Study of Antimicrobial Activity of Vegetable Alcoholic Extracts Obtained from *Vinca Minor* L.

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Abstract

Important natural antimicrobial compounds derived from *Vinca minor* L. plant contain a wide variety of secondary metabolites which are useful for brain health (increasing blood circulation in the brain, supporting brain metabolism, preventing memory loss and problems with concentration and premature aging of the cell), and externally they play a role in tissue repair and skin lesion healing. The testing of the antimicrobial activity of the alcoholic plant extracts obtained from *Vinca minor* plant was performed on two types of pathogenic germs: a Gram-positive strain, *Staphylococcus aureus* ATCC 25923 and a Gram-negative strain, *Escherichia Coli* ATCC 25922, as well as on a yeast strain pathogenic, *Candida albicans* ATCC 900288. Quantitative evaluation of antimicrobial activity was performed by a variant of the Kirby-Bauer diffusimetric method, a simple and fast method that allows the determination of the spectrum of sensitivity / resistance to antibiotics of the microorganism. Experimental studies have shown that all samples of alcoholic extract of 70% concentration, in volumes of 7µL, exerted a total antibacterial inhibitory action against the reference strains *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 and moderate against the strain *S. aureus* ATCC 25923, results that agree with the literature. Regarding the alcoholic plant extracts with a concentration of 40% ethyl alcohol, obtained from the leaf and stem of *Vinca minor* plant, they did not show antimicrobial activity, which suggests that 40% ethyl alcohol does not extract bioactive compounds with antimicrobial activity.

Keywords: Vinca minor, bioactive substances, antimicrobial activity, Staphylococcus aureus, Escherichia Coli, C. albicans

Introduction

It is known from the literature that the plant material of *Vinca minor* L. plant is a valuable source of antimicrobial compounds, and alcoholic extracts obtained from this plant have antibacterial and antifungal activities on a variety of bacterial and fungal cultures [Kwiecinski J, 2009]. Natural plant products are a useful source of substances with antimicrobial properties, active mainly on bacteria and fungi. Given that many of these compounds may be a source of new drugs, herbal extracts may become useful therapeutic tools [Savoia D. 2012]. Important natural antimicrobial compounds derived from *Vinca minor* L. plant contain a wide variety of secondary metabolites, which are useful for brain health (increasing blood circulation in the brain, supporting brain metabolism, preventing memory loss and problems with concentration and premature aging of the cell) and externally they play a role in tissue repair and healing of skin lesions [Hadjiakhoondi A., 2011, Farahanikia B., 2011].

In addition, the natural substances present in *Vinca minor* L. plant are a useful source of antimicrobial compounds active against bacteria and fungi. Numerous studies provide a wealth of information regarding the antibacterial [Mehrab S., 1995, Phillips JD, 1992] and antifungal [Ferrieres L, 2007] activities of compounds. bioactives from various plant extracts of the species *Vinca minor* L [Rongai D., 2012], Recent findings indicate that phenol and natural phenolic compounds existing in this plant have antibiotic effect on biofilm formation [Jagani S., 2009]. It has been concluded that these polyphenolic compounds can interfere with the formation of bacterial biofilm, preventing its formation, because bacteria possess sophisticated systems for regulating metabolic activities depending on population density [Huber B. 2010]. Research into plant extracts with antimicrobial activity is an important alternative strategy in the control of infectious diseases, caused by antibiotic-resistant pathogens. The natural antimicrobial capacity of plant extracts has been the basis of many applications, including raw materials and preservation of processed foods, pharmaceuticals, cosmetics, in alternative medicine and natural therapies [Martınez-Tome M. 2011].

In this study, the antimicrobial activity against the pathogenic bacterial strains *S. aureus*, *E. coli* and *C. albicans* mentioned, was for 4 alcoholic plant extracts from the leaf and stem of *Vinca minor* plant, obtained in ethyl alcohol of 40% and 70% concentration (two leaf extracts and two stem extracts). In choosing these microbial strains, we took into account the fact that all strains are common commensal species in the normal microbiota of the skin and mucous membranes, but which, under certain conditions of imbalance, following associated diseases, immunocompromise, old age, etc., can become pathogenic.

Staphylococcus aureus is a Gram-positive bacterium that causes suppurative infections or sepsis, skin infections, causing secondary wound infection [Martınez-Tome M. 2011].

Escherichia coli is a Gram-negative, commensal bacterium in the digestive tract of mammals and humans, frequently detected in a wide variety of food sources: water, raw meat, vegetables and dairy products. It is an important indicator of sanitation, its presence on the skin showing a fecal contamination of animal and / or human nature [Andrews J. M., 2005].

Candida albicans is an opportunistic fungus, which is found in the normal microbiota of the skin, intestines and genitourinary tract, but which can turn into a pathogen under conditions of low immunity or due to an imbalance of the bacterial microflora [Wilson C.L, 1997, BermanJ, 2012].

There is a difference in the architecture of the bacterial cell wall, which is complex in Gram-positive bacteria, by the presence of peptidoglycan (murein), its own constituent of the bacterial cell, a giant molecule, a heteropolymer with three-dimensional network, composed of glycan and a peptide, compared to Gram-negative bacteria, in which the architecture is simpler, the wall being thinner, with only two layers. This architecture will condition the selective permeability of the bacterial coating and its synthesis mechanisms, with consequences in terms of sensitivity / resistance of bacteria to various bioactive compounds, with potential antibiotic effect [Moldoveanu D., 2004]. In yeast, the cell wall has a complex structure and plays an important role in the relationship with the external environment, the host or the substrate on which the fungus is attached and in the defense of the yeast cell. Thus, C. albicans has complex structures consisting of chitin, (1–3) -D-glucan, (1, 6) beta-glucans, lipids and peptides, embedded in a protein matrix [Andrews J. M., 2005].

Material and Methods

Plant materials

The materials used to analyze the antimicrobial activity are represented by:

40% and 70% alcoholic plant extracts obtained from the leaf and stem of *Vinca minor* plant

bacterial and fungal pathogenic strains: *Escherichia coli* ATCC 25922; *Staphylococcus aureus* ATCC 25923; *Candida albicans* ATCC 10231.

To perform microbiological analyzes, the microbial strains were revitalized and maintained by culturing on the Plate Count Agar (PCA) culture medium (Fig. 1.) At 37 ° C ± 0.5 for 22 ± 2 hours.

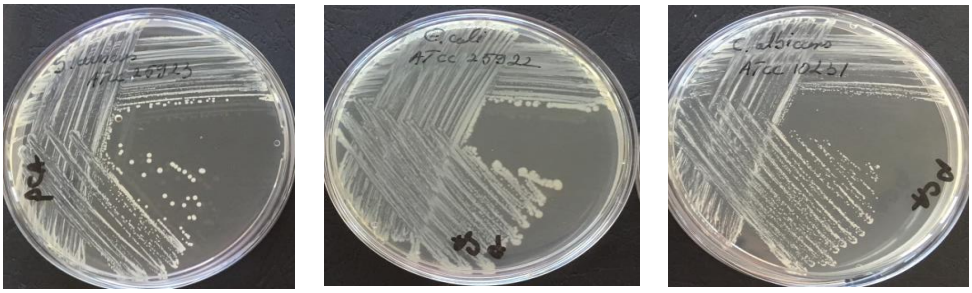

Fig. 1. Bacterial strains grown on Plate Cound Agar (PCA) culture medium

Preparation of plant extracts

The dried and crushed plant material was extracted with ethyl alcohol: 40%, 70%, 96% alcoholic extractive solutions obtained from the leaf and stem of the *Vinca minor* plant were prepared according to the 10th edition of the Roman Pharmacopoeia. This process involved crushing 10 g of plant product from the leaf and stem of the *Vinca minor L.* plant, to which was added ethyl alcohol of 40%, 70% and 96% to 100 mL, respectively (Ratio 1:10). The extracts were left for 10 days in optimal conditions, away from light and moisture, in a cool place. During the 10 days, the extracts were carefully monitored and shaken 2-3 times a day. At the end, they were filtered using cotton cloth filters so that they could be separated from the plant material. The liquid collected on filtration was stored in sterile, dark containers.

Diffusimetric method

Quantitative evaluation of antimicrobial activity was performed by a variant of the Kirby-Bauer diffusimetric method [Moldoveanu D., 2004], a simple and fast method that allows the determination of the spectrum of sensitivity / resistance to antibiotics of the microorganism, variant in which the microtablets with classical antibiotics were replaced with quantities of 5 μL and 7 μL respectively of the test samples, arranged by micropipetting on the surface of the PCA medium inoculated with the test microorganism (Fig. 1.). This method was used both for the extracts obtained from the stem and for those obtained from the leaf of *Vinca minor* plant.

For the preparation of the inoculum, the method of direct homogenization of the colonies was used, consisting in the homogenization in sterile physiological serum of 3-5 colonies from the culture plate, in the stationary growth phase, after 18 hours, to obtain a standardized turbidity. A 0.5 McFarland standard was used to control the density of the inoculum (optical density at λ of 550 nm is 0.125), as well as the Densimat digital densitometer.

The suspensions were homogenized using a Vortex stirrer for 15–20 seconds. Calibration of the suspension was performed by adding a larger or smaller amount of isotonic chlorinated solution, until the desired optical density was obtained. 1/10 dilution was made from the initial suspension. The plates were sown no later than 15

minutes after the preparation of the inoculum, by the technique of sowing "in cloth", by flooding. On the surface of the agarized Muller-Hinton medium, 500 µL of the 1/10 dilution of the 0.5 McFarland suspension were dispersed with the help of a Drigalski rod. After sowing, the plates were left for 3-5 minutes to absorb the inoculum. The extracts obtained from the leaf and stem of *Vinca minor* plant were applied in a spot of 5 µL and 7 µL. To assess the diameters of the inhibition zones, the extracts were pipetted into wells, practiced using sterile durham tubes, in the medium inoculated in cloth with the test microorganism, with a diameter of 5 mm and 7 mm, respectively. After 15 minutes from the placement of the spots, respectively of the volumes in the wells, the plates were thermostated at 35 ± 2 ° C, for 16-18 hours, under aerobic conditions. The reading and interpretation of the results was done only qualitatively, measuring the diameter of the zones of inhibition in the reflected light, on the back of the Petri dish, with a graduated ruler, the presence of any zone of inhibition being interpreted as sensitivity (S), and its absence as resistance (R).

Results and Discussions

After the expiration of the incubation time, the population density of the culture in each spot was assessed, compared to the control of ethyl alcohol in a concentration of 40% and 70%. In Table 1. are represented the results obtained following the antimicrobial analysis of the alcoholic extracts of 40% and 70% concentration obtained from the leaf and stem of *Vinca minor* L. plant, by the diffusimetric method.

Table 1 shows that the values of inhibition rays obtained when testing plant extracts against the selected reference strains (*S. aureus*, *E. coli*, *C. albicans*) are close in order of size, both for alcoholic extracts in concentration 40% and 70 % prepared from leaves, as well as for those from the stems of *Vinca minor* L. plant, which indicates the presence of bioactive compounds with similar antimicrobial activity in the extracts obtained from both studied plant materials.

Experimental studies have shown that all samples of alcoholic extract of 70% concentration, in volumes of 7µL, exerted a total antibacterial inhibitory action against the reference strains *E. coli* ATCC 25922 and *C. albicans* ATCC 10231 and moderate against the strain *S. aureus* ATCC 25923, results consistent with the literature [6]. From the data obtained by other researchers it was found that the efficiency of ethanolic extracts obtained from *Vinca minor* plant on microorganisms is influenced by certain factors, such as: the source from which the plant was harvested, but also their seasonality. Another important factor that can influence the efficiency of antimicrobial activity is the type and concentration of the solvent used in the preparation of extracts (water, alcohol, acetone), because inhibition rates can differ greatly depending on the chosen solvent [Kwiecinski J, 2009, Rongai D, 2012].

Vegetable alcoholic extracts with a concentration of 40% ethyl alcohol, obtained from the leaf and stem of *Vinca minor* plant did not show antimicrobial activity, which suggests that 40% ethyl alcohol does not extract bioactive compounds with antimicrobial activity.

Table 1. Results obtained from antimicrobial analysis of plant alcoholic extracts obtained from *Vinca minor* L. plant by diffusimetric method

Samples of vegetable alcoholic extracts	Tested volume	Φ Inhibition area expressed in mm		
		S. aureus ATCC 25923	*E. coli ATCC 25922*	*C.albicans ATCC10231*
F40	5µl	0	0	0
T40	5µl	0	0	0
F70	5µl	0	7	8
T70	5µl	0	7	7
Alcohol witnes	5µl	0	0	5
F40	7µl	0	0	0
T40	7µl	0	0	0
F70	7µl	7	9	12
T70	7µl	7	8	12
Alcohol witnes	7µl	0	0	10

In Figures. 2. 3 and 4 is the comparative study of the antimicrobial activity of plant alcoholic extracts of 40% and 70% concentration obtained from the leaf and stem of *Vinca minor* plant, tested on strains of S. aureus, E. coli and *C. albicans*. It is observed that ethyl alcohol in a concentration of 70% used in the preparation of extracts is the solvent with the highest extraction power of compounds with antimicrobial activity, highlighted by the appearance of halos of inhibition of microbial growth (right plates on figures).

In figures 5 and 6 are presented the inhibition halos formed as a result of the antimicrobial activity exerted by the alcoholic plant extracts of 70% concentration obtained from the leaf and stem of *Vinca minor* plant, on the strains of the bacteria *E. coli*, *S. aureus* and *C. albicans*.

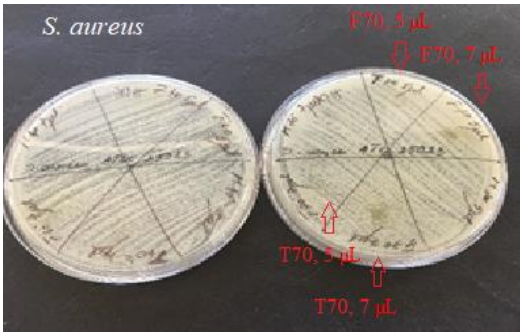

Fig. 2. Antimicrobial activity of alcoholic plant extracts of concentrations 40% and 70% obtained from the leaf and stem of *Vinca minor* L. on *S. aureus*

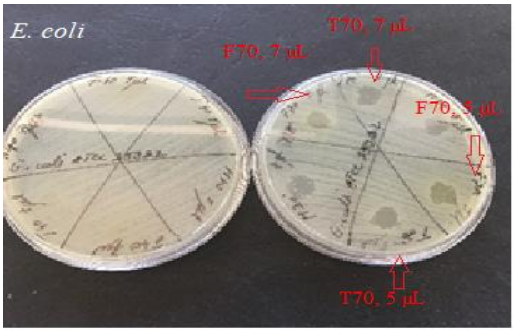

Fig. 3. Antimicrobial activity of alcoholic plant extracts of 40% and 70% concentration obtained from the leaf and stem of *Vinca minor* L. on *E. coli*

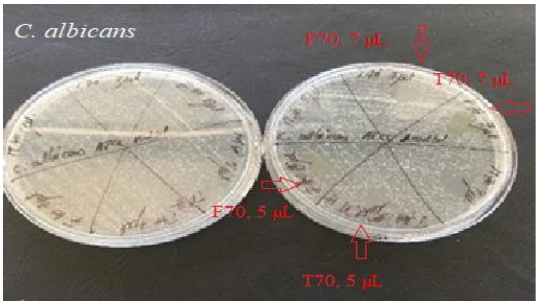

Fig. 4. Antimicrobial activity of alcoholic plant extracts of concentration 40% and 70% obtained from the leaf and stem of *Vinca minor* L. on *C. albicans*

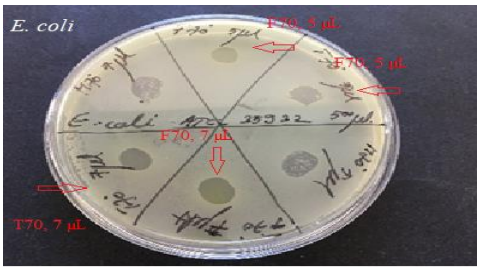

Fig. 5. Antimicrobial activity of alcoholic plant extracts of 70% concentration obtained from the leaf and stem of *Vinca minor* L. (F70 and T70) on *E. coli*

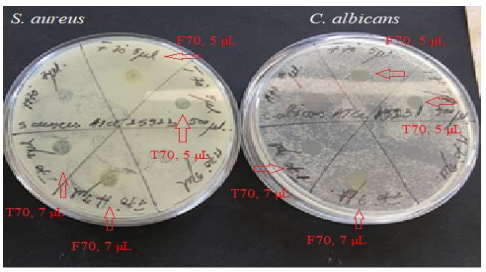

Fig. 6. Antimicrobial activity of alcoholic plant extracts of 70% concentration obtained from the leaf and stem of *Vinca minor* L. (F70 and T70) on *S. aureus* and *C. albicans*

Following the results obtained for the alcoholic extracts studied from *Vinca minor* L., it was observed that their antimicrobial action was influenced by factors such as: concentration of solvent used, volume of solution used and the type of microorganism for which these extracts showed antimicrobial properties.

The study performed for alcoholic extracts in a concentration of 70% highlighted the antibacterial properties of this extract on the tested microorganisms. The results obtained showed that all samples of alcoholic extract of 70% concentration, in volumes of 7 µL, exerted a total antibacterial inhibitory action against the reference strains E. coli and C. albicans and moderate against the strain S. aureus, results that agrees with the literature [Berman J., 2012]. The obtained results show that the alcoholic extracts in concentration 70% are close in value both for those obtained from the leaf of *Vinca minor* L. and for those obtained from the stem, which indicates the presence of compounds with similar antimicrobial activity for the extracts obtained from both studied plant materials.

Conclusion

In this study were analyzed by diffusimetric method, the antimicrobial properties of plant alcoholic extracts in a concentration of 40% and 70% obtained from the leaf and stem of *Vinca minor* plant. Testing antimicrobial activity was performed on two types of reference bacteria positive*: S. aureus* and one Gram-negative: *E. coli* and a species of yeast (*C. albicans*). Studies performed for alcoholic extracts of 70% concentration have demonstrated the efficacy and antibacterial character of these extracts on various microorganisms tested, such as: two types of bacteria: Gram-positive: *S. aures* and Gram-negative: *E. coli* and a species yeast: *C. albicans*, using the diffusimetric method. The results obtained underlined that all samples of alcoholic extract of 70% concentration, in volumes of 7 μL, exerted a total antibacterial inhibitory action against the reference strains *E. coli* and *C. albicans* and moderate against the strain *S. aureus*, results that agrees with the literature. For plant extracts obtained in alcohol of 40% concentration, the results obtained from antimicrobial testing showed that they do not show antimicrobial activity. For alcoholic plant extracts with a concentration of 70%, the results obtained showed close values for both those prepared from the leaf and those prepared from the stem of *Vinca minor* plant, which highlights the presence of compounds with similar antimicrobial activity for extracts obtained from both studied plant material.

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