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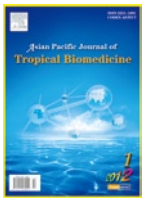

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In vitro antibacterial activity and phytochemical analysis of *Catharanthus roseus* (Linn.) G. Don.

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ABSTRACT

Objective: To identify the antibacterial activity and phytochemical analysis of *Catharanthus roseus* (*C. roseus*) and also screening of leaf, stem, flower and root extracts of the plant for its antibacterial activity. **Methods:** Antibacterial activity of *C. roseus* was performed with different solvents (ethanol and methanol) against various human pathogens viz., *Bacillus subtilis* (*B. subtilis*) , *Escherichia coli* (*E. coli*) , *Staphylococcus aureus* (*S. aureus*) , *Salmonella thphi* (*S. thphi*) and *Pseudomonas aeuroginosa* (*P. aeuroginosa*). Preliminary phytochemical analysis was carried with potent ethanolic extract. **Results:** The ethanolic extract showed a maximum zone of inhibition (21.15±1.64 mm) against *S. typhi* and minimum zone of inhibition (06.24±0.69mm) with ethanolic extract against *S. aureus*. Further, the methanolic extract observed in maximum (15.61±1.35 mm) against *S. typhi* and minimum (05.20±0.8 6mm) zone of inhibition against *E. coli*. In addition to the phytochemical analyses were showed the presence of soluble sugar, reducing sugar, protein, amino acids, lipids, total chlorophyll, phenol and ortho-dihydroxyphenols in the ethanolic extract. **Conclusions:** The ethanolic extract of *C. roseus* can be used as potential antibacterial sources.

1. Introduction

Herbal plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat contagious diseases [1-3]. The *Catharanthus roseus* (Vinca rosea) (*C. roseus)* is an important medicinal plant belongs to the family Apocynaceae, is an erected procumbent herb or under shrub containing latex. Traditionally, *C. roseus* has been used in folk medication to take care of diabetes, high blood pressure and diarrhea [4-6]. Though, in modern medicine alkaloids and chemotherapeutic agents form *C. roseus* are known for their anticancer pain-relieving and properties. The plant is recognized to control major diseases such as leukemia and diabetes [7-9]. It is cultivated mainly used for its alkaloids, which are having anticancer activities [10]. The present study aims to assess the antibacterial unique bio-active compounds against human pathogenic bacteria.

2. Materials and methods

2. 1. Plant material

The fresh plant (white coloured flower species) materials of *C. roseus* plants were collected in May of 2011 from coastal region of Thondi (Lat: 9 ° 44.03′N; Long: 79 ° 0.97′E) located at School of Marine Science, Alagappa University, Thondi Campus. Plant materials were washed separately under running tab water and distilled water for removing soil and other dust particles and finally washed with sterilized and de ionized water excess of water was removed from the plant material were shade dried, for other experimental purpose in the laboratory.

2. 2. Extract preparation

The different shade dried plant materials were powdered in a mixer grinder. Then this powdered is soaked with (10 g/100 mL) in different solvent (ethanol and methanol), for over night

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at room temperature. Soxhelt apparatus are used for the extraction [11]. The extract from three consecutive soaking are pooled and evaporated under pressure to give dark brown gummy mass and the resultant residues were stored at 4 ℃ for further analyses.

2. 3. Antibacterial assay

The following bacterial strains were used in this study viz., *Bacillus subtilis (B. subtilis)* , *Escherichia coli (E. coli)* , *Staphylococcus aureus (S. aureus)* , *Salmonella thphi (S. thphi)* and *Pseudomonas aeuroginosa (P. aeuroginosa)*. Disc diffusion assay method was carried out by using standard protocol [12]. Overnight bacterial culture (100 μL) was spread over Muller Hinton Agar (HiMedia Laboratories Private Limited Mumbai, India) plates with a sterile glass L-rod. 100 μL of the each extracts were applied to each filter paper disc Whatman No.1 (5 mm dia.) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate and the plates were inoculated at 37 ℃ for 24 hours. After incubation, the diameter of inhibition zones was measured.

2. 4. Phytochemical analysis

Plant extract was subjected to preliminary phytochemical tests and antibacterial activity. The triplicate samples were taken for analysis and calculated the mean value and standard error. Preliminary phytochemical screening was done by the standard methods described by Harborne. Recently, we used the following different methods for phytochemical analysis viz., Protein Kumar *et al* [13], Amino acids Kumar *et al* [14], Reducing sugar Brindha *et al* [15], Soluble sugar Ayoola *et al* [16], Total chlorophyll Lichtenthaler and Welburn [17]. Phytochemical analysis are followed the crude Phenols and Tannins Krishnaiah *et al* [18] Flavonoids Mattila and Hellstrom, [19] and Yamunadevi Mariswamy *et al* [20] Saponins Mallikharjunah *et al* [21].

3. Results

The results of the antibacterial activity suggested that, the maximum zone of inhibition *Catharanthus roseus* was observed in leaf ethanolic extract against *S. typhi* followed by *B. subtilis, S. aureus, P. aeruginosa* and minimum zone of inhibition was observed in ethanolic extract against *E. coli.* Further, the maximum zone of inhibition was observed in methanolic extract against *S. typhi* additionally *B. subtilis, S. aureus, P. aeruginosa* and minimum zone of inhibition was observed in methanolic extract against *E. coli.* The maximum zone of inhibition was observed in ethanolic extract against *S. aureus* followed by *B. subtilis, S. typhi* and minimum zone of inhibition was observed in ethanolic extract against *P. aeruginosa.* Moreover, the maximum zone of inhibition was observed in stem methanolic extract against *B. subtilis* additionally *S. typhi* and minimum zone of inhibition was observed in methanolic extract against *S. aureus.* In addition

Table 1
Antibacterial activity in different solvents of *C. roseus.*

S. No	*C. roseus*		Test organisms and zone of Inhibition diameter (mm ±S.E)				
	Plant parts	Extract (75%)	*B. subtilis*	*E. coli*	*S. aureus*	*S. typhi*	*P. aeuroginosa*
1.	Leaf	Ethanol	16.82±1.62	8.46±0.46	14.71±1.26	21.15±1.64	11.32±1.00
		Methanol	10.25±0.74	7.22±0.59	09.10±0.94	15.61±1.35	7.30±0.80
2.	Stem	Ethanol	9.27±0.99	–	11.44±1.13	8.66±1.69	6.73±0.37
		Methanol	12.82±1.85	–	6.24±0.69	7.46±0.61	–
3.	Root	Ethanol	7.32±0.68	10.03±0.81	15.19±1.40	18.51±1.06	12.80±0.86
		Methanol	–	5.20±0.86	8.21±0.51	13.43±0.92	7.66±0.33
4.	Flower	Ethanol	7.80±0.61	–	14.84±1.44	15.23±1.76	7.16±0.84
		Methanol	–	–	–	–	–

(–) denotes absent

Table 2
Quantitative (%) estimation of different phytochemical compounds in *C. roseus*.

S. No	Phytochemicals components	Quantitative % (±SD)
1.	Soluble sugar	82.18±2.51
2.	Reducing sugar	61.38±2.43
3.	Proteins	70.10±3.39
4.	Amino acids	32.73±1.66
5.	Lipids	42.43±1.05
6.	Total chlorophyll(mg ml-1)	01.40±0.37
7.	Phenols	21.83±1.43
8.	Ortho-dihydroxy phenols	16.83±1.54
9.	Steroids	–
10.	Saponins	–

(–) denotes absent

to the maximum zone of inhibition was observed in *C. roseus* roots extract against *S. thphi* followed by *S. aureus*, *P. aeruginosa*, *E. coli* and minimum zone of inhibition was observed in ethanolic extract against *B. subtilis* (Table 1). Further, the maximum zone of inhibition was observed in methanolic extract against *S. typhi* followed by *S. aureus*, *P. aeruginosa* and minimum zone of inhibition was absorbed in methanolic extract against *E. coli*. Maximum zone of inhibition was observed in *C. roseus* flowers ethanolic extract against *S. thphi* additionally *S. aureus*, *B. subtilis* and minimum zone of inhibition was observed in ethanolic extract against *P. aeruginosa*. Further, the no activity flower of methanolic extract (Table I). In addition, the quantitative phytochemical estimation were showed that, the highest percentage of soluble sugar followed by proteins, reducing sugar, lipids, amino acids, phenols, Ortho-dihydroxy phenols, total chlorophyll (mg/mL) and absence of saponins and steroids was observed in ethanolic extract (Table 2).

4. Discussion

India has a rich and diverse flora of flowering medicinal plants. Plants have been used as a same of medicine by all cultures from arced times to the recent days. Medicinal plants play a vital role in human health care, about 80% of the world population role on the use of traditional medicine, concomitantly based on plant materials. The World Health Organization (WHO) predicts that the number of cases worldwide for diabetes is now 150 billion, which will double by the year 2025. The results of the maximum antibacterial activity was identified with ethanolic and methanolic leaf extract of *C. roseus* against *S. typhi* and the antimicrobial activity of the ethanolic extract might be due do the presence of unique phytochemical constituents. Similarly, Dash *et al* [22] reported the antibacterial with methanol and acetone extract against *Trigonella foenum* and *Coriandrum sativum* against *Pseudomonas sp. Shigella dysentiriae*, *Salmonella typhi* and *E. coli*. *Abdul Viqar Khan et al* [23] reported the antibacterial activity with the Melia azedarach against *S. aureus*, *Staphylococcus epidermidis*, *Streptococcus sp*, *Enterococcus faecalis*, *B. subtilis*, *E. coli*, *Edwardsiella tarda*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhi*, *Shigella boydii*, *Shigella dysenteriae*, *Shigella flexneri* and *Plesiomonas shigelloides*. The present learn also made an effort to identify the phytochemical constituents analysis and the results showed the presence soluble sugars, reducing sugar, amino acid, proteins, lipids, chlorophyll, phenol, ortho-dihydroxy phenols and this phytochemical constituents previously reported with several biological properties [1,24]. Similarly Dipak Koche *et al* [25] reported the phytochemical analysis with the *Ocimum sanctum*, *Hyptis suaveolens*, *Croton viscose*, *Malachra capitata*, *Physalis minima*, *Cleme viscose*, *Galphimia glauca* and *Tephrosia villosa* and *Iqbal Hussain et al* [26] reported the phytochemical analysis through the Ranunculus arvensis, Equisetum ravens, Carathamus lanatus and Fagonia critica. Moreover, several species of Apocynaceae family plants has been widely used as main ingredient in traditional medicine. Hence, the presently studied *C. roseus* plant extract could be of considerable infers to the development of new life saving drugs. However, further research in required to isolate the bioactive principle of this species as well as further studies on its bio efficiency against human pathogens.

It can be concluded from the present findings that, the ethanolic extract of *C. roseus* collected from the Thondi coastal region was showed potential antimicrobial activity source for various infects. Further, studies is need to be conform identify the particular compounds to use as a drug as main ingredient in the traditional medicine.

Conflict of interest statement

We declare that we have no conflict of interest.

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