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Short Communication Article

Determination of antimicrobial and anthelminthic activity of the leaves of the plant *Catharanthus roseus* Linn.

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ABSTRACT

Aim and objective: The present study was aimed to evaluate the antimicrobial efficacy and anthelminthic activity of both the ethanol and aqueous extract of the leaves obtained from *Catharanthus roseus* Linn.

Material and Method: The leaves of *C. roseus* were extracted incorporating the conventional methods of using ethanol and water as menstrum. The extract obtained was subjected for lyophilization to obtained freeze dried mass. The dried extract obtained was evaluated for antimicrobial activities incorporating disc diffusion methods over various bacterial strains viz. *Bacillus subtilis* (MTCC No-441), *Escherichia coli* (MTCC No-40), *Pseudomonas aeruginosa* (MTCC No-424) and *Staphylococcus aureus* (MTCC No-87). Anthelmintic activity was examined on stain of earth worm *Pheretima posthuma.*

Results and conclusion: Zone of inhibition values were compared with standard ciprofloxacin in concentration 50 and 100 µg/mL. The anthelminthic activity was determined using *Pheretima posthuma* as an experimental model against the standard Albendazole in concentration 50 mg/mL. The results revealed that the ethanol leaf extract obtained from C. roseus showed significant antimicrobial activity against gram positive bacterial as compared to gram negative bacteria and also significant anthelminthic activity against the flat worm.

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1. Introduction

Catharanthus roseus is a medicinal perennial herbaceous plant belongs to the family Apocynaceae native and endemic to Madagascar. Vinca alkaloids were discovered in the year 1950 by two Canadian scientists Robert Noble and Charles Beer. Commonly known as Periwinkle and Nityakalyani and is regarded as a rich source of pharmaceutically important terpenoid indole alkaloids. It is a circular flowering plant with 30-100 cm height, and composed of five petals (Koul et al., 2013; Parameswari et al., 2015). The leaves are glossy, oblong –elliptic, acute, rounded apex and the flowers are several colors, including white, red, pink and purple, in terminal or auxiliary cymose clusters, hairy follicle, many seeded, 2- 3 cm long; seeds oblong, minute fragrant. The plant is also known by different names such as *Vinca rosea*, *Ammocallis rosea* and *Lochnera rosea*. The genus Vinca comprises of different varieties of the species like *Vinca differmis, Vinca erecta, Vinca herbacea, Vinca major, Vinca minor, Vinca pubescent* and *Vinca soneri* (Parameswari et al.,2015; Tolambiya et al., 2016).

C. roseus which is a potent medicinal plant and posseses many of the pharmacological actions such as antimicrobial, antioxidant, anthelminthic, antisterility, antidiarrheal, antidiabetic, hypotensive, wound healing effect etc. that is used to treat many of the fatal diseases. The plant mainly possesses anticancer activity due to the presence of two major alkaloid constituents like vincristine, vinblastine and some effect also due to another constituent vindoline (Koul et al., 2013; Parameswari et al., 2015; Tolambiya et al., 2016).

In the present investigation both the ethanolic extract and aqueous extract of the leaves were subjected for study of antimicrobial activity against the different bacterial strains *Bacillus subtilis* (MTCC No-441), *Escherichia coli* (MTCC No-40), *Pseudomonas aeruginosa* (MTCC No-424) and *Staphylococcus aureus* (MTCC No-87 by disc diffusion method and anthelminthic activity by *Pheretima posthuma* as an experimental model.

2. Material and Methods

2.1 Collection and extraction procedure

The fresh leaves of *Catharanthus roseus* were collected in the month of January 2018, from the local area of Berhampur, Odisha. The collected aerial part with complete herbarium was authenticated at Department of Pharmacognosy, Royal college of Pharmacy and Health Sciences, Berhampur by Dr. G.H. Panda. The collected leaves (180 g) of *C. roseus* were then washed off under running tap water to remove dust. The plant samples were then air dried for the removal of moisture. The leaves constituents are extracted by grinding process with 50 mL of water and 50 mL of ethanol separately. The solution then filtered with the help of what man filter paper of 0.45 micron size to get a clear solution.

2.2 Conversion into sulphate form

The two different extracts i.e aqueous extract and ethanolic extract were converted to their sulphate form by adding 50 ml of 0.1 N sulphuric acid to all of them. Then again the two extracts were filtrated and store in a refrigerator for further analysis.

2.3 Lyophilization (Getting freeze dried product)

Ethanol and aqueous extracts of *C. roseus* were frozen and lyophilized using lyophilizer (Yorco, YSI- 250) for 24 hr (-40°C). Then the freeze dried samples were stored in refrigerator and further used for analysis (Lavakumar et al., 2013; Jadhav et al., 2015)

2.4. Determination of zone of inhibition by disc diffusion method

The antimicrobial activity in terms of zone of inhibition of both the ethanol and aqueous extract was determined against 4 different bacterial strains (Table 2) and the results were compared with Ciprofloxacin as standard. All the dilutions for preparation of test and standard drug were done in double distilled sterile water. The nutrient agar plates were prepared and incubated at 37°C for 24 hours and then checked for any sort of contamination. An overnight grown peptone water culture of the bacterial strains to be tested (at concentration 106 colony forming units, cfu/mL) was spread on the solid media plates with a sterile swab. Filter paper discs 6 mm diameter was impregnated with two concentrations each of test and standard drug (Test drug- 50 and 100 µg/mL, Standard drug-50 and 100 µg/mL) were placed at the centers of the inoculated plate marked as quadrants and each petriplates receive two concentrations each of standard and test drugs. After refrigerating the plates at 4°C for 2 hours, the plates were incubated at 37±2°C for 24 hours (Mishra et al., 2017; Rajalakshmi et al., 2013; Wagay et al., 2013). The antibacterial activity was measured as a diameter (in mm) of inhibitory zones on the agar plates. The experiment was repeated in triplicate and average value was represented in Table 1.

2.5. Evaluation of anthelminthic activity

The anthelminthic activity was performed according to the standard method. Study of leaves extract of *C. roseus* showed potent anthelminthic activity in experimental adult earthworm. In the study, the control drug Albendazole used at concentration of 50 mg/ ml showed more potent anthelminthic activity compared to the ethanol and aqueous extract. *Pheretima posthuma* was placed in petridish or beaker containing 3 different concentrations (200 mg/ml, 250 mg/ml and 300 mg/ml) of ethanolic and aqueous extract of the leaves of *C. Roseus*. Each of them was placed with 3 to 4 worms and observed for paralysis and death. Mean time for paralysis was noted (table 2) when no movement of any sort could be observed and the time death of worm (min) was recorded after ascertaining that worms neither moved when shaken nor when given external stimuli(dipped in hot water, 50°C). Here 0.9% NaCl is used as control which is having no effect toward the worms (Parihar et al., 2017; Renjini et al., 2017; Gajalakshmi et al., 2013).

3. Results and Discussion

The observation of the zone of inhibition (ZOI) of the extract and its comparison with standard antibiotic ciprofloxacin (50µg/mL and 100 µg/ mL) was recorded in Table 1.1 and 1.2. The ethanolic and aqueous extract of *C. roseus* showed the significant antibacterial activity in the following decreasing order; *Bacillus subtilis* > *Pseudomonas aeruginosa* > *Escherichia coli* > *Staphylococcus aureus* (for ethanol extract) and *Escherichia coli* > *Pseudomonas aeruginosa* > *Bacillus subtilis* > *Staphylococcus aureus* (for aqueous extract). From the result of ZOI values and their competition to that of standard ciprofloxacin, it evident that the ethanol extract is more active against gram positive and gram negative bacteria than the aqueous extract. The study of anthelminthic activity has been tabulated in Table 2.1 and 2.2 and it was found that the plant possess effective anthelminthic activity against the flat worm. The ethanolic extract showed the anthelminthic activity at a concentration of 250 mg/ml with death time of 48.63 min and The aqueous extract showed the anthelminthic activity at a concentration of 300 mg/ml with death time of 49.76 min whereas the standard drug (Albendazole) at 50 mg/mL was found to show the death time of 40.12 min.

Figure 1: *Catharanthus roseus* plant.

Table 1.1: Determination of zone of inhibition of ethanol extract of *C. roseus* by disc diffusion method

Sl. No.	Name of bacteria	Ethanol extract (µg/mL)		Ciprofloxacin (µg/mL)	
		50	100	50	100
1.	*B. subtilis* (MTCC No-441)	13	18	19	22
2.	*E. coli* (MTCC No-40)	10	12	13	15
3.	*P. aeruginosa* (MTCC No-424)	12	13	13	16
4.	*S. aureus* (MTCC No-87)	9	12	18	23

Table 1.2: Determination of zone of inhibition of aqueous extract of *C. roseus* by disc diffusion method

Sl. No	Name of bacteria	Aqueous extract (µg/mL)		Ciprofloxacin (µg/mL)	
		50	100	50	100
1.	*B. subtilis* (MTCC No-441)	7	9	16	21
2.	*E. coli* (MTCC No-40)	10	13	12	15
3.	*P. aeruginosa* (MTCC No-424)	8	10	10	14
4.	*S. aureus* (MTCC No-87)	6	8	15	20

Table 2.1: Determination of the anthelminthic activity exhibit by the ethanol extract of *C. roseus*

Sl. No	Extracts	Mean paralysis time (in min.)		Mean death time (in min.)	
		200 mg/ml	250 mg/ml	200 mg/ml	250 mg/ml
1.	Control	-	-	-	-
2.	Ethanol extract	49. 54	41.12	58.21	48.63

Table 2.2: Determination of the anthelminthic activity exhibit by the aqueous extract of *C. roseus*

Sl. No.	Extracts	Mean paralysis time (in min.)			Mean death time (in min.)		
		200 mg/ml	250 mg/ml	300 mg/ml	200 mg/ml	250 mg/ml	300 mg/ml
1.	Control	-	-	-	-	-	-
3.	Aqueous extract	61.65	52.54	41.87	69.65	60.10	49.76

Conclusion

From the study it was found that the ethanol extract was found more effective than the aqueous extract of the plant in both the cases of antimicrobial and anthelminthic activity. So, the present investigation offers a scientific support to the ethanomedicinal uses of the plant by the traditional healers

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Conflict of Interest

Authors report no conflict of interest

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