Open Access Journal of Toxicology
ISSN 2474-7599

Juniper Publishers
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Research Article
Volume 4 Issue 1 - April 2019
DOI : 10.19080/OAJT.2019.04.555626

Open Acc J of Toxicol

Phytochemical Study and *In Vitro* Antibacterial Activity of Two Traditional Medicinal Plants (Vinca Rosea and Vinca Difformis) from Libya

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Submission: March 04, 2019; **Published:** April 02, 2019

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Abstract

Vinca rosea and *Vinca difformis* are magnificent plants growing in the Libyan woodlands and are utilized beautifully as an embellishing in gardens. Aqueous and Ethanol extracts leaf of both species were preliminary phytochemically screened and tested against some pathogenic bacteria. Qualitatively analysis revealed Tannin, Saponin, Flavonoids and Terpenoids gave positive results and phlobatanins and Steroids gave negative results. Quantitative analysis revealed that the percentage yield of bioactive constituents is present more in *Vinca rosea* than in *Vinca difformis*. In addition, the crude extracts of *Vinca rosea* and *Vinca difformis* were tested (using the Disc Diffusion Method) for their antimicrobial activity against the bacterial pathogens. The influences of aqueous and ethanol extracts on some pathogenic: two strains of gram-positive bacteria include *Staphylococcus aureus* and *Streptococcus spp.*, and two strains of gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumonia*, and the sensitivity of the microorganisms to the extracts of plant's species were compared with each other and with designated antibiotics by measuring the diameter of the inhibition zones, After incubation, the zone of inhibition was measured in mm, a good inhibition of more than 6 mm were observed indicating the effective antibacterial activity of the bioactive compounds in both of the plants leaves extracts. Moreover, the including results of the antibacterial activities of plants leaves extracts were discussed regarding their phytochemical components which exhibited that the *Vinca rosea* and *Vinca difformis difformis* have a good inhibition zone against two tested bacterial strains.

Keywords: *Vinca rosea; Vinca difformis*; Bioactive Constituents; Qualitative and Quantitative analysis; Antibacterial activities

Introduction

Medical plants are usually used in conventional medicine as treatments for several diseases and contagious illnesses. Utilize herbal plants own fewer side consequences and including its cost is lower. Herbal plants are a group of flower plants that play an important role in the process of public and private parks, which are multi-color, shapes, sizes plant. *Vinca* is one of the grass groups of high coordination value for its numerous forms. It has multiple uses in the garden and can be used in designing and streets to withstand extreme external conditions. *Vinca rosea* and *Vinca difformis* are magnificent plants growing in the woodlands and utilized beautifully as an embellishing in gardens, they have a blooming season from late winter to early spring and are often evergreen. *Vinca rosea* has purplish red flowers while *Vinca difformis* has whitish-blue flowers. The *Catharanthus roseus,* ordinarily known as *Vinca rosea* and recognized as the *rose periwinkle, rosy periwinkle* or *Madagascar periwinkle.* Though the *Vinca difform is* commonly called the intermediate periwinkle, is a species of flowering plant in the dogbane family *Apocynaceae. Catharanthus roseus* original and endemic to Madagascar were bout species are growing in Europe, North Africa and south-west Asia, and used as a scenically and medicinal plant, as a source of the drugs which used to treat cancer. Because of presence bioactive ingredients which are existing in plants such as phenolic, tannins, alkaloids and flavonoids are vital and characterized as a natural's antioxidants owing to existing these substantive useful composites, and which effect on improvement of the performance of humane body organs, adding, are entered in modifiable of lowering or raising certain excretion of hormones. Moreover, *Vinca rosea* assistance in cumulative the insulin creation which helps in healing diabetes, furthermore, the major influence complete is around the antidiabetic potential of this plant through using crude extracts rather more than the pure bioactive compounds [1,2].

Materials and Methods

Collection and extraction

The fresh and healthy samples of leaves of *Vinca rosea* and *Vinca difformis* were collected from wild of Al-Khums, Al-Khums, Libya. They were washed by tap water then by distilled water, later that dried in shade, drying then was finished in an oven at 45 °C. Samples were powered by the electrical blender. 20g of finely

powdered leaves of the *Vinca rosea* and *Vinca differmis* whereas each sample was soaked with 400ml appropriate solvent (distilled water and ethanol, separately), into flasks which were fixed on the shaker and then settled at a room temperature 25±2 °C for 72 hours. The solvent was filtered then removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark brown residue of water extract and a viscous dark green residue of ethanol extract.

Qualitative phytochemical screening

The finely powdered leaves of the *Vinca rosea* and *Vinca differmis* and the prepared crude extracts were carried out for the existence of bioactive composites by via standard methods [3-7].

Steroid

5ml of crude extract was mixed with 2ml of chloroform and concentrated sulphuric acid was added carefully along the side of the test tube. A red color formed in the lower chloroform layer indicated the presence of steroids.

Sterols and triterpenes

5g of the finely powdered leaves was sited in a test tube and 20ml of 50% alcohol was added, the tube was then heated for 3min in a water bath. It was then allowed to cool to room temperature and filtered. The filtrate was then evaporated in an evaporating beaker to dryness and about 10ml of petroleum ether was added to the beaker and stirred for 5min, the petroleum ether portion was then discarded. 15ml of chloroform was then added and stirred for about 5min, it was then transferred into a test tube and about 1mg of anhydrous sodium sulphate was added and shaken gently and filtered, the filtrate was then divided into two test tubes and used for the following tests

a. **Salwoski's test:** To the first test tube: 2 to 3 drops of concentrated sulphuric acid was added to form a lower layer. Reddish-brown color at the interphase indicates the existence of a steroidal ring.

b. **Lieberman-Burchard's reaction:** To the second test tube: an equal volume of acetic anhydride was added and gently mixed. Then 1ml of concentrated H_2S0_4 was added down the side of the tube. The appearance of a brownish-red ring at the contact zone of the two liquids and a greenish color in the separation layer indicates the existence of sterols and triterpenes.

Carbohydrates

Fehling's test: 2ml of Fehling A and B reagents (Equal volume were mixed together) was added to 5ml of crude extract and gently boiled. A brick-red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Benedict's: 2ml of Benedict's reagent was mixed with 5ml of crude extract and boiled, a reddish brown precipitate formed which indicated the presence of the carbohydrates.

Molisch's test: 2ml of Molisch's reagent was mixed with 5ml of Crude extract and the mixture was shaken accurately. Afterwards, 2ml of concentrated H_2S0_4 was poured prudently along the side of the test tube. The appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Iodine test: 5ml of the crude extract was mixed with 2ml of iodine solution. A dark blue or purple colouration indicated the presence of the carbohydrate.

Flavonoids

Shinoda test: 5ml of the crude extract was mixed with few fragments of magnesium powder. Concentrated HCl was added dropwise. Pink scarlet color appeared after a few minutes which indicated the presence of flavonoids.

Alkaline reagent test: 5ml of the crude extract was mixed with 2ml of 2% solution of NaOH. An intense yellow color was formed which turned colorless on the addition of a few drops of diluted HCl acid which indicated the presence of flavonoids.

Saponins

5ml of distilled water was mixed with extract in a test tube and was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Alkaloids

5g of the finely powdered leaves were boiled in a water bath with 50ml of 5% H_2S0_4 in 50% ethanol. Then was cooled and filtered. A portion was set aside. One more portion of the filtrate was put in 200ml of separating funnel and the solution was made alkaline by adding 3 drops of concentrated ammonia solution. Formerly Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was collected into a second separating funnel. The ammoniacal layer was set aside. The chloroform layer was extracted with two quantities each of about 15ml of dilute sulphuric acid. The various extracts were then used for the following test:

a. **Wagner's test:** 2ml of the filtrate was added 1ml of Wagner's reagent in dropwise. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

b. **Dragendorff's test:** 2ml of the filtrate was added a 1ml of Dragendorff's reagent in dropwise. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

c. **Mayer's test:** 2ml of the filtrate was added a 1ml of Mayer's reagent in dropwise. Formation of a greenish colored or cream precipitate indicates the presence of alkaloids.

Tannins and phenols

5ml of the crude extract was mixed with 1ml of 2% solution of $FeCl_3$. A blue-green or black colouration indicated the presence of phenols and tannins.

Proteins and amino acids

a. **Millon's test**: 5ml of crude extract was mixed with 2ml of Millon's reagent; a white precipitate appeared which turned red upon with gentle heating that indicates the presence of protein.

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b. Ninhydrin test: 5ml of crude extract was boiled with 2ml of 0.2% solution of Ninhydrin, a violet colour appeared to indicate the presence of amino acids and proteins.

Anthraquinones

0.5g of the crude extract was dissolved in 5ml of 1% HCl and boiled then filtered. The filtrate was shaken with 5ml benzene and the benzene layer was decanted. 10% Ammonium hydroxide was added and the color in the alkaline phase was observed. Formation of pink-violet or red color indicated the presence of anthraquinones.

Quantitative Analysis:

Quantitative Analysis for the alkaloids, Flavonoid, Saponin and total phenols which present in the extracts is carried out by following the procedure [7-12]:

1. **Alkaloid determination:** 10g of the soft plant sample was weighed into a 500ml beaker and 400ml of 10% acetic acid in ethanol was added and covered and allowed to settle for 4h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution could stand and the precipitated was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed [8].

2. **Flavonoid determination:** 5g of the soft plant sample was extracted with 50ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper 125 mm. The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [7].

3. **Saponin determination:** 10g of plant sample was mixed within 100ml of 20% ethyl alcohol. The suspension was heated over a water bath for 4h with continuous stirring at about 50°C. The mixture was filtered, and the residue re-extracted with another 100ml of 20% ethyl alcohol. The combined extracts were reduced to 20ml over a water bath at about 80°C. The concentrate was transferred into a 250ml separating funnel and about 10ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification procedure was repeated. 30ml of n-butanol extracts were washed twice with 5ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven into a constant weight. The saponin content was calculated in percentage [8].

4. **Determination of total phenols:** 10g fine powdered sample was weighed into a 500ml titration flask and 200ml n-hexane was added twice for 4h each; the filtrates were discarded for fat-free sample preparation. Then, 100ml of diethyl ether was added twice, heated for 15min each, then cooled up to room temperature and filtered into a separating funnel. About 100ml of the 10% NaOH solution was added twice and shook well each time to separate the aqueous layer from the organic layer. It was washed three times with 50ml deionized water. The total aqueous layer was acidified up to pH 4.0 by adding 10% HCl solution and 100ml dichloromethane (DCM) twice to acidify the aqueous layer in the separating flask. Consequently, the organic layer was collected, dried and then weighed [9-11].

5. **Antibacterial Activity:** The aqueous and ethanolic crude extracts of the *Vinca rosea* and *Vinca differmis* were tested against four strains of human pathogenic bacteria were obtained from Analysis Laboratory of Al-Khums Teaching Hospital, Al-Khums Libya, and were intended for the examinations of two strains of gram-positive bacteria include *Staphylococcus aureus* and *Streptococcus spp.*, and two strains of gram-negative bacteria including *Escherichia coli* and *Klebsiella pneumonia*, and the sensitivity of the microorganisms to the extracts of bout plant's species were compared with each other and with designated antibiotics by measuring the diameter of the inhibition zones (The antibiotic discs such as Ciprofloxacin (Cipr), Gentamycin (Gent), Ampicillin (Amp) and Penicillin (Pinc) were placed on the surface of the plates used as positive control while the aqueous and ethanol solvent used as negative control. The plates were incubated at 37 °C for 24 hours and after incubation the diameter of the inhibition zones were measured in mm and recorded), (Sterilized discs paper 6mm in size, and each sterile disc was saturated separately with 50µl of crude extracts using micropipette, and they were allowed to dry, then immediately placed into Muller Hinton Agar plates were prepared and cultivated by the appropriate test organisms were swabbed over the surface of agar plates using sterile cotton swab), After incubation for 24 hours at 37 °C the diameter of inhibitory zones formed around each disc were measured (mm) recorded, a good inhibition of more than 6mm were observed indicating the effective antibacterial activity of the bioactive [12,13].

Results and Discussion

Quantitative Analysis

a. **Calculation of Yields (%) leaves of *Vinca rosea:***

Fresh Weight of the Plant leaves = 50.653gm.

Weight of dry powder used for aqueous extraction = 20.315gm. Weight of powder after Soxhlet extraction (aqueous) = 17.132gm, thus real weight loose = 3.183gm.

Weight of dry powder used for ethanolic extraction = 20.105gm. Weight of powder after Soxhlet extraction (Ethanolic) = 18.431gm, thus real weight loose = 1.674gm.

b. **Calculation of Percentage Yields (%) leaves of *Vinca differmis*:**

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Fresh Weight of the Plant leaves = 50.203gm.

Weight of dry powder used for aqueous extraction = 20.201gm. Weight of powder after Soxhlet extraction (aqueous) = 16.924gm, thus real weight loose = 3.183gm.

Weight of dry powder used for ethanolic extraction = 20.014gm. Weight of powder after Soxhlet extraction (Ethanolic) = 17.213gm, thus real weight loose = 2.801gm.

As shown in Table 1 the percentage yields of each chemical constituent's present in *Vinca rosea* and *Vinca difformis* leaves were 84.331 and 83.778 % of aqueous and ethanolic extracts, while percentage yield of each alkaloid, Flavonoids, Saponins and Phenols in *Vinca rosea* were 44, 25, 31 and 36% correspondingly, and *Vinca difformis* 30, 22, 29 and 33% respectively. These results which may be reflected for such these plants as major in folk medication for diseases treatment and give importance for nowadays to considering it as a source for extracting such chemical constituents as a benefit in drugs manufacturing. Also, it provides a moreover conception for a specific investigation to provide some biochemical basis for ethnopharmacological utilization of these species in the medication of diabetes. Additionally, the leaves have tetrhydrolastonine, coronaridine, vindoline, lochnerine, catharanthamine, leurosin, Cartharanthine, Vincarodine which are an effective chemical constituent [14].

Table 1: Results of the quantitative analysis of the *Vinca rosea* and *Vinca difformis* leaves.

Plants Names	Percentage Yields (%)					
	Chemical Constituents		Alkaloids	Flavonoids	Saponins	Phenols
Vinca rosea	Aqueous	Ethanolic				
	84.331	91.673	44	25	31	36
Vinca difformis	83.778	86.004	30	22	29	33

Qualitative analysis

Table 2: Results of the pphytochemical screening results of crude extracts of leaves of Vinca rosea and Vinca difformis:

Name of the Compound	Crude Extracts of Leaves of *Vinca Rosea*		Crude Extracts of Leaves of *Vinca Difformis*		The Tests Names and Resulted in Colours
	H_2O	EtOH	H_2O	EtOH	
Steroid	+	+	+	+	Chloroform: Red
Sterols & Triterpenes	+	+	+	+	Salkowski: Reddish-brown
	+	+	+	+	Lieberman-Burchard: Brownish-Red ring & green
Carbohydrates	+	+	+	+	Fehling: Brick red precipitate
	-	-	+	+	Benedict: Reddish Brown Precipitate
	+	+	+	+	Mulish: Violet Ring
	+	+	+	+	Iodine: Dark Blue or Purple
Flavonoids	+	+	+	+	Shinoda: Pink Scarlet
	+	+	+	+	Alkaline Reagent: Yellow
Saponin	+	+	+	+	Foam: Stable Foam
Alkaloids	+	+	+	+	Wagner: Reddish-Brown Precipitate
	+	+	+	+	Dragendorff: Reddish-Brown Precipitate
	+	+	+	+	Mayer's: Creamy Precipitate
Tannins & phenols	+	+	+	+	Ferric Chloride: Blue- Green or Black
Proteins and amino acids	-	-	+	-	Millon's: Red
	+	+	+	+	Ninhydrin: Violet
Anthraquinones	-	+	+	+	Ammonium hydroxide: Pink-Violet or Red

+ = Present, - = Absent, EtOH = Ethyl Alcohol, H2O = Distilled water

Table 2 reveals the phytochemical screening of various chemical ingredients of selected plant species beneath study on a qualitative reason, were the phytochemical screening revealed the presence of most of the compounds such as Steroid, Sterols and Triterpenes, Carbohydrates, Flavonoids, Saponins, Alkaloids, Tannins and Phenols, Proteins and amino acids while Anthraquinones not present in the crude extracts of leaves of *Vinca rosea* and *Vinca difformis.* The existence of these ingredients could be responsible for the performance as antidiabetic activities. It proffers possible an additional requirement for a thorough investigation to obtain a specific biochemical source for ethnopharmacological purposes of this plant in the treatment of diabetes. The natural organic compounds such as alkaloids are mostly contained nitrogen atoms. These groups are characterized by weakly acidic properties,

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further involves some related composites with neutral. Certain synthetic composites of the like structure are likewise named alkaloids. *Vinca rosea* and *Vinca difformis* are rich in the alkaloids which own an extensive enormous of pharmacological effects, Besides that, alkaloids involving atoms such as nitrogen, hydrogen, carbon further sometimes involving sulfur and elements like to phosphorus and chlorine which that played an important role in this effectiveness. Also, these chemical ingredients such as terpenoids are useful in the therapy and inhibition of various illnesses, as well as cancer, and the terpenoids own Antihyperglycemic, anti-allergenic, antiparasitic, antimicrobial, antiviral, antifungal and anti-inflammatory properties [15,16]. While steroids act responsibly for cholesterol-decreasing characteristics and assists in regulating the immune response [17]. Traditionally, in Libya *Vinca* plant is used in the system of conventional medicine, commonly, such as a blood glucose lowering agent and essentially anticancer treatments. The existence of proteins in these plant's species should not be ignored and considered it as nutritional strength and as protein complements [18]. Tannins were found in the whole extracts of selected medicinal plants, wherever tannins have good properties such as infected mucous membranes and increasing of wounds healing. However, flavonoids improve in effecting diabetes-induced oxidative tension. Therefore, from the present study medicinal properties of these species plant could be identified based on the ingredients present in them.

Antibacterial Activities: Vinca rosea and Vinca difformis

Table 3: Antibiotic and Antibacterial activity of aqueous and ethanol extract of Vinca rosea and Vinca difformis by the disc diffusion method.

Human Pathogenic Bacteria	Zone of Inhibition (in mm.)							
	Vinca Rosea Extracts		*Vinca Difformis* Extracts		Antibiotics			
	Aqueous	Ethanol	Aqueous	Ethanol	Cipr	Gent	Ampi	Pinc
S. aureus	10	14	9	9	20	23	25	23
S. spp.	13	12	11	11	21	22	26	24
Esch. coli	10	11	8	10	23	21	20	25
K. pneumonia	13	14	12	11	23	24	21	26

mm = millimeter, Staphylococcus aureus (S. aureus), Streptococcus spp. (S. spp.), Escherichia coli (Esch. coli) and Klebsiella pneumonia (K. pneumonia).

The results are presented in Table 3 of antibacterial activity of each of *Vinca rosea* and *Vinca difformis* extracts and which were analyzed against specific of humane pathogenic bacteria, were the maximum antibacterial activities was observed in ethanol extract of *Vinca rosea* 14mm against *S. aureus* then 13mm aqueous extract against *S. spp.* and *K. pneumonia*, followed by 12mm formed from ethanol extract against *S. spp.,* and 11mm was formed from the ethanol extract of *Vinca rosea* against *Esch. Coli* and 10mm was aqueous extract against each of *S. aureus* and *Esch. Coli.* While for the *Vinca difformis* extracts were 9, 11, 8 and 12mm formed from the aqueous extract against *S. aureus, S. spp, Esch. Coli* and *K. pneumonia*, correspondingly, where the ethanol extract was 9, 11, 10 and 11mm against each of *S. aureus, S. spp, Esch. Coli* and *K. pneumonia* correspondingly. The presence of the chemical constituents such as tannins, saponins and flavonoids were found this specific plant might acting against these sorts of bacteria and are beneficial when it's used in both protective and remedial of the human body. Likewise, this study was conducted to realizing that this medicinal plant's species ordinarily employed in traditional treatment were effective to the following strains of bacteria *Staphylococcus aureus, Streptococcus spp., Escherichia coli* and *Klebsiella pneumonia*. Moreover, the including results of the antibacterial activities of plants leaves extracts were discussed with respect to their phytochemical components which exhibited that the *Vinca rosea* and *Vinca difformis* have a good inhibition zone against the four tested bacterial strains. *Vinca rosea* causes a slowdown of the central nervous system and leads muscles to relax and acts on lowering blood pressure for analgesic and used for natural tranquillizing. The alkaloids have influences on the human's biological activity, where producing various stages of psychological and physiological replies, mainly by intervening with neurotransmitters. Also, the alkaloids function as interference with membrane transportation, protein synthesis or extra processes and if used in enormous amounts will lead to highly toxic fatal while if used in small amounts, may have pain killers, chemotherapy and corrective advantage.

Conclusion

The phytochemical ingredients of the leaves of the *Vinca rosea* and *Vinca difformis* were examined. The leaves have Steroid, Sterols & Triterpenes, Carbohydrates, Flavonoids, Saponin, Alkaloids, Tannins & phenols, Proteins, amino acids and Anthraquinones. The leaves of this plant used in traditional medicine for the treatment for diabetic and as an anticancer treatment. Correspondingly, from this research study were observed that all extracts of the plant were concerned a good effect in the antimicrobial activity.

Acknowledgement

We would like to thank Department of Chemistry, Science College, Department of Chemistry, Education College, El-Mergib University Al-Khums Libya Department of Microbiology Laboratory at Al-Khums Teaching Hospital, Al-Khums, Libya, Institute of Organic Chemistry and Technology Faculty of the Chemical Technology. University of Pardubice Czech Republic for providing the necessary facilities.

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DOI: 10.19080/OAJT.2019.04.555626

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