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ANTIMICROBIAL ACTIVITIES AND HEMATOLOGICAL STUDY OF *CATHARANTHUS ROSEUS* L. LEAF EXTRACT

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ABSTRACT : Diseases with a bacterial cause are among the most important challenges facing scientists to reduce disease infections and the most important aspects that scientists have followed is the use of plant extracts that give effectiveness against bacteria and reduce the side effects of chemical properties. We have used in this study the effect of two concentrations of 50% and 100% of the cold extract. The leaves of the planted eye of the bazin plant in Iraq, which maintains the effectiveness of natural active compounds against human pathogens, including bacteria *Pseudomonus aeruginosa*. The extract showed a concentration of 100% high effectiveness against bacteria and the same two concentrations worked effectively in prolonging the time of blood clotting outside the body through study. That was performed on humans and mice, as well as the increase in the time of bleeding within a living body and a marked increase in the number of platelets.

Key words : *Catharanthus roseus* L, leaf extract, antimicrobial activities.

INTRODUCTION

Infections are one of the prominent symptoms of infection of the living organism in bacteria, which occurs as a result of the separation of toxic substances from bacteria and their transmission through the bloodstream and spread to the rest of the organs, which caused damage and the appearance of the disease in the organism (AL-Rajab *et al*, 2018). *Catharanthus roseua* is herb that is characterized by the presence of the substance Milky in its tissues, erect and evergreen and it has simple facing thick leaves and its individual flowers in a violet color with an eye shape, so the plants are called a little bright eye (Asma *et al*, 2016). Belongs to the oleander family or is sometimes called the dogbane family worldwide, especially in the tropics and mainly in the permanent rain forests, as well as in the semi-tropical and temperate regions as well (Dacie *et al*, 1984). It consists of about 18 genera and 1500 species, its dendritic or semi-dendritic plants with milky substance (Duncan, 1955). It contains 16 chromosomes and is a good example of chromosomal studies and cellular divisions a derivative called *Catharanthus* L.G. Don from Katharos in the sense of pure and anthos in the sense of flowers (Hayati *et al*, 2012). It is one of the most important plants containing alkaloids, as it has been studied and researched in recent decades and includes 130 types of alkaloids in different parts of it, and includes eight types of the most important *C. roseus* grow in warm and temperate regions. (Hayati *et al*, 2012). *C. roseus* contains indole alkaloids that have reached 100 species and most of them are of real estate efficacy (Issa *et al*, 2020) and one of the most important benefits of alkaloids is their anti-cancer effectiveness, anticancer, as Vinblastine is effective against leukemia in mice (Jayanthi *et al*, 2010). Plant extract has also been used to treat diarrhea and skin infections caused by microbes. It is effective against pathogenic microorganisms and is a vermifuges in the intestine, oral treatment for diabetes, wound healing and diuretic antidiabtic (Kartlni *et al*, 2019).

MATERIALS AND METHODS

Plant collection

The plant was obtained from nurseries, and it was confirmed and diagnosed based on the classification keys provided in the plant was dried and then ground and preserved with dark pots (Mohammed *et al*, 2011).

Biological study

This study was conducted on bacterial strains obtained from the laboratories of the maternity hospital, Erbil. The purity of isolates was confirmed and then diagnosed with the help of specialists. Bacterial culture was active in test tubes containing 5 ml of sterile Nutrient

broth medium.

Preparing the cold aqueous extract

Weigh 100 gm of vegetable powder and add 500 ml of sterilized distilled water boiled in a clean and sterile glass flas and leave it for a period of 24 hours while continuing to shake for such a long time to allow With the extraction process, then pass over several layers of fine sterile fabric to filter it and then dry the liquid in an incubator at a temperature of 37°C and for a period of 2-3 days until it is a powdered powder. Then collect the powder and store in a clean and sterile glass bottle, and store it at room temperature

Determination of MIC by Broth dilution method

The plant extracts with a final concentration of bacterial suspension were diluted at (1.5×108) cells / ml in sterile test tubes according to the law (first concentration × first volume = second concentration x second volume) and preserved with a temperature 37°C for a period of (24) hours as the MIC value of the raw vegetable extract is against the strains of bacteria, as it is the minimum dilution that inhibits bacterial growth after a lap time (24) h, for the eye extract of ethyl ether, and then the bacterial plankton is planted on the medium of nourishing nests (Muhammad *et al*, 2009).

Clotting time

Tube method was used for lee and white using small glass tubes of similar size 1 cm diameter washed with distilled water and sterilized as they were internally coated with concentrated grades of alcoholic gradient extract (50 and 100) mg/ml for dry plant extract. Then, it was left in the laboratory atmosphere to completely dry out, then blood was drawn from the radial vein using a (syringe) from a donor person, as the venipuncture must be done quickly and in a good way and the clock began setting as soon as the blood entered the cyanosis. Fill each tube (0.5) ml with blood, then close the tube with cotton, then put it in a water bath at a temperature of (37 °C) then tilt the tube at an angle of (45) degrees to see if the blood has coagulated, and observe 15 seconds until the thrombus is seen, with adjust the time and record the clotting time (Prajakta *et al*, 2010).

Hematological test *in vivo*

In this study, 12 laboratory mice of the Balb.c strain were used for males only, and their weights were (30 ± 5) gm at the age of about three months, divided into three, each group includes 4 mice and mice were placed In clean laboratory cages in the animal house of the Department of Biology, College of Biotechnology, Gyhan University, Erbil, at a temperature of 30±2°C. It was prepared with a special food ration and quantities of water that included the need. The groups were numbered and dosed in the two concentrations of the raw hot water extract, the dry plant (50 and 100) mg / ml, with a volume of 0.4 ml once during the day, while the third group was administered with distilled water of the same size and represented the control group.

Measurement of clotting time outside the living body

According to Ramya *et al* (2008) method, the mouse guilt was wound after sterilizing with alcohol and using a clean and sterile scalpel to obtain blood drops and collection in the capillary tube and after 30 seconds the formation of fiberin was observed as a red thread.

Measuring bleeding time inside the living body

Measure the time of bleeding according to the Duke method, as sterilizing the guilt of the mouse, then making an incision in it using a sterile scalpel, as the blood must flow easily without the need to compress the guilt of the mouse, and to set the time when the blood drops, and after 30 again, I picked up the first drop of blood on the filter paper and completed the procedures (Singh *et al*, 2001).

Platelet count

Set the number of platelet count according to the visual method for whole blood, as detailed in Stearmn (1972).

Statistical analysis

Followed the Multiple range and Multiple F method test at three iterations at a probability level (0.05) as stated in Verpoorte *et al* (2007).

RESULTS AND DISCUSSION

The results shown in Table 1 showed the effect of cold water *C. roseua* extract with great effectiveness on pathogenic bacteria, as the results showed a difference in the rates of inhibition that have a direct relationship in the sensitivity of each type of bacteria, the type of extract and especially the cold is important in the quality and quantity of metabolism compounds The secondary active in the dry plant, and the results shown by *C. roseua* extract are counter-effective for some different bacteria types at a concentration of 100%, especially on *P. aeruginosa*, where the rate of inhibition of bacterial growth was 23 mg ml^{-1} and the lowest inhibition region in *Bacillus subtilis* when The concentration was 50% and was 10 mg ml^{-1} (Table 1). The effectiveness of this extract is due to the fact that it contains most of the active anti-bacterial compounds that are positive and positive for a colorant such as: turbines, phenols, flavonoids,

Table 1 : Minimum inhibitory concentration MIC of cold extract of *C. roseua.*

Tested bacteria	MIC(mg ml^{-1})		Antibiotic
	50	100	Erythromycin
Pseudomonus aeruginosa	22	23	6.7
Staphylococcus auras	11	14	8.6
Escherichia coli	13	19	7.3
Klebeslla sp.	14	20	4.7
Bacillus subtilis	10	12	3.7

LSD 5% = 2.103

Significant differences were observed at the level of <0.05> p, as the clotting time for experimental animals was recorded 4-6 minutes for the groups, at a concentration of 100 mg/ml while, the results of the control were 1.77 minutes, while the time of bleeding was 90 minutes, while in the control the result was 7 minutes and the normal number of blood platelets had (5.7) cells /mm^3, while the readings of giving the alcoholic extract increased by 712000 Cell/mm^3 at same focus above. We note the direct relationship between the studied

Table 2 : Heterogeneity after treatment with cold extract at a concentration of 50 mg and 100 mg of *C. roseua*.

Blood parameters	Control	Group 1		Group 2		Group 3	
		50	100	50	100	50	100
Clotting Time	3.18	8	9	6.3	7.3	4.2	4.6
Bleeding time inside	7	90.7	91.7	53.5	55.7	20	21
Clotting time outside	1.77	3	4	4	5	7	6
Platelet count	5.73	670000	760000	696000	712000	554000	652000
LSD 5%	3.66	3.98		2.77		0.43	

Saponins and volatile oils. It liberates the active compounds as an organic solvent (Zhu *et al*, 2014).

The effectiveness of this extract is attributed to the fact that it contains most of the active anti-bacterial compounds that are negative and positive for the gram test . Some of the secondary antimicrobial active compounds in the plant, such as the active compounds, are destroyed by heat, so it is preferable to take them as a cold extract, as is the case with the effective antimicrobial compounds in spices that are variable in temperature. Thus, effectiveness was lost during 20 minutes at 100°C . The plant also shows a high oxidative efficacy that has an effect on fighting infections caused by pathogenic bacteria.

The normal blood clotting time for control was recorded 3 minutes, as the normal blood clotting time for a person ranges (3-7) minutes. The time of blood clotting in humans was recorded when using laboratory tubes coated from the inside with concentrations 50 and 100 mg/ml of cold aqueous extract, as the lowest time at concentration 50 was recorded for most groups, as we notice an increase in clotting time with an increase in concentration as shown in Table 2. Since, *C. roseua* affects the platelet aggregation and inhibits its aggregation. Thus, inhibition of thrombus formation as the effect of Aspirin, Warfarin. Table 2 also shows the results of giving the aqueous extract of *C. roseua* plant to the first, second and third group of study mice by dose for a period of seven consecutive days; it was noted an increase in clotting time, bleeding time and the number of blood platelets compared to the control group. characteristics and the increase in concentration and it was noted that the number of thrombocytopenia fits with the time required for the process of clotting and bleeding, as platelets participate in the formation of blood clot by grouping and adhering to the threads of the fibrin network The *C. roseua* has stimulated the Stem cells, which are the main source in the formation of platelets, as well as the formation of other blood components from red and white blood cells according to Unitarian theory and it has the ability to increase liquidity Blood by interfering with platelet function. Thus, it increases the time of bleeding and clotting time and this efficacy may be attributed to the eye of the *C. roseua* in preventing the formation of a blood clot and inhibiting the platelet aggregation to alkaloids and flavonoids that reduce the platelet aggregation as well as the presence of glycoside and Resins compounds.

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