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In vitro antibacterial activity of *Catharanthus roseus* (Linn.)

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ABSTRACT

This study investigated the antimicrobial activity of ethanol and hot water extracts of Catharanthus roseus (Vinca rosae L.) In vitro antimicrobial activity of extracts was studied on *E.coli*, ***Pr. velgaru***s ***P. aeruginos S. aureus***.Testing was performed by well method and minimum inhibitory concentration (MIC) were determined. The maximum antibacterial activity was observed in Ethanolic extract of Catharanthus roseus against *Staph. aureus*

INTRODUCTION

Many products of Medicinal plants prove to be very useful in reducing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and achieving positive health care system (Kaushik *et al*.,2002).

Herbal plants used in traditional medicine contain a wide range of bioactive compounds that can be used to treat contagious diseases (Kiruba et al.;2011) .

The Catharanthus roseus (Vinca rosea)) 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 (Appidi et al.;2008). 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 (Chattopadhyay et al .;1992). The major alkaloid is vincamine and its closely related semi synthetic derivative widely used as a medicinal agent, known as ethyl- apovincaminate or vinpocetine, has vasodilating, blood thinning, hypoglycemic and memory- enhancing actions. Rosinidin is an anthocyanidin pigment found in the flower of C.roseus. The genus Vinca (Apocynaceae) comprises about seven species in the world. The leaves are oblong-ovate to elliptical, approximately 2-5 cm long and 1-2.5 cm wide, with petioles 1-3 mm long. The flower is perfect and without odor. It is showy, purple to blue and occasionally white, borne singly in an axillary position on a 1-1.5 cm pedice. The fruit is a non-fleshy follicle, approximately 2–2.5 cm long. It releases three to five seeds (DARCY *et al*., 2002).

In folk medicine, it is used internally for circulatory disorders, cerebral circulatory impairment and brain's metabolism support (FARAHANIKIAA et al, 2011). Numerous literature data describe the compounds isolated from this plant. Phenols have been investigated for its antioxidant properties (NISHIBE et al., 1996). Also flavonoids were tested for the anti-inflammatory (BAHADORI et al., 2012) and antimicrobial effects (SZOSTAK and KOWALEWSKI, 1975). Extracts from V. minor species showed a pronounced antioxidant activity and the ability to reduce lipid peroxidation (NISHIBE et al., 1996) as well as the ability in treatment of cardiovascular and neurodegenerative diseases (KHANAVI et al., 2010; FERNANDES et al., 1996). There is plenty of information about antibacterial (YILDIRIM et al., 2012; MEHRABIAN et al., 1995; TURKER *et al*., 2009) and antifungal (WILSON *et al*., 1997; DOMENICO *et al*., 2012) activities of V. minor ethanolic extract. In the available literature there are no data about the activity of V. minor diethyl ether extract. Considering that V. minor species is from the Balkans and has not been studied with these aspects, the aim of this study was to investigate the antibacterial and antifungal activities as well as phytochemical analysis with antioxidative activity of ethanolic and diethyl ether extracts of this plant.

Material and Methods

Plant material in October 2017 leaves of species V. rosea L. were collected at the time of flowering. The leaves were harvested at noon . The collected plant material was air-dried in darkness at ambient temperature. The dried plant material was cut up and stored in paper bags until needed.

Preparation of plant extracts

Dried ground plant material was extracted by maceration with ethanol and hot water. 20 g of plant material was soaked with 200 ml of solvent for 24 h at room temperature. The resulting extract was then filtered through filter paper (Whatman No.1) then evaporated to dryness using oven at 40 °C. The obtained. The extracts were kept in sterile sample tubes and stored at -20 °C.

Determination of antimicrobial activity

Test microorganisms Antimicrobial activity of ethanol and hot water extracts were tested against Escherichia coli ,Staphylococcus aureus , Pseudomonas aeruginosa and, Proteus velgarus .The r microorganisms were provided from the Microbiology Laboratory, veterinary medicine University of A lQadisiyah. Well diffusion assay method was carried out by using standard protocol. Suspension preparation Bacterial was prepared by the direct colony method. The turbidity of initial suspension was adjusted by comparing with 0.5 McFarland's standard (ANDREWS, 2005). Initial bacterial suspensions contain about 10^8 colony forming units (CFU)/ml. 1:100 dilutions of initial suspension were additionally prepared into sterile 0.85 % saline. . Over night bacterial culture(l) was spread over Muller Hinton Agar plates with a sterial glass L-rod of the different extracts(Ethanol and Aqueous) were loaded on wells the agar plate. different extracts(Ethanol and Aqueous) was tested in triplicate and the plates were inoculated at 37°C for 24 hours. After incubation the millimetre of inhibition zones was measured. Microdilution method Antimicrobial activity was tested by determining the minimum inhibitory concentration (MIC) by using microdilution (SARKER et al., 2007. The inoculated plates were incubated at 37 °C for 24 h for bacteria.

Results and Discussion

The present study reveals the antimicrobial activity of ethanol leaf extracts of Catharanthus roseus.The antimicrobial activity of Catharanthus roseus leaf extract was tested against four pathogenic bacteria, *Staphylococcus aureus Pr. velgaru*s *P. aeruginos* and E.coli shown in table 1. The tested extract showed very strong antimicrobial activity against these pathogenic microorganisms. The antimicrobial activity was evaluated by measuring the zone of inhibition. Table 1,3 illustrate the zone of inhibition for bacteria . The maximum zone of inhibition catharanthus roseus was observed in leaf and flower ethanolic extract against *S.aureus*, followed by *E.coli*, ***Pr. velgaru***s and *P.aeuroginosa* at 100 mg/ml leaf extract(15,14,10,8 mg/ml respectively) (table 2). The results of this work are in agreement with other studies that the leaf extracts of this plant was more bioactivity against Gram positive than Gram negative (Grujić et al.;2014) also agreement with Kabesh et al.;(2015) whose reported that ethanolic extract was more inhibitor than aqueous extract .This study illustrate that a Gram-positive bacteria were more susceptible to this extract as compared to Gram-negative bacteria species. This is probably due to the differences in chemical composition and structure of cell wall of both types of microorganisms (Pankaj et

al.2008). Results of this work showed that the extraction of antimicrobial substances by organic solvents is better as compared to aqueous extracts (Thongson *et al*. 2004). The polarity of antibacterial compounds make them more readily extracted by organic solvents, and using organic solvents does not negatively affect their bioactivity against pathogenic bacteria species.

Table 1: Antibacterial activity of aqueous extract

bacteria	***Zone of inhibition in mm***			
	25mg/ml	**50mg/ml**	**100mg/ml**	**Gentamycin**
E. coli	**6.3**	**8**	**10**	**17**
Pr. velgarus	**6**	**6.4**	**7**	**16**
P. aeruginosa	**-**	**6.1**	**6.6**	**12**
S. aureus	**7**	**9**	**10.3**	**22**

Fig. 2: Antibacterial effect in ethanol extract of *C. roseus*

bacteria	***Zone of inhibition in mm***			
	25mg/ml	**50mg/ml**	**100mg/ml**	**Gentamycin**
E. coli	**8.5**	**12.3**	**14.**	**17**
Pr. velgarus	**6.5**	**9**	**10.**	**16**
P. aeruginosa	**6**	**6.3**	**8.**	**12**
S. aureus	**9**	**12.6**	**15.**	**22**

Table 3: Antibacterial activity of aqueous extract

bacteria	***MIC***	
	Ethanol extract	**aqueous**
E. coli	**125**	**625**
Pr. velgarus	**125**	**625**
P. aeruginosa	**250**	**375**
S. aureus	**125**	**125**

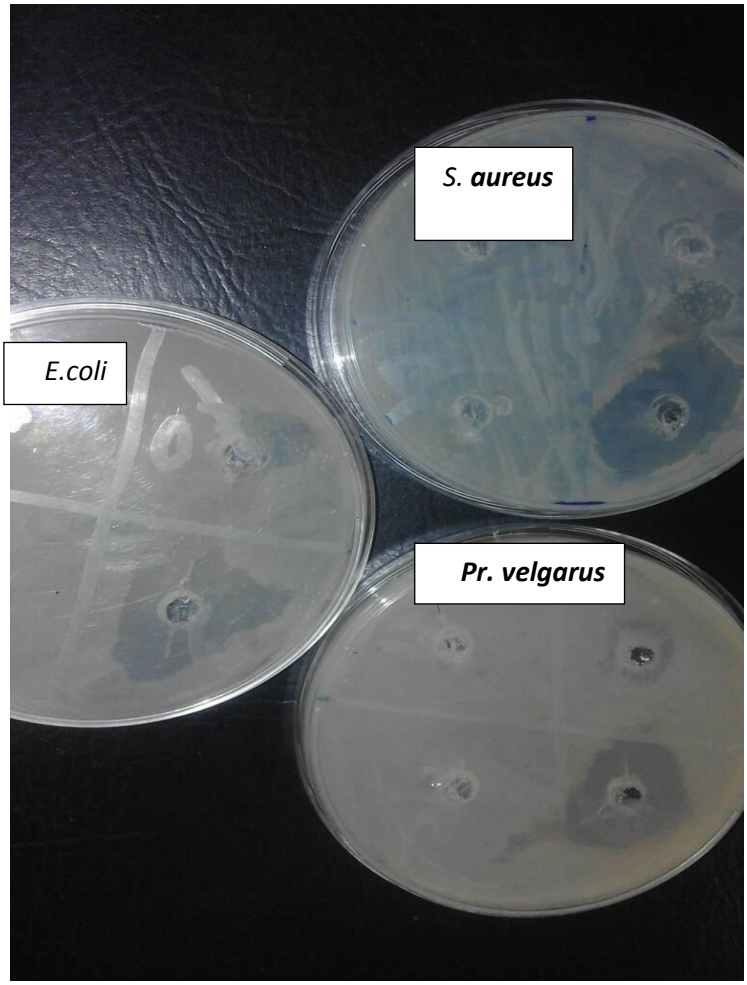

Fig.1 : Antibacterial effect in ethanol extract of *C. roseus*

Conclusion

The results of antimicrobial activity indicate that tested extracts showed different degree of antimicrobial activity in relation to the tested species. Extracts of *V.rosea* demonstrated more potent inhibitory effects on the growth of ***S. aureu***s than to the other tested bacteria. Therefore, the flowers of this plant can be a potential source of antibacterial substances Further research is needed for the isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

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