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Research Article

Antioxidant and Antimicrobial Activity of Flavonoids Fraction Extract from *Arnebia Decumbens* (Vent) Growing in South East Algeria

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ABSTRACT
This study intends to investigate plants that grow in southeast Algeria namely *Arnebia Decumbens (Vent.)* which are commonly used by medical science for a treatment. More especially this study shed light on the antioxidant effect and biological activity of the plant whereby extraction of the active ingredients flavonoids is taken into account. The active ingredients were identified through the scanning device using High-performance liquid chromatographic (HPLC). In-order to get deep insight into the body of knowledge towards extraction process; current study utilized both qualitative and quantitative approach. The data was analyzed separately on the antibacterial activity and oxidation of the extracts flavonoids. The obtained results revealed a significant effect on the proliferation of some bacterial strains and free radical. In addition, extracts of flavonoids have shown an active effect on bacteria; Staphylococcus aureus ATCC 6816 and Staphylococcus aureus methicillin resistant and a greater efficacy than the antibiotic chosen "polymyxin B". Hence, based on the empirical evidences it can be stated that from various concentrations approach; the sensitivity of each type of bacteria against each extract can be determined.

Keywords: *Arnebia Decumbens (Vent.)*, flavonoids, DPPH, antioxidant activity, antimicrobial.

INTRODUCTION

Arnebia Decumbens (Vent.) it called in the region of south east Algeria in the name *« homaire »*, it is a small red pigment which is usually found in the crust of the roots of an herbaceous plant in the region of south Algeria. The size of the homaire does not exceed 25 cm. It is covered with stiff bristles of the latter turn into a semblance of thorns thin when they reach the plant and the beginning of drying *Arnebia Decumbens (Vent.)* elongated leaves and do not have a clear neck. The flowers are yellow in color and collects in nurat apical dense[1]. Herbal treatment for curing certain medical diseases is a common practice in Africa, statistically it is estimated that over 80% of the total population produced a wide array of phytochemical most of which are used from the plant. The main reason for preferring herbal treatment is to avoid the undesirable secondary effects that are commonly known as unwanted side effects of some synthetic chemical drugs[2]. Moreover, research has indicated that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease[3]. Two synthetic antioxidants namely butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) which are more used in the food industry and also considered as a major contributor towards liver damage and carcinogenesis or toxic[4,5,6]. Thus, in order to avoid certain unhealthy circumstances, it is necessary to focus on others natural antioxidant extracts from plants. Several chemical compounds extracted from plant leaves, however, the most important is the flavonoids. Flavonoids are secondary metabolites ubiquitously distributed in all higher plants[7]. Flavonoids (or bioflavonoids) (from the Latin word flavus meaning yellow, their color in nature). Chemically, they have the general structure of a 15-carbon skeleton, which consists of two phenyl rings (A and B) and heterocyclic ring (C). This carbon structure can be abbreviated C6-C3-C6[8,9]. Contemporary studies confirmed the antimicrobial activity of flavonoids occurring in vegetable foods and medicinal plants. These antimicrobial activities facilitate in diverse ways such as anti-allergic, antimicrobial, anti-inflammatory, vasoprotector and anti-tumour agents respectively[10]. The fruits date is rich with phytochimicals like phenolic acids, sterols, procyanidins, flavanoids, carotenoids and anthocyanidin. Research has also revealed that the Arnebia decumbens (Vent.) Coss et Kral are beneficial in a biological and pharmacological viewpoint. Because Arnebia decumbens (Vent.) Coss et Kral contains antiviral, antibacterial, anti-inflammatory, antitumor, these activities strength the immunity system,

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Table 1: Gradient elution

Temps (min)	Solvent A (%)	Solvent B (%)
0	10	90
5	20	80
10	30	70
30	50	50
40	60	40
45	70	30
50	90	10
55	50	50
60	10	90

also it contains anti-oxidant activity[11-15]. Although all studies conducted in appointments, in our knowledge, there is no scientific information and empirical evidence on the study of the plant, the ability of anti-oxidation of bacterial activity on the Arnebia decumbens (Vent.) Coss et Kral plant. There the current the study was conducted to estimate the chemical composition, the effect of an anti-microbial and anti-oxidant from the extraction of natural products effective "flavonoid" of Arnebia decumbens (Vent.) Coss et Kral plant that grows in the southeast of Algeria. It is expected that the obtained results can be taken as a guideline and might considered as a new source of agent antioxidant and antimicrobial.

MATERIAL AND METHODS

Chemicals and reagents

Methanol, ethanol absolute, chloroform ($CHCl_3$), n-butanol, ethyl acetate and ultra pure water were purchased from VWR Merk (France), Diphenyl-1 picrylhydrazyl (DPPH), BHT and chlorogenic acid were procured from Sigma–Aldrich Inc (Paris, France). All other chemicals and reagents were analytical-reagent, sodium carbonate (Na_2CO_3), gallic acid, sodium nitrate ($NaNO_2$), aluminium chloride ($AlCl_3$), sodium hydroxide (NaOH), catechin, hydrochloric acid (HCl), quercetin, linoleic acid, sodium phosphate, ferric chloride ($FeCl_3$), sulfuric acid (H_2SO_4) and ammonium molybdate. The following reagents were used for the microbial activity namely Nutrient agar and sabouraud dextrose agar. *Plant material*The aerial parts of *Arnebia decumbens (Vent.)* in March 2014 from Douilatte located in Wilaya of El-Oued south east Algeria (33° 07" 00" N 7° 11' 00" E). This species was identified by Pr. Gerraf Noureddine; Laboratory of Biomolecules and Plant, Breeding, Larbi Ben M'hidi University, Oum El Bouaghi. Prior using the extraction of the plant for medical purpose, the leaves were dried in well-ventilated spaces at room temperature. After, powdered and sifted in a sieve (0.75 µm).

Extraction of flavonoids

After drying in a dry, ventilated area, away from sunlight, the plant is crushed and then be weighted (100 g). Plant material obtained is then maceration in a hydro alcoholic mixture (methanol /water; 80/20; V/ V). This maceration process repeated in three times with solvent renewed every after forty-eight hours. After went through the process of filtration and concentration in vacuum, the methanolic extract is then diluted with water distilled at 50 ml per 100 g of dry matter, the rest is left in solution overnight then filtered. After filtration, the solution has undergone successive liquid-liquid extractions type using solvents of increasing polarity starting with chloroform and ethyl acetate and finally with n-butanol[16-19].

Analyses using High performance liquid chromatography (HPLC)

Flavonoids compounds have been separated and identified by liquid chromatography system high performance reverse phase mark (Agilent Technologies 1260, Germany) equipped with a UV diode array detector (DAD) and equipped with a chromatographic column filled with a grafted silica gel, octadecyl type ZorbaxEclipse XDB- C18 (4.6 x 100 mm, 3.5 microns). For the various extracts, a conventional chromatographic condition is usually adapted. Indeed, the detector (DAD) is adjusted to a scan of scanning from 200 to 400 nm, whereby the column temperature was maintained at 25 ° C. The volume injected is 20 µl and the mobile phase used is made up of two solvents A and B: Solvent A (Methanol), Solvent B (water containing 0.1% formic acid). The speed of this phase is set at 0.4 ml / min. The separation method adopted is the gradient elution in which the program is shown in the Table 1. Identification of flavonoids compounds was performed by comparing the retention times of peaks obtained for those flavonoids standards injected in the same chromatographic conditions.

Determination of total flavonoids

Two reagents were used namely sodium nitrite colorless solutions ($NaNO_2$, 5%) and aluminum chloride ($AlCl_3$, 10%). The principle of the method is based on the oxidation of the flavonoids by these reagents; it leads to the formation of a brownish complex, which absorbs at 510 nm. Comparing the OD observed to that obtained by a known concentration of catechin standard used to evaluate the total content of flavonoids. The total flavonoids are measured calorimetrically in a flask of 10 ml were introduced successively 250 µl of extract of known concentration in leaves and 75 µl of a solution of $NaNO_2$ (5%). After 6 minutes was added 150 µl of $AlCl_3$ (10%) and 500 µl of NaOH (1N) and in 1525 µl of distilled water was added to the mixture successively. A calibration curve is prepared at different concentrations with standard solutions of catechin. The absorbance of

Table 2: Mass yield and flavonoids content EC mg /g DM) of leaves extract obtained by methanol 80% of *Arnebia Decumbens (Vent.)*. Results are expressed as the mean and ±standard deviation of three independent experiments.

	Yield (%) w/w	Total antioxidant activity mg GAE/g DW	Flavonoids content
Arnebia Decumbens (Vent.)	10,25± 0,07	129.45 ± 3.43	9.181 ±0.23

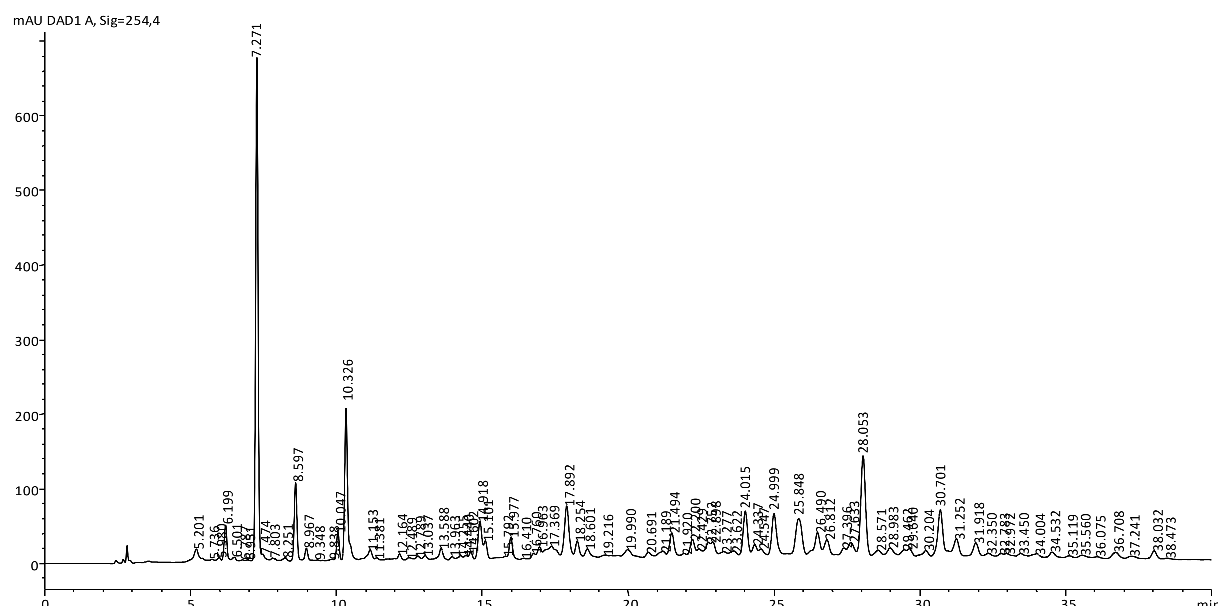

Figure 1: HPLC profile of leaves extract from *Arnebia Decumbens (Vent.) r*ecorded in UV at 254 nm

Table 3: Quantification of flavonoids compounds identified in methanolic 80% leaves extract from *Arnebia Decumbens (Vent.)*.

Retention time (min)	Pic surface	Identification	Quantification µg/ml
8.597	690.4	Gallic acid	63.676
7.271	3992.9	No identified	-
10.326	1493.3	Catechin	122.584
24.015	625.6	p-comaric acid	101.451
25.848	715.8	Coumarine	82.478
30.701	783.5	Isoquercetrin	557.084
28.053	1502.3	No identified	-
12.622	255	Querecetin	20.30

the mixture obtained is directly measured by UV-visible spectrophotometer at 510 nm and the results are expressed in mg catechin equivalent /g of dry matter (EC /g DM). The data was analyzed with three separate experiments. The obtained correlation coefficient of the calibration curve was $R^2 = 0.988$ while the result of the obtained result is presented as mean (± SEM)[20-24].

Determination of the antioxidant activity

The measurement of antioxidant potential was carried out by determining the products resulting from the oxidation or by assessing the ability to trap reaction models radicals. The first mode requires prior knowledge of the compounds from oxidation. Indeed these methods seek some functional groups (aldehydes, ketones, dicarbonyl) in the derivatives of the original components. The second mode links the amount of trapped radicals of used antioxidant. These dual modes and forms of expression are preferred to use the percent inhibition (IC) and / or equivalence standards polyphenols obtained by UV-Visible spectroscopy. The percent inhibition for assessing antioxidant activity of a sample is determined using the following formula: $IC (\%) = [(A-B) / (A)] \times 100$

Where a = absorbance of the oxidized solution in the absence of antioxidant agents, b = absorbance of the oxidized solution in the presence of antioxidant agents. Evaluation of the ability of the compound (extract) to trap free radicals is therefore to measure its ability to scavenge free radicals and therefore slow or inhibit the creation of free radicals. In the case of the evaluation of the antioxidant activity according to the equity standard polyphenols, the method comprises of comparing the absorbance of targeted samples to that of a calibration straight line, which connects the absorbance to the concentration in standard. The types of radicals that are used to evaluate the antioxidant activity of extracts of the leaves are reducing power, the radical ABTS test, the radical OH, and the DPPH radical[25-28].

Total antioxidant activity

During this test, hydrogen and electron are transferred from the reducing compound (extract-antioxidant) to the oxidizing complex. This transfer phenomenon depends on the redox potential of the medium pH and on the structure of the antioxidant compound. The test is based on the reduction of the molybdenum of the oxidation stage (VI) the oxidation state (V). This reduction is materialized by forming a greenish complex (phosphate / Mo (V)) at an acidic pH that measures the decrease in the coloration of molybdenum (VI) complex in the presence of antioxidant. Unlike other tests, this test makes it possible not only to quantify the contribution of the antioxidant activity of the polyphenols but also other antioxidants such as vitamins. The method comprises introducing into an Eppendorff

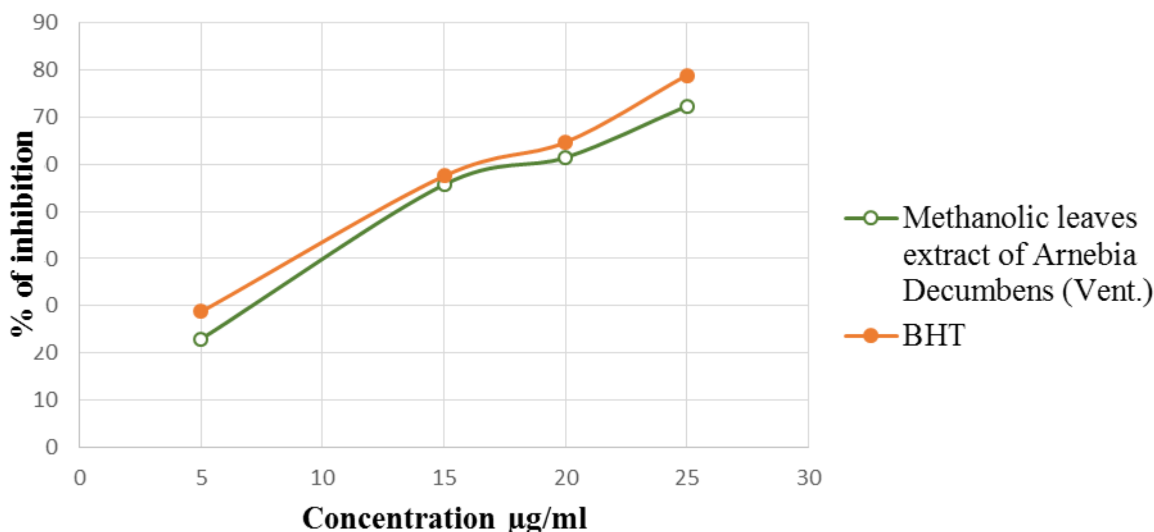

Figure 2: DPPH scavenging radical activity of leaves extract from *Arnebia Decumbens (Vent.)*.

Table 2: Antibactrial activity of methanolic extracts leaves of *Arnebia Decumbens (Vent.)*

Microorganisms	Diameter of zone inhibition	
Bacteria	extract	polymyxine B
Staphylococcus aureus ATCC 6816	13 ± 0.6	12,5 ± 0,5
Staphyloccocus aureus	14 ± 0.5	11,0 ± 0,4
Bacillus cereus ATCC 14579	8 ± 0,2	8,5 ± 0,1

tube 100 µl of the extract of the leaves mixed with 1 ml of a reactive compound of H_2SO_4 (0.6 M), NaH_2PO_4 (28 mM) and ammonium molybdate (4 mM), the tube incubated at 95 °C for 90 min. After cooled, the absorbance is measured at 695 nm, the control consisted of 100 µl of methanol mixed solvent with 1 ml of the reagent mentioned above[29,30]. For the calibration curve and controls are incubated under the same conditions. The results are expressed in mg of gallic acid equivalents per gram of dry matter (EAG mg / g DM). The calibration curve is established with a correlation coefficient R^2 = 0.998.

DPPH assay

DPPH test (2, 2-diphenyl-1-picrylhydrazyl) is a method widely used in the analysis of an antioxidant activity. Indeed, the DPPH is characterized by its ability to generate stable free radicals. This stability is due to the relocation of the free electrons in the molecule. The presence of DPPH radicals resulted in a dark purple color of the solution, which absorbs at about 517 nm. The reduction of DPPH radicals by an antioxidant agent results in a discoloration of the examined solution. Evaluation of the antioxidant capacity is performed as follows, in 250 µl of DPPH solution (7.8 mg DPPH in 100 ml of methanol) was mixed with 1 ml of leaves extract. The resulting mixture was then kept sheltered from the light at room temperature for 30 minutes. The absorbance is measured at 517 nm. Sample preparation and the control is carried out under the same operating conditions. The decrease in the absorbance is measured spectrophotometrically and IC% (percent inhibition) is calculated using the formula below: $\% IC = [(At_0 - At_{30}) / At_0 \times 100)]$

Where At_0: absorbance of control (containing no antioxidant) 30 minutes At_{30}: excerpts absorbance measured after 30 minutes. The activity of radical scavenging is usually expressed in IC_{50} (µg/ml), the dose radical scavenging required to cause 50% inhibition. All results presented are averages (± SEM) and analyzed with three repetitions. By varying the concentration of the extracts and calculating the percentage concentration for each corresponding IC, a linear regression was determined between the different concentrations and percentage of inhibition. Prediction also leads towards deduction of the corresponding IC_{50} value[31,32].

Antimicrobial activity assays

Microorganisms

In total, three bacterial cells were used in a study. The bacterial cells assayed: Staphylococcus aureus ATCC 6816, Staphyloccocus aureus, Bacillus cereus ATCC 14579. All strains were obtained from the Laboratory of Bioactive Substances, Center of Biotechnogy, Borj Cedria (CBBC), and BP 901- Hammam Lif - 2050, Tunisia[33-35].

Incubation conditions

Nutrient agar was used culture medium for bacteria which was incubated for 24 h at the temperature of 37 °C and yeasts were cultured in sabouraud dextrose agar (SDA; 4% dextrose, 2% neopeptone and 1,7% agar) for 24-48 hours at the temperature of 30°C[36,37].

Disc diffusion assay

Methanol water extracts *Arnebia Decumbens (Vent.)* were dissolved in methanol-water 50% for a final concentration 10 mg/ml and filter-sterilized through a 0.45 membrane filter. The antimicrobial activity was estimated by method of disc diffusion, 100 µl of suspension for each microorganism 108 colony-forming units (CFU)/ml containing 20 ml of nutrient agar for bacteria, after were placed in the petri sterilized filter paper disc (6mm in diameter). Later it was soaked with 15 µl of the 50 mg/ml of each methanolic extracts (150 µg/disc). The methanol 50% was used as a negative

control whereas polymyxine B was taken as the positive control. However, both positive and negative control was prepared with the same procedure. The detailed described on the procedure was mentioned-earlier except for the methanol extract which was substituted by 15 µl of positive control at 50 mg/ml. The diameter of the inhibition zone around each disc was measured for three replicates.

Statistical analysis

Data was analysed using statistical tests whereby the obtained results were presented in mean values, and standard deviations (SD). Since, all measurements were carried out in three experiments therefore, the all the analyses in the present study were analyzed three times determinations). Statistical calculations were carried out by OriginPro version 8 software (Prolab), correlations were obtained by Pearson correlation coefficient using bivariate correlations test. P value were set at 0.05. Therefore, the obtained value less P value (0.05) was regarded as a statistically significant and P values < 0.01 was regarded extremely statistically significant.

RESULTS AND DISCUSSION

Extract yield

The methanol is a solvent extract significant amount of flavonoids compounds and recently used in several studies. It is considered as the best solvent of antimicrobial substances compared with the other solvents and given an elevated antioxidant activity. The results of extract yield of *Arnebia Decumbens (Vent.)* are mentioned in Table 3, which shows the extraction yield (g/100 g dry weight), the mass yield obtained for methanolic water extract of leaves from *Arnebia Decumbens (Vent.)* is found 10,25%.

HPLC analysis

The identification of compounds flavonoids Extract the majority of plant extract *Arnebia Decumbens (Vent.)*. HPLC was carried out based on the comparison of their retention times with those obtained for the same standard compounds. This comparison allowed us to confirm the presence of two major flavonoids isoquercitrin with a retention time of 30 701 min, and catechin with a retention time 10 326 min. Show in Figure 2 and table 4 with rates respectively 557 084 mg / 100 g of plant extract and 122 584 g / 100 g of plant extract, and in very small, we find a Querecetin compound with a retention time 12.622 min. Minor peaks were also recorded with retentions times ranging from 8597 min probably are phenolic compounds gallic acid with a rate of 63 676 µg / 100 g and compounds coumarine p-coumaric acid 24.015 min a time of retention, and coumarine at retention time of 25 848 min. with rates respectively 101.451µg / 100g and 82.478µg / 100 g of plant extract. Flavonoids rates are determined in plant extracts according to the calibration curve (peak areas as a function of the concentration of the standards).

Flavonoids content

The results obtained are expressed as mg catechin equivalents per gram of dry mass (EC mg / g DM). The results of the quantitative analyzes of flavonoids in the extracts of the leaves of *Arnebia Decumbens (Vent.)*. These results indicate that the extract from the leaves of *Arnebia Decumbens (Vent.)*, the extract is rich in flavonoids; the results are shown in table 2. The reference compound used in the preparation of this curve is catechin. The curve is calculated with a correlation coefficient $R^2 = 0.988$.

Total antioxidant activity

The total antioxidant activity of methanolic 80% leaves extracts from *Arnebia Decumbens (Vent.)* is 129.45 ± 3.43 mg GAE/g DW. These results exhibit strong values and confirmed the high antioxidant activity of leaves extract of *Arnebia Decumbens (Vent.)* founded in DPPH reducing power. Study estimated the total antioxidant capacity of Korkobb, Tunisian date fruit. The authors suppose that the highest level of flavanoid in this variety is responsible for the higher total antioxidant capacity[36], the result are presented in Table 2.

DPPH radical scavenging activity

The DPPH radical scavenging activity of methanolic water extract of *Arnebia Decumbens (Vent.)* is represented in Figure 2. The crude extract of leaves displayed the high value (IC_{50}=12.05±0.08 µg/mL). The antioxidant capacity of leaves extract is higher than the positive control BHT (IC_{50}= 14.46 ± 0.06 µg/mL)**.** The phenolic compounds were considered to be the most active antioxidant derivatives in plants and are well known as antioxidant and scavenger agent against free radicals[111]. In living system, the free radicals are constantly generated and their associated with oxidative extensive damage to tissues. Different therapeutic approaches can be used to decrease the oxidative stress including scavenging of free radicals. Inhibition of these radicals produces enzymes and enchains antioxidant system by targeting the signaling routes. Many synthetic drugs protect against oxidative damage but they have adverse side effects[37]. The present study showed that the leaves methanolic water extracts of *Arnebia Decumbens (Vent.)* have good antioxidant as well as free radicals scavenging properties.

Antimicrobial activity

The diameter inhibition of leaves extract from *Arnebia Decumbens (Vent.)* is 13±0.6 mm. Then we can say that the bacteria Staphylococcus aureus ATCC 6816 sorting sensitive to flavonoids. The results showed that the diameter inhibition 12.5 ± 0.5 mm for Polymyxin B are against the bacteria Staphylococcus aureus ATCC 6816. Through reading the results, we conclude that the most effective flavonoids to polymyxin B because the diameter of inhibition is greater. The diameter of inhibition of leaves extract from *Arnebia Decumbens (Vent.)* is 14 ± 0. 5 mm, then we can say that the methicillin-resistant Staphylococcus aureus bacteria sensitive to the extracts flavonoids. The results give inhibition diameter 11.0 ± 0.4 Polymyxin B for against bacteria Staphylococcus Aureus Methicillin resistant. Through reading the results, we conclude that flavonoids are more effective than polymyxin B against bacteria Staphylococcus aureus methicillin resistant. The diameter of inhibition of leaves extract from *Arnebia Decumbens*

(Vent.) is 8 ± 0, 2 and from these results we can say that the bacteria Bacillus cereus ATCC 14579 Medium Sensitivity against flavonoid. Through reading the results, we conclude that flavonoids are less effective than polymyxin B against to bacteria Bacillus cereus ATCC 14579.

CONCLUSION

We think that the present study is the first investigation and comparing the phytochemical composition, antioxidant and antimicrobial activity of extracts of three varieties of Arnebia decumbens (Vent.) growth in Southeast Algeria. This study showed that considerable variance exists between the three extracts of leaves for plant Arnebia decumbens (Vent.) Cosset Kral" flavonoids". We found highest amount of flavonoids. On the other hand, the results of antioxidant activity tests present the strong capacity of extracts of leaves for plant Arnebia decumbens (Vent.), higher than the standards antioxidants (BHT). Finally, all extracts Appears the high antimicrobial activity for the Microorganisms tests (bacteria) exceeded most of the time the positive control. A strong correlation was found between activity and phytochemical contents indicates that the effects observed could be attributed to flavonoids compounds. This data Suggest a strong potential of this extracts as a natural source of flavonoids compounds, antioxidant and antimicrobial and may be considered in future to replace synthetic preservatives and drugs in pharmaceutical and food industry. After the results obtained in the previous study, from various concentrations, we can determine the sensitivity of each type of bacteria against each extract. As conclusion, we can say that they Extracts flavonoids to have an active effect on bacteria; Staphylococcus aureus ATCC 6816 and Staphylococcus aureus methicillin resistant and have shown greater efficacy than the antibiotic chosen "polymyxin B".

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