soaked into the plant extract and kept onto agar plates pre-inoculated with the fungal inoculum and incubated at 27°C for 48 hrs. After completion of incubation, the diameter of zone of inhibition was measured.

Results and Discussion

Phytochemical analysis

Phytochemicals like Alkaloids, Phenols and tannins, Flavonoids, Glycosides, and Quinines are found for both the *C. roseus* variants viz. Rosea and Alba in methanol as well as chloroform extract (Table 1). Saponins, Steroids, Terpenoids and Phytosterol were present in both variants but observed in only methanol extract. Anthocyanin and Betacyanin were not found in any of the extract.

Table 1: Presence/Absence of phytochemicals in *C. roseus* (Rosea and Alba)

Test of Phytochemical	Methanol extract		Chloroform extract	
	Alba	Rosea	Alba	Rosea
Alkaloids	+	+	+	+
Saponins	+	+	-	-
Phenol and Tannins	+	+	+	+
Flavonoids	+	+	+	+
Glycosides	+	+	+	+
Steroids	+	+	-	-
Terpenoids	+	+	-	-
Quinines	+	+	+	+
Anthocyanin and Betacyanin	-	-	-	-
Phytosterol	+	+	-	-

+ Present; - Absent

Thin Layer Chromatography

After spraying of Dragendroff's reagent shown different colored bands on TLC plate (Fig. 1). Rf value was calculated for the identified band. The methanolic extract of *C. roseus* (rosea) contains highest Rf value 0.7 and showed grayish colour which indicates presence of vindoline. In rosea chloroform extract highest Rf value was 0.885 which showed orange colour which indicates presence of vinblastine. The chloroform extract of *C. roseus* (alba) with Rf value 0.930 showed Bluish gray colour which indicates presence of vindoline. The methanol extract of *C. roseus* (alba) contains highest Rf value 0.875 and showed Orange colour which indicates presence of vinblastin.

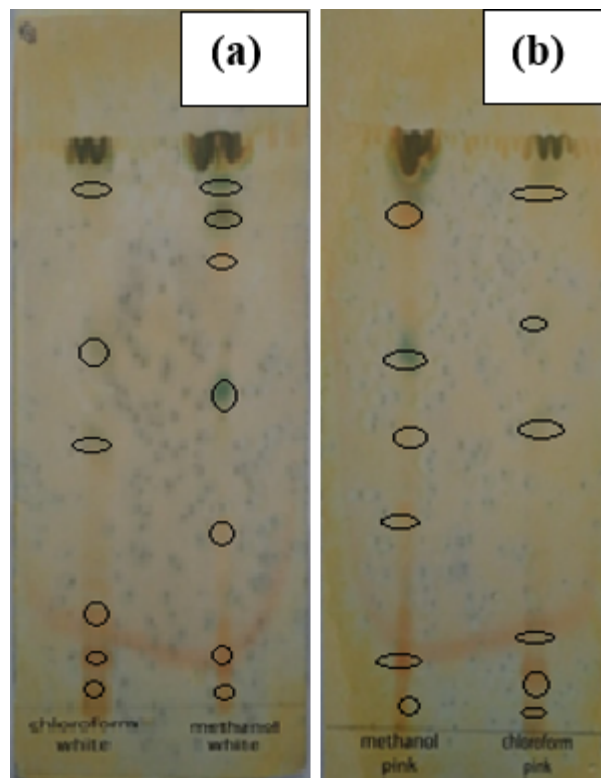


Fig 1: Thin Layer Chromatography (a) Alba and (b) Rosea

Antimicrobial activity

Inhibition executed by methanol extract of Alba towards *B. subtilis*, *S. typhi* and *P. aeruginosa* and that of Rosea towards *P. aeruginosa* was comparable with standard (Table 2). In case of chloroform extract, only Rosea extract could inhibit *S. typhi* comparable to standard.

Table 2: Inhibitory reaction of *C. roseus* (Rosea and Alba)

Name of bacteria	Inhibition zone (mm)					
	Methanol extract			Chloroform extract		
	Standard	Alba	Rosea	Standard	Alba	Rosea
<i>S. aureus</i>	26	9	10	28	6	9
<i>S. typhi</i>	20	16	11	20	11	16
<i>B. subtilis</i>	18	14	8	26	8	9
<i>E. coli</i>	23	8	8	28	8	8
<i>P. aeruginosa</i>	9	7	7	17	8	7

Antifungal activity

The antifungal activity had been tested against *A. Niger*. None of the extract could show a clear inhibition zone against the growth of *A. niger*.

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