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Chemical Characterization and Biological Study of the Species *Senecio Cineraria*

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ABSTRACT

Senecio cineraria is a perennial shrub of Mediterranean origin, belonging to the family of Asteraceae, it has been used in pharmaceutical preparations and in homeopathy. This investigation has been a scientific contribution to the determination of certain phytochemicals, as well as the study of some in vitro biological activities of the methanolic extract of the plant. The analysis of the extract by colorimetric tests revealed the presence of flavonoids, alkaloids and tannins. Qualitatively, the TLC analysis of the extract showed the presence of a multitude of flavonoid varieties. The flavonoid assay showed a significant content on the order of 60.16 mg EQ / g E. The study of the antioxidant power by the DPPH method showed a concentration which traps 50% of the DPPH• (IC50) radical was 0.35 mg / ml. Methanol Extract of Senecio cineraria (MESC) revealed a significant anti-hemolytic effect compared to the positive control. This was proportional to the concentration of the extract used during the test. The antibacterial potential of the extract was confirmed on strains: Staphylococcus aureus, Listeria monocytogenes and Klebsielaoxytoca, with MICs of 10 mg / ml, 20 mg / ml and 2.5 mg / ml; respectively. Chronometric coagulation tests (TCK and TQ) showed that the extract has significant anticoagulant activity.

Keywords: *Senecio Cineraria, Methanolic Extract, Colorimetric Tests, Flavonoids, Alkaloids and Tannin, TLC Analysis*

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1. INTRODUCTION

Algeria, thanks to its particular geographical situation, its vast surface and its relief, benefits from a very varied range of climates and soils, favouring the development of a rich and diversified flora. Indeed, the Algerian territory covers important plant resources. This richness and originality leads to the study of "fundamental" scientific interest for the knowledge and know-how in the field of ethnobotany, traditional pharmacopoeia, but also an "applied" scientific interest in the field of the valorization plant genetic resources.

The cinerary maritime or the Cinnamon Sorrel, is a plant which belongs to the family of Asteraceae. This family has been the largest angiosperm with 23600 species, widespread in temperate regions (Dupont and Guignard, 2012).

The present work has been dedicated to the phytochemical study and the evaluation of biological activities of the methanolic extract of the plant Senecio cineraria, it aimed to extract the bioactive molecules, with the determination of certain groups, as it aimed to test the biological activities of the methanolic extract especially the antioxidant, antimicrobial, anticoagulant and antihemolytic activity.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1. Plant material

It consisted of the methanolic extract of the aerial part of the medicinal plant *Senecio cineraria* (MESC) harvested in the region of el kalla wilaya of Tarf- Algeria during the month of May 2016 and identified by botanist Dr. Nabila SUILAH from the laboratory for the unit of natural resources, bioactive molecules and physicochemical and biological analyses, at Mentouri Constantine University.

2.1.2. Bacterial strains

In order to test the antibacterial potential of (MESC) in vitro, five bacterial strains were used; two Gram positive: *Staphylococcus aureus (ATCC 25923)* and *Listeria monocytogenes (ATCC 7644)* and three Gram-negative: *Escherichia coli (ATCC 25922), Klebsiella pneumonia (ATCC 700603)* and *Pseudomonas aeruginosa (ATCC 27853)* were

used. The strains came from the Institute of Nutrition Food and Agro-Food Technology of Constantine (INATAA).

2.1.3. Blood samples

Six blood samples were collected from healthy individuals for the evaluation of anti-coagulant and anti-hemolytic activity.

2.1.4. Medication

Four drugs were used as a positive control:

1. Antibiotics:"penicillins" and "ofloxacin" of 10 IU concentration and 5µg respectively for antibacterial activity;
2. The anticoagulant drug "Lovenox" of concentration 2000 IU for anticoagulant activity;
3. Antihemolytic drug "Dicynone" concentration 250 mg for antihemolytic activity.

2.2 Methods

2.2.1. Preparation and phytochemical study of the methanolic extract of the medicinal plant *Senecio cineraria*

2.2.1.1. Preparation of the methanol extract

The aerial parts (leaves, blossoms and stems) of the plant have been well cleaned and dried at room temperature and away from the moisture and sunlight. Finally, the dry plant was pulverized using an electric grinder to obtain a fine powder so that it was ready for use. *The Markham method* (Markham, 1982) was followed for the preparation of methanolic extract; 950 g of the vegetable powder was introduced into a beaker which contained the hydroalcoholic mixture; methanol / H_2O (7: 3) overnight (alcoholic maceration). This maceration was repeated 3 times with the renewal of the solvent, it lasted in each case for 24 hours. The methanol was then removed from the filtrate by the evaporation under reduced pressure at 55 ° C. in a Rotavapor to obtain the crude methanolic extract.

2.2.1.2. Phytochemical characterization of the plant

Phytochemical screening tests were physicochemical reactions that made it possible to characterize the presence of the main categories of natural chemicals contained in a plant and responsible for the pharmacological properties; they were made based on the precipitation or characteristic colorations.

A. Identification of flavonoids

A few drops of $AlCl_3$ (1%) were added to 05 ml of the extract, the appearance of a yellow color indicated the presence of flavonoids (Kumaran SP et al., 2005).

B. Identification of tannins

2 to 3 drops of the 2% $FeCl_3$ solution were added to 2 ml of the crude methanol extract. The solution obtained was rested for a few minutes. The test was considered positive if there was the appearance of a blue-black color and a precipitate (Middleton et al., 2004).

C. Identification of saponins

5 ml of the crude methanol extract were mixed with 10 ml of the distilled water for 2 minutes. The formation of a persistent foam after 15 min confirmed the presence of saponosides (Middleton et al., 2004).

D. Identification of alkaloids

5ml of 1% HCl were added to 1 ml of extract. The mixture was heated in a water bath, and then treated separately with the Bouchardat reagent (iodine-iodide reagent) which gave a reddish brown precipitation (Pastre and Priymenko, 2003).

2.2.1.3. Determination of flavonoids

The flavonoids of the extract were quantified by the aluminum trichloride method (Boharun, 1996)**, (**1 ml of the extract (prepared in methanol to have suitable concentrations) was added to 1 ml of the $AlCl_3$ solution (2%, in methanol). After 10 minutes of the incubation, the absorbance was read at 430 nm. The concentration of flavonoids in the extracts was calculated from a calibration curve (**y = ax + b)** established with the quercetin at different concentrations (0-40 µg/ml, each prepared in methanol) performed in the same operating conditions as the extracts would be used for the quantification of flavonoids.

2.2.1.4. Thin Layer Chromatography (TLC)

This method was based on the separation of the different constituents of an extract according to their migration force in the mobile phase, which was generally a mixture of solvents, adapted to the type of separation to be searched for, and their affinity with respect to the stationary phase which might be a silica gel or polyamide gel. Chromatographic techniques were not sufficient to identify a product but they provided information (frontal report -R_f- and coloring) likely to guide a hypothesis of structures.

The TLC analyses were carried out with silica gel plates, on a rigid aluminum support. The extract was deposited using a micropipette (2 µl) at reference points, 1.5 cm from the lower edge of the plate. Then, the plates were placed in the development tanks in which there was a suitable solvent system called mobile phase, about 0.5 cm in height. The migration was carried out by the use of seven solvent systems:

System 01: Ethyl Acetate-Formic Acid-Acetic Acid- Distilled Water (100/11/11/26).

System 02: Butanol-Acetic Acid-Distilled Water (04/01/05).

System 03: Chloroform-Acetone-Ammonia10% (80/40/18).

System 04: Acetone-distilled water (01/01).

System 05: chloroform-methanol-distilled water (65/45/12).

System 06: Butanol-Acetic Acid-Distilled Water (02/03/05).

System 07: Acetate of Ethyl-Acetic Acid-Formic acid (100/11/11).

For each spot, the retention factor was calculated which was equal to **the distance travelled by the constituent / distance travelled by the solvent**. This factor made it possible to mention the preliminary information on the structure of flavone substances.

After development, the plates were dried and then visualized separately by a UV lamp at 254 and 365 nm. The frontal ratios (R_f) of the spots resulting from the separation were calculated according to the following ratio:

R_f = d / D

d: Distance travelled by the substance, **D:** Distance travelled by the solvent

2.2.2. Evaluation of in vitro biological activities of (MESC)

2.2.2.1. Evaluation of antioxidant activity

The ability of the extract to trap free radicals was determined by a colorimetric method, simple and fast; the Koleva method (Koleva et al., 2002) which uses the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) which, in the stable state, has a dark violet color which absorbs at 517 nm, was applied. The reduction of the DPPH radicals with an antioxidant caused the solution to fade to pale yellow. (Figure 1)

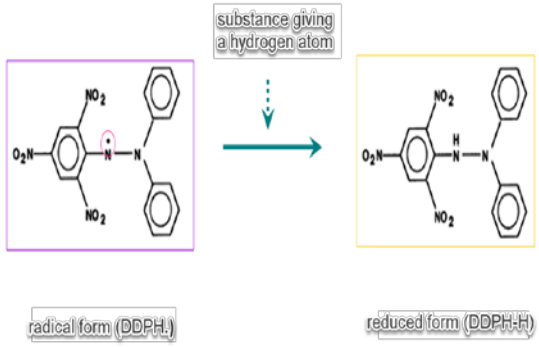


Figure 1: Reduction of DPPH • by an antioxidant (Molyneux, 2004)

The DPPH2,2-diphenyl-1-picrylhydrazyl ($C_{18}H_{12}N_5O_6$) was solubilized in absolute methanol to obtain a 100 µM solution. From a methanolic stock solution of C_1 = 10 mg / ml of each extract, the following dilutions were prepared: C_2 = 100 mg / ml, C_3 = 70 mg / ml, C_4 = 50 mg / ml, C_5 = 30 mg / ml, C_6 = 20 mg / ml, C_7: 15 mg / ml, C_8 = 10 mg / ml, C_9 = 05 mg / ml.

To each volume of 1.5 ml of the methanolic solution of DPPH, a volume of 15 µl of each prepared concentration of the extract was added. After stirring and incubation at room temperature for 15 minutes, the optical densities of the reaction mixtures were measured by the spectrophotometer at 517 nm against a blank prepared under the same conditions by methanol.

The antioxidant activity was compared to that of a commercial flavonoid which was quercetin which was in turn determined in the same way as that of the extract. The results were expressed as **the % inhibition (DPPH% = [(A_{Blanc} $A_{Extrait}$) / A_{Blanc}] x 100),** and the IC50 parameter or the effective concentration of the extract which caused the 50% loss of DPPH activity, the lower this concentration was, the higher the antioxidant effect would be (Brand-Williams et al., 1995).

2.2.2.2. Evaluation of antibacterial activity

The antibacterial activity of *Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa, Echerichia coli* and *Klebsiella pneumoniae* was evaluated by the diffusion technique in agar media on petri dishes. For the results showing a positive effect against the bacterial strains tested, the minimum inhibitory concentration (MIC) was determined on a liquid medium.

2.2.2.2.1. Disk Method -Tests of Efficiency-

A. Preparation of the inoculum

The different bacterial strains were subcultured by the streak method, and incubated at 37 ° C for 18 to 24 hours to obtain a young culture and well isolated colonies that were subsequently used to prepare the inoculum. The bacterial inoculum of each strain was prepared in 5 to 10 ml of sterile physiological saline from pure culture.

B. Seeding

After adjusting the turbidity of the inoculum suspension, a swab was deceived into the suspension, and its contents were spread over the entire surface of the Mueller-Hinton agar three times. After each application, the dish was rotated about 60°C in order to ensure a homogeneous distribution of the inoculum.

C. Preparation of aromatogram disks

Sterile Wattman Number 3 paper disks 5 mm in diameter, each impregnated with the extract taken up in dimethyl sulphoxide (DMSO) at a rate of 100 mg/ml, were deposited using forceps at the surface of the agar medium. The Petri dishes were first left for 30 minutes at room temperature for prediffusion of the substances, before being incubated at 37 °C in an oven for 24 hours.

Negative controls (disks impregnated with DMSO) and positive antibiotic discs were tested. Ofloxacin (OFX) and Penicillin (P) were the antibiotics chosen because of their broad spectrum of action and their frequent use in hospitals for the treatment of infections caused by most germs in this study. The experiments were performed in three repetitions.

D. Incubation and reading

The diameters of the zones of inhibition (ZI) were measured around the disks after a preincubation of 30 minutes at room temperature followed by the incubation in an oven at 37 °C for 24 or 48 hours depending on the seed.

The effect of the extract results in the appearance around the disc of a translucent halo corresponded to the absence of bacterial growth. The larger the diameter of this zone, the more sensitive the strain was (Jehl, 2014)

Indeed, the results were expressed on average ± SD (standard deviation). The strains having a diameter D< 8mm, 9 ≤D ≤ 14mm, 15≤D ≤ 19mm, D>20mm were considered respectively resistant (-), sensitive (+), highly sensitive (++), extremely sensitive (+ ++).

2.2.2.2.2. Determination of Minimal Inhibitory Concentration (MIC)

MIC was determined using micro-dilution technique with Muller Hinton broth using U-bottom microplates (NCCLS, 1997).

The MIC of the (MESC) was determined according to the following protocol:

90 µl of Muller Hinton broth were distributed in all wells of the microplate, then 2. 100 µl of (MECS) were distributed in the first wells of the microplate (A_1, A_2, A_3), and then the successive dilutions (100 µl of A_1 in B_1 and so on) were carried out. The bacterial inoculum of *Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains was prepared. The microplates were inoculated with 10 µl of the bacterial suspension (diluted 1/10) to obtain a final volume of 200 µl; A_6, A_7, A_8 and A_9 wells have been reserved for antibiotics (positive controls). The A_{11} and A_{12} wells respectively represented the culture control of the bacteria and the sterility control of the MH culture medium (negative controls); the prepared microplates were incubated at 37 ° C for 24 hours. After incubation, the bacterial growth (expressed as turbidity) was examined in each well.

The MIC of an (MESC) with respect to a given strain would be the smallest of the concentrations showing no visible growth of germ (olou et al., 2011).

2.2.2.2.3. Synergy between the plant MESC studied and antibiotics

The synergistic effect of the extract with antibiotics was evaluated by the disk diffusion method described above. The antibiotic disks were impregnated with 10 µl of (MESC), then gently deposited on the MH agar seeded beforehand with a bacterial suspension of the strains tested. After the incubation at 37 ° C for 24 hours, the diameters of the inhibition zones

around the disks were measured in mm. The inhibition zones observed for the combinations were compared to the average of the zones of inhibition for the antibiotics tested alone. A significant difference (≥ 5mm) showed a synergistic effect (Saffidine, 2015).

2.2.2.3. Evaluation of in vitro anticoagulant activity (Brummel et al., 2002)

The anti-coagulant activity of the (MESC) was evaluated in vitro with respect to the exogenous and endogenous coagulation pathway, and this on a pool of normal plasmas was released using 2 global and chronometric tests, the Quick time (TQ) or also referred to as Prothrombin Rate (TP), and Kaolin Cephalin Time (TCK).

2.2.2.3.1. Plasma (standard) plasma pool preparation

The dislocated plasma pool was a mixture of plasma removed from the untreated healthy adult volunteers, whose TQ and TCK were normal and comparable.

The blood of each volunteer was collected by venipuncture in a plastic tube on the anticoagulant solution of 3.2% sodium citrate and at a rate of 1 volume for 3 volumes of blood. The blood was then centrifuged for 10 min at 3000 rpm to obtain a platelet-poor plasma.

2.2.2.3.2. Evaluation of anticoagulant activity vis-à-vis the exogenous route

Anticoagulant activity against the exogenous coagulation pathway was assessed using a Quick (TK) coagulation test or prothrombin time (PT) which allowed for a global exploration of the factors of the exogenous pathway of the coagulation pathway coagulation.

A. Principle of the test

This test consisted of measuring the coagulation time at 37 °C of a platelet-poor plasma in the presence of a mixture of tissue factors and phospholipids (thromboplastin). The factors of the exogenous pathway were thus activated, and the time which elapsed until the formation of the clot was measured. An extended coagulation time compared to that of the negative control explained that the (MESC) exerted an anticoagulant effect vis-à-vis this coagulation pathway.

B. Experimental protocol

The effect of (MESC) on the exogenous pathway of coagulation was evaluated according to the following steps; 10 µl of the (MESC) prepared in DMSO (0.5 mg / ml) was added to 90 µl of the standard plasma, the mixture was incubated at 37 °C for 15 min. After the incubation, coagulation was triggered by the addition of 200 µl of thromboplastin preincubated at 37 ° C for 15 min. The time elapsing until the clot formation was then measured visually using the stopwatch (Brummel et al., 2002).

2.2.2.3.3. Evaluation of anticoagulant activity vis-à-vis the endogenous pathway

The Cephalin Kaolin Time Test (TCK) was a test that could be used to explore the activity of endogenous pathway plasma factors.

A. Principle of the test

This test consisted of measuring the coagulation time at 37 °C of a platelet-poor plasma in the presence of a mixture of tissue factors and phospholipids (thromboplastin) and calcium. The time that elapsed until the clot formation was measured. An extended coagulation time compared to that of the negative control explained that the (MESC) exerted an anticoagulant effect vis-à-vis this coagulation pathway.

B. Experimental protocol

The effect of (MESC) on the endogenous pathway of coagulation was evaluated according to the following steps:

10 µl of the (MESC) prepared in DMSO (0.5 mg / ml) was added to 90 µl of the platelet-poor plasma which was then incubated at 37 °C for 15 min. After the incubation, 100 µl of a cephalin solution Kaolin were added, and the mixture was then re-incubated at 37 °C for 3 min, and the coagulation was then triggered by the addition of 100 µl of an aqueous solution of 0.025 M $CaCl_2$ (Brummel et al., 2002).

The time elapsing until clot formation was then measured visually using the stopwatch.

An anticoagulant drug (Lovenox 2000 UI) dissolved in DMSO was used as a positive control.

2.2.2.4. Evaluation of in vitro antihemolytic activity

The antihemolytic effect of EMSC was performed according to the method of (Yang et al., 2005)

2.2.2.4.1. Preparation of red blood cells

5 ml of a healthy person's blood were collected in EDTA-treated tubes, and centrifuged for 5 min at 1000 rpm. The supernatant was removed, and the pellet was washed three times with phosphate buffered saline (PBS) (0.2 M and pH 7.4) (Appendix n) and resuspended in saline (4%). The washing operation consisted of a series of centrifugation at 1000 rpm for 5 min of the pellet suspension in PBS. After the last centrifugation, 0.4 ml of the pellet was added to 9.6 ml of phosphate buffered saline (0.2 M at a pH of 7.4) to obtain an erythrocyte solution of 4% hematocrit.

2.2.2.4.2. Preparation of the extract

Different concentrations of (MESC) (1 mg / ml, 0.5 mg/ml and 0.25 mg/ml) were prepared in PBS.

2.2.2.4.3. Expression of results

The percent inhibition of hemolysis was calculated according to the following formula (Miwa et al., 1987)

% inhibition = (Ac - Aext / Ac) 100

Ac: Absorbance of the negative control.

Aext: absorbance of the extract.

2.3 Statistical analysis

All experiments were done in triplicate. The results were expressed as mean ± (standard deviation (n = 3)).

3. RESULTS AND DISCUSSION

3.1. Results of the chemical characterization of the (MESC)

3.1.1. Phytochemical Screening

Phytochemical tests consisted of detecting different families of compounds existing in the studied part of the plant by qualitative characterization reactions. These reactions were based on the precipitation or staining by reagents specific to each family of compounds.

The phytochemical screening carried out on (MESC) revealed the presence of several metabolites whose results have been presented in Table 1.

Table 1: Results of phytochemical tests of the plant *Senecio cineraria*

Métabolites secondaires	L'observation	Résultat
Flavonoïdes	Appearance of a yellow color	++
Tanins	Appearance of a black-blue color. Absence of a precipitate	+
Saponines	Absence of a foam	-
Alcaloïdes	Appearance of a brown brown precipitate	++

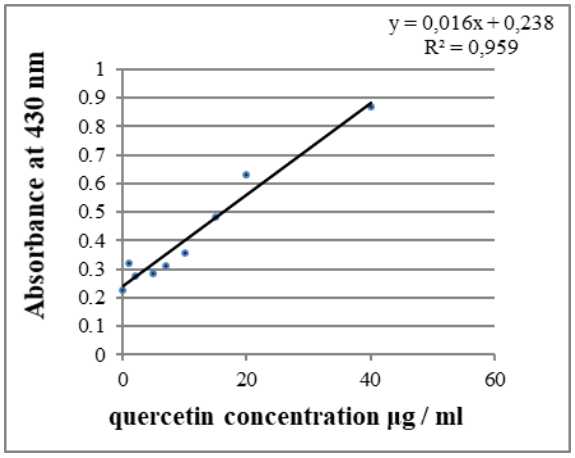
(+): weakly positive test, (++): strongly positive test, (-): negative test

Phytochemical tests carried out on the (MESC) revealed the presence of flavonoids, tannins, and alkaloids. **It is important to note that this has been the first identification of secondary metabolites for the *Senecio cineraria* plant.**

3.1.2. Determination of flavonoids

The quantitative study of the extract by means of spectrophotometric assays, according to the $AlCl_3$ aluminum trichloride method, was aimed at determining the total content of the flavonoids. A calibration curve (Figure 2) was drawn for this purpose, established with quercetin. Optical density measurements were made at 430 nm. The amounts of the corresponding flavonoids have been reported in milligram equivalents of quercetin per gram of the extract and determined by the equation:

Y = 0.016 X + 0.238.



Each point of the curve represents the average

Figure 2: Calibration curve of Quercetin.

The result of the determination of flavonoids in the methanolic extract of S. cineraria was estimated at a significant content in the order of 60.16 mg EQ / g E. The choice of quantifying flavonoids among the different phytochemicals was resulted from the fact that flavonoids had very important biological activities (Beta et al., 2005).

This considerable value in flavonoids has been responsible for their analgesic, antifungal, antioxidant, antimicrobial and haemostatic properties (Kabran et al., 2012).

3.1.3. Thin layer chromatography (TLC)

TLC has been one of the methods for separating and rapidly identifying the constituents of a given plant extract. This technique informs about the flavonoid content of the plant extracts. To obtain a better separation system, several systems were used of which 07 gave results. The results of TLC have been shown in Table 2.

Table 2. EMSC TLC Result by Different Solvent Systems

N° System	N° spots	R_f	The colors of the compounds	Results
System 1	02	- $R_{f=}$ 0.22 - R_{f2} = 0.85	-Blue fluorescent white -Orange	-Terpenes, flavonoids -Flavones, quercetin
System 2	02	- R_{f1} = 0.09 - R_{f2} = 0.7	-Yellow -Dark Purple	- Myrcétine, flavones, flavonols, -Myrcétine, flavones, flavonols, substituted flavonols
System 3	01	- R_f = 0.13	-Violet or mauve	-Gallic acid, phenol acid
System 4	01	- R_f = 0.93	-Orange	-Catechin, myrcétine
System 5	01	- R_f = 0.96	-Orange	Anthocyanidin 3,5 diglucoside
System 6	02	- R_{f1} = 0.53 - R_{f2} = 0.82	-Violet - Yellow	-Flavones -Myrcétine
Système 7	01	-Rf = 0.26	- Yellow green	-Flavonols

Two spots were segregated from the EMSC deposits by the solvent systems: 1 (ethyl acetate-formic acid-acetic acid-distilled water), 2 (butanol-acetic acid-distilled water) and 6 (butanol-acetic acid). distilled water), while a single spot was obtained by systems 3 (chloroform-acetone-ammonia) 4 (acetone-distilled water) (chloroform-methanol-distilled water) and 7 (ethyl acetate-acetic acid) -formic acid-distilled water).

According to the literature, most flavonoids have not been visible on the chromatography gel (cellulose) after the migration, with the exception of anthocyanins appearing in orange spots and chalcones, aurones and 6-hydroxyflavonols appearing in yellow. For this reason, the chromatograms must have been viewed under UV light, and the flavonoids became visible (fluorescent) under this light (Markham, 1982; Hamlat, 2006)

Based on this literature (Table 3) and according to the spot colors obtained on the chromatogram of the extract (Table 2), the existence of the following flavonoid types could be deduced: terpenes, flavonoids, flavones, quercetin, flavonols, catechin, myrcetin, substituted flavonols, gallic acid, phenol acid, anthocyanidin 3,5 di glucoside.

Table 3: Interpretation of Spot Colors in Different Classes of Flavonoids

Flavonoid Rf	Types of Flavonoids	References
0.06-0.9	terpenes	(Mamyrbekova et al., 2013)
0.03-0.97	flavonoids	(Mamyrbekova et al., 2013)
0.09	catechin	(Kholkhal, 2014)
0.23		(Meziti, 2009)
0.03-0.58	quercetin	(Mamyrbekova et al., 2013)
0.09-0.59		(Meziti, 2009)
0.35-0.41		(Kholkhal, 2014)
0.80-0.97	Anthocyanidine 3,5 diglucoside	(Said and Aouina, 2016)
0.13-0.64	Phenol acid	(Kholkhal, 2014)
0.00-0.5	Substituted flavonols	(Said and Aouina, 2016)
0.13	Gallic acid	(Kholkhal, 2014)
0.38		(Meziti, 2009)
0.23-0.85	Flavones	(Kholkhal, 2014)
0.07-0.75		(Said and Aouina, 2016)
0.5-0.75	Flavonols	(Madjour, 2012)
0.15-0.78		(Meziti, 2009)
0.04-0.41		(Kholkhal, 2014)

3.2. Results of biological activities

3.2.1. Determination of the antioxidant activity of (MESC) by the method of DPPH•

Since the main mechanism of the antioxidant action of plant polyphenols has been the trapping of free radicals, several methods have been developed to evaluate the antioxidant activity of plants by trapping synthetic free radicals in solution in polar solvents such as methanol at temperature room. The most frequently used radicals included 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-O-azino-bis (3-ethylbenzolin-6-sulphonic acid) (ABTS).

In this study, the DPPH method was chosen to evaluate the antioxidant activity of the plant extracts because it has been one of the simplest, fastest, and most effective methods because of the great stability of the DPPH radical (Bozin et al., 2008).

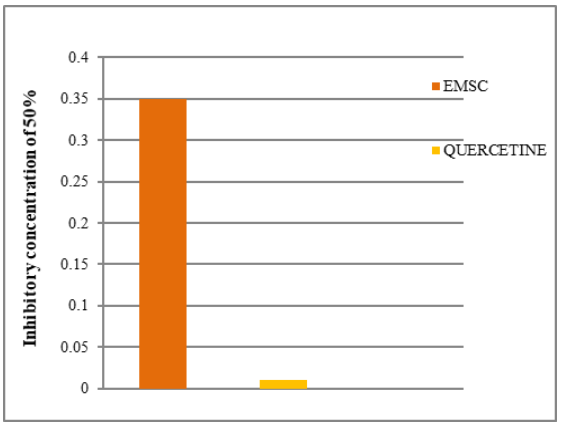
In the DPPH test, the kinetics of decolorization of this radical was monitored at 517 nm, which was due to its reduction to a non-radical form DPPH-H, by the antioxidants (AH) hydrogen donors present in the extract. As shown in the following equation:

$$DPPH^{\bullet} + AH\ (\text{Flavonoïde}) \rightarrow DPPH - H + A^{\bullet}\ (\text{radical flavonoxy})$$

DPPH has a dark purple color when trapped with hydrogen-containing antioxidant substances present in the plant extract, the reduced form conferred on the solution of a pale yellow color, the turn towards this coloration and the intensity discoloration of the color of the free formed in solution depends on the nature, concentration and potency of the anti-radical substance. For comparative purposes, quercetin was used as a standard antioxidant.

The results of the antioxidant activity exerted on the free radical DPPH• by the (MESC) have been expressed by the parameter IC50 (Figure 3). This parameter was used by several groups of researchers to present their results, it defined the effective concentration of the substrate that caused the loss of 50% of the activity of DPPH• (IC50),

The lower this concentration, the higher the antioxidant effect was (Brand-Williams et al., 1995). The results of this activity have been shown in Figure 3.



Each value represents the mean ± SD (n = 3).

Figure 3: Concentrations of EMSC that inhibit 50% of the radical DPPH •

According to the results recorded, the extract had a significant antioxidant activity, their IC50 was 0.35 mg / ml, but relatively lower than that of quercetin whose value of their IC50 was of the order of 0.01 mg / ml. The difference in inhibitory activity might be due to the difference in antioxidant content (polyphenols and flavonoids). In the case of phenolic compounds, the main mechanism of action was the entrapment of free radicals by the transfer of the H atom on the DPPH •, then transformed into a stable molecule DPPH-H (Halliwell, Barry, 1990)

3.2.2. Evaluation of anti-haemolytic activity

Red blood cells were among the mostly used cells in the evaluation of toxicity because of their availability, and the ease of their monitoring during lysis through the release of haemoglobin. In addition, the presence of a very high concentration of polyunsaturated fatty acids on the membrane and the role of oxygen transport played by hemoglobin molecules made erythrocytes a preferred target for free radicals (Surveswaran et al., 2000)

The interaction of a cytotoxic compound with the membrane of red blood cells causes the loss of their integrity which leads to their lysis. Hydrogen chloride has been widely used in evaluation tests for anti-haemolytic activity. It causes the degradation of the cell membrane of red blood cells by releasing the cytoplasmic content and particularly haemoglobin (Urquiaga, Leighton, 2000)

Figure 4 shows the percentage inhibition of haemolysis in the presence of different concentrations of the methanolic extract, compared to a positive control containing intact red blood cells (in the presence of PBS).

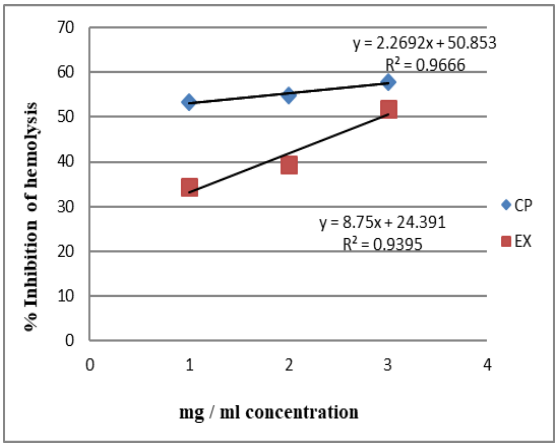


Figure 4: Curves of percent inhibition of hemolysis versus different concentrations.

According to the results obtained (figure 5), the (MESC) induced a significant anti-hemolytic activity compared to the positive control. This was proportional to the concentration of the extract used during the test.

The anti-haemolytic activity was probably due to the presence of a small amount of terpene compounds which at low concentrations played an anti-haemolytic role. These molecules promoted the interactions with proteins and phospholipids through their affinity with the latter which led to a protective effect against oxidants (Sylvie, 2017)

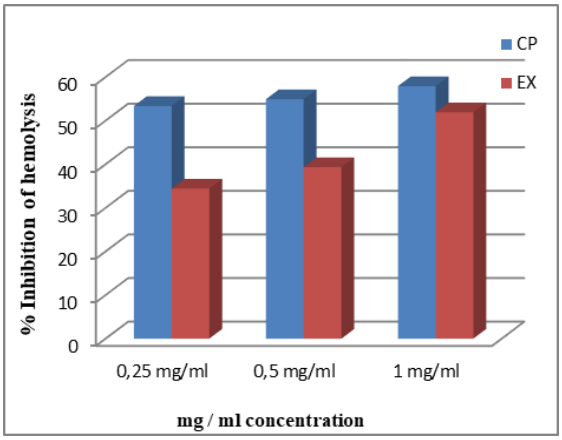


Figure 5: Histogram of percentage inhibition of hemolysis in the presence of different concentrations.

3.2.3. Evaluation of antibacterial activity

A. result of antibacterial activity tested by the disc method

The results of the evaluation of the antibacterial activity of the extract have been presented below in the tables (Table 4 and 5).

Table 4: Determination of the zone of inhibition (ZI) of the extract of Senecio cineraria.

strains	MESC	CN	CP P	CP Ofl
Escherichia coli	7.5±0.7	00	00	25
Staphylococcus aureus	09±2.8	00	14±2.8	21±0.7
Pseudomonas aeruginosa	5.25±0.35	00	11.5±2.12	20
Klebsiela oxytoca	10±1.4	00	10	25±1.4
Listeria monocytogenes	09±1.4	00	17	23.5±0.7

The results obtained above showed an antibacterial power observed for the strains: *Staphylococcus aureus, Listeria monocytogenes* and *Klebsiela oxytoca.*

Table 5: Minimal inhibitory concentration

strains / Concentrations	Staphylococcus aureus			Klebsiela oxytoca			Listeria monocytogenes		
	MESC	P	Ofx	MESC	P	Ofx	MESC	P	Ofx
40	-	-	-	-	-	-	-	-	-
20	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-
05	-	-	+	-	-	-	+	-	-
2.5	+	+	+	-	-	-	+	+	-
1.25	+	+	+	-	-	+	+	+	+
0.625	+	+	+	+	+	+	+	+	+
0.3125	+	+	+	+	+	+	+	+	+

CN: disks impregnated by DMSO. CP: antibiotic discs

The inhibitory potential of the extract was confirmed by the MIC determination tests. The results in the table above show that the MIC value was 1.25 mg / ml for the *Klebsiela oxytoca* strain, 05 mg / ml for *Staphylococcus aureus* and 10 mg / ml for the *Listeria monocytogenes* strain. The most sensitive bacteria were Gram-positive bacteria. However, *Klebsiela oxytoca*, which was a gram-negative bacterium, had a low MIC value (2.5 mg / ml). The structural organization of the cell wall of Gram-positive bacteria was less complex than that of Gram-negative bacteria. Of the strains studied, *Pseudomonas aeruginosa* and *Escherichia coli* were resistant (00 <ZI <06 mm).

The antibacterial activity of (MESC) against *Staphylococcus aureus*, *Listeria monocytogenes* and *Klebsiela oxytoca* might be due to the presence of alkaloids, flavonoids and tannins (Brantner et al., 1996), whose presence was demonstrated in the methanolic extract of this plant. Phenolic compounds and quinones derived from oxidation contributed to the plant's defence against the aggression as an antibacterial by binding to proteins and inactivating the enzymatic activities of microorganisms (Harborne and Williams., 2000) For example, the action of tannins that could inhibit the enzymatic machinery of microorganisms, had a lytic action by direct effect on their membrane or complexation certain metal ions essential for their survival, in particular iron (Milal et al., 1996). The negative result for the other bacterial species could mean the resistance of the latter vis-à-vis the (MESC) knowing that certain microorganisms could also degrade the phenolic compounds which then served as carbon substrates, and thus promoted their growth.

B. Test of synergy

Combination studies of medicinal substances in general, and antibiotics in particular, have been more and more often described in the literature (Saffidine, 2015) This strategy has been indeed of great interest for potential clinical applications, since it makes it possible to reduce the possible side effects of current treatments by reducing the dose of the compound used (Saffidine, 2015), thus also limit the development of resistance phenomena. It has been with this in mind that the (MESC) was tested in combination with penicillin and with ofloxacin on the five strains: *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiela oxytoca*. The results have been shown in Table 6.

Table 6: Results of the zones of inhibitions of the synergy EMSC / Antibiotic.

strains	Extract + Penicilline		Extract + Ofloxacine	
	R_1	R_2	R_1	R_2
Staphylococcus aureus	14	14	24	22
Listeria monocytogenes	12	14	24	24
Escherichia coli	00	00	00	00
Pseudomonas aeruginosa	14	16	23	23
Klebsiela oxytoca.	11	14	26	26

There were potentiating effects, and the extract / antibiotic combination was effective. With this combination, the necessary concentrations of penicillin and ofloxacin for the inhibition of strains were markedly reduced, as was the extract concentration, whereas the extract was only slightly active when used alone.

3.2.4. Evaluation of anticoagulant activity

The anticoagulant potency of the (MESC) was evaluated in vitro against the endogenous and exogenous coagulation pathways using two time tests, TP and TCK; respectively.

The TCK test was activated by the contact between the factor XII and the electronegative surface of the activator which was kaolin (substitute for collagen and connective tissue in vivo). This interaction induced the activation of factor XII and consequently the sequential activation of factors XI, IX, X and thrombin (factor II) (Gerotziafas, 2007). An extended

coagulation time compared to the negative control where the sample was replaced by DMSO reflected an anticoagulant activity of the tested material. The results of anticoagulant activity have been shown in Table 7 and Figure 6.

Table 7: Results of anticoagulant activity

	TQ (s)	TP (%)	TCK (s)	TCK (%)
CN	15	66.9	13.6	90.7
CP	23.3	32.16	20.3	39.9
Tests	43.6	51.3	27	26.7
Témoins	14	86.9	14.3	85.4

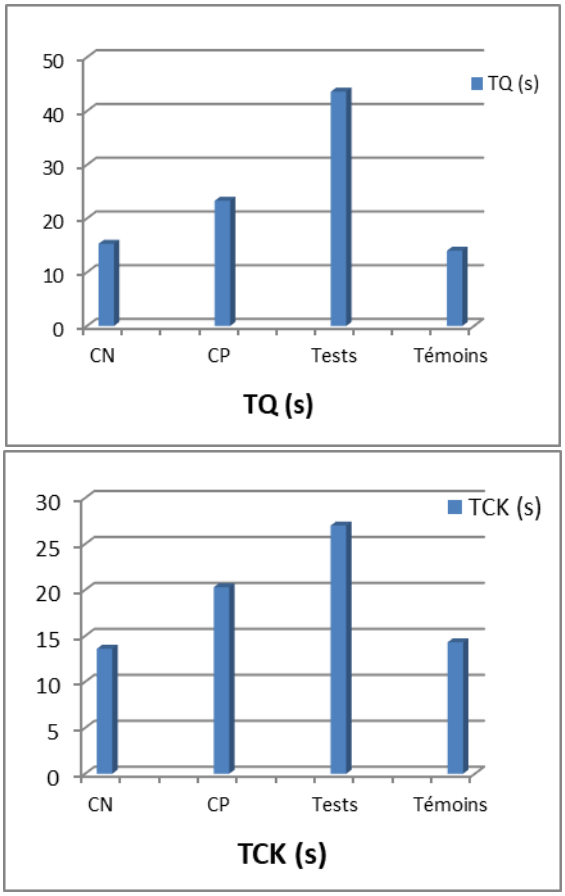


Figure 6: Histograms of TCK and TQ.

These results showed that the difference was significant with the negative control. The TCK times were between 29 and 24s for the extract, and the negative control time was between 13 and 15 s.

The time of Quick (TQ) or the prothrombin rate (TP) was the test which made it possible to explore globally the exogenous pathway of coagulation where the tissue factor was the trigger of this pathway (Tripodi, 2009). The normal TQ was between 12 and 14 seconds depending on the reagents used (Caquet, 2004), and an elongation relative to the negative control reflected an anticoagulant activity of the test material vis-à-vis the exogenous pathway of coagulation.

The difference was significant between the extract and the negative control, the TQ time was between 40 and 49 (s) for the extract, and the negative control time was between 14 and 16 (s).

Overall, the evaluation of the anticoagulant capacity of (MESC) established by the two coagulation timekeeping tests, TCK and TQ, showed that the extract exerted a significant anticoagulant activity with respect to the two coagulation pathways.

4. CONCLUSION

At present, medicinal plants are still the first reservoir of new drugs. They have been considered a source of raw materials essential for the discovery of new molecules necessary for the development of future drugs. For this purpose, secondary metabolites have been the subject of lot of research studies.

The objective of this work was the study of some characteristics of the medicinal plant Senecio cineraria by the phytochemical study, the evaluation of their contents in flavonoids, the thin layer chromatography as well as the in vitro estimation of the anticoagulant activities, antibacterial, antioxidant and antihemolytic of this plant, and the analyses were carried out on the methanolic extract.

The results of this study indicated the richness of this plant in different active secondary metabolites, whereas the quantitative analysis in flavonoic compounds and the qualitative analysis by TLC proved the richness of this plant in different classes of flavonoids which were endowed with an antioxidant power.

The antimicrobial activity of plant extracts on bacterial strains was important, as this plant showed remarkable activity on the three strains tested.

The study of antioxidant power by the DPPH test revealed a strong antioxidant power. The study of the anticoagulant activity in vitro by the two chronometric tests, the TCK and the TQ revealed a strong anticoagulant power.

According to the results obtained, the methanolic extract of *Senecio cineraria* induced a significant antihemolysis activity. This was proportional to the concentration of the extract used during the test.

As a result of these results, it would be interesting to extend the range of biological tests as well as the isolation and characterization of the active compounds in the various extracts in order to identify the different molecules responsible for the different biological activities of this species of plant.

All these results obtained in vitro have been only a first step in the search for the naturally occurring biologically active substances, an in vivo study is desirable to obtain a more in-depth view of the biological activities of the extracts of this plant.

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