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Research Article

Determination of antioxidant activity by chemical and electrochemical methods of extracts of *Launaea resedifolia* from the Algerian Sahara plant

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Abstract – The aim of this study is evaluating the antioxidant activity of the *Launaea Resedifolia* aerobic parts' extracts, which grows in Bashar region (Algeria). The antioxidant properties of methanolic extract and the organic fractions of *L. resedifolia* have been estimated using the chemical and electrochemical methods. Where the chemical method is the superoxide scavenging assays, while the electrochemical method is the cyclic voltammetry assay. Further, both methods depend on the inhibition of superoxide anion radical. The results indicate that the extracts showed an inhibition of superoxide anion radical with an amount of I% ranging between (81.8181 ± 0.001) % and (66.6667 ± 5.2486) % in chemical method and IC_{50} between 3.2814 g/l and 1.7990 g/l in electrochemical method. Thus, the rate inhibition (I%) in the chemical method were found best in butanol fraction (BF) then chloroform fraction (CF) and ethyl acetate fraction (AF) then crud extract (CE). Both the effective scavenging concentration (IC_{50}) in the electrochemical method were found best in ethyl acetate fraction (AF) then butanol fraction (BF), crud extract (CE) and chloroform fraction (CF).

Keywords – Launaea resedifolia; Antioxidant activity; superoxide; cyclic voltammetry.

I. INTRODUCTION

Increasingly, researchers are interested in the role of free radicals where the latter are constantly generated and created in the human body due to the metabolism (metabolism or metabolism) as well as infection. On the one hand, Oxygen is an important and essential element of air organisms, but on the other hand it has a negative effect associated with free radical figuration [1], which cause oxidative damage to cells and biological molecules [2, 3]. As a consequence, attention is focused on the study of antioxidants because they are the system that protects organic from free radical damage [1 - 5]. Here, the search has been initiated about natural antioxidant resources and to assess their impact. In recent years, increasing attention has been given to the role of human health diet. Where epidemiological studies have shown that consuming

fruits and vegetables is beneficial in human health as well as reducing diseases risk [6]

II. Materials and Method

2.1. Chemicals and reagents

All chemicals and reagents used in the study were analytical grade purchased from Biochem. The N, N dimethylformamide extra dry, and the tetrabutylammonium hexafluorophosphate Bu_4NPF_6 of electrochemical grade were purchased from Fluka. Pyrogallol, phosphate buffer, gallic acid and ascorbic acid, were obtained from Sigma - Aldrich and Biochem.

2.2. Plant material

The aerial parts of *L. resedifolia* were collected in May 2017 from Bashar region, south-west of Algeria. The identification was done on the basis of Quezel and Santa [7] by Dr. Halis Youcef researcher in Touggourt's Scientific and Technical Research Centre for Arid Areas.

2.3. Sample preparation and extraction

The aerial parts of L. resedifolia were dried and crushed. Then, hundred grams (100 g) of plant powder was macerated with MeOH - H_2O (70 / 30, v/v) for 48 hours at room temperature. The procedure was repeated three times. After filtration, the filtrate was evaporated, recovered with warm distilled water and partitioned using organic solvents with different polarity successively chloroform, ethyl acetate, and n-butanol. The extract, also the remaining water fraction, were concentrated under reduced pressure at 40 °C and then get samples dry and preserved at 4 °C.

2.4. Antioxidant activity assays

2.4.1. Superoxide scavenging assay

The rate of pyrogallol autoxidation was measured according to the method of Marklund and Marklund [8, 9], with slight modifications. The 0.5 ml of diluted extracts of *L. resedifolia* or water were added to 4.5 ml of phosphate buffer (50 mM, pH 8.2) and 10 µl of 45 mM pyrogallol at room temperature. The absorbance of the mixture was recorded at 320 nm every 30 s. The inhibition rate for the effect of extracts on pyrogallol autoxidation was calculated as we plot the absorbance as a function of time and from these curves we deduce the slope which expresses the speed of the autoxidation of pyrogallol.

2.4.2. Cyclic voltammetry assay

Voltalab PGZ 301 made up of a Potentiostat-Galvanostate equipped with Volta Master 4 software was used for voltammetry experiments. The electrochemical cell containing three electrodes immersed in a solution containing the analyte and an excess of supporting electrolyte, as contains a syringe for injecting extracts without opening the closed system. A saturated calomel electrode (SCE) was used as reference electrode, a platinum wire as the auxiliary electrode and a glassy carbon electrode (Ø_3.0 mm) as the working electrode respectively. Before each experiment, the working electrode was polished with silicon carbide 4000 paper in, then rinsed with acetone and dried with a dry tissue paper. Further, all experiments were conducted at ambient temperature. As well as, potentials were measured with respect to a saturated calomel electrode.

25ml of an extra-dry DMF solution containing the supporting electrolyte 0.0516 M Bu_4NPF_6 was saturated by dry air during 15 min. The cyclic voltammogram (CV) of the oxygen reduction was then recorded at a scan rate $0.1Vs^{-1}$. However, the applied potential range was from 0 V to -1.8V versus SCE.

Addition ally the superoxide radical scavenging activity of the different extracts of *L. resedifolia* were determined by using cyclic voltammetry method of Bouvellec et al [10 - 12], with some modifications. The effect of extracts was examined by the method of the proportioned additions and the successive addition of 200 µl of initial solution of crud extract, ethyl acetate and Butanol fractions and 40 µl of **c**hloroform fraction to the 25 ml oxygen solution in order to get an antioxidant substrate concentration in the range [0 - 1.9551] g/l, [0 - 1.2734] g/l, [0 - 1.3432] g/l, [0 - 1.9175] g/l and [0 - 2.6950] g/l, respectively. After each aliquot addition, CV of the oxygen solution was recorded at a scan rate $0.1Vs^{-1}$. As a result, the total antioxidant activity of extracts determined in comparison with gallic acid and ascorbic acid. Hence, the superoxide

radical inhibition rate was calculated as relationship:

$$I\% = \left(\frac{I^0_{pa} - I^s_{pa}}{I^0_{pa}}\right) \times 100 \ldots\ldots\ldots\ldots\ldots (1)$$

Where I^0_{pa} and I^s_{pa} are the anodic peak current of $O_2^{\bullet-}$oxidation with and without the extracts.

2.5. Statistical analysis

Thus, the results of chemical method were presented as the mean averages ± SD. the measurements of them were replicated three times, means while, correlation analysis between of antioxidant activities were carried out using the correlation. All determinations were carried out by means of software Origin Pr-08 the data analysis and graphing workspace. Farther, a Microsoft Excel 2016 was used to calculate IC_{50} from linear regression.

III. RESULTS AND DISCUSSION

3.1. Superoxide scavenging assay

The superoxide anion radical is one of representative oxygen species generated in the body. The capacity of the crude extract and fractions of *L. resedifolia* to remove superoxide anion was inquired by using the autoxidation reaction of pyrogallol occurring under alkaline conditions. The inhibition rate reflects the ability of extracts to remove superoxide anion from the testing system; however, Figure 1 shows the rate of autoxidation of pyrogallol in the presence of crude extract and fractions of *L. resedifolia* and gallic acid. The highest inhibition rate was recorded in the butanol fraction (81.8181 ± 0.001) %, then the chloroform fraction and ethyl acetate fraction with a value equal to (75.7576 ± 10.4973) % however the lowest activity was found in the crude extract (66.6667 ± 5.2486) % (Table1). As a result, the fractions showed better inhibition rate than the gallic acid where I% was achieved at (40.9090 ± 6.4282) %.

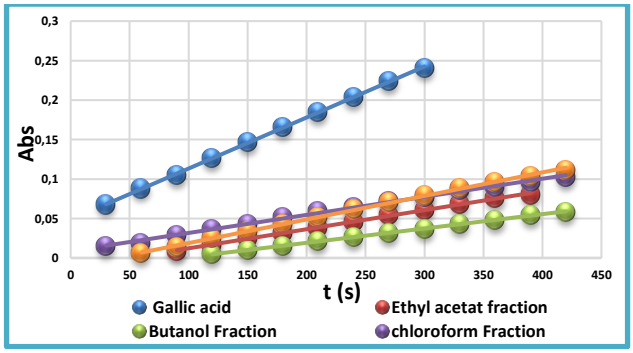
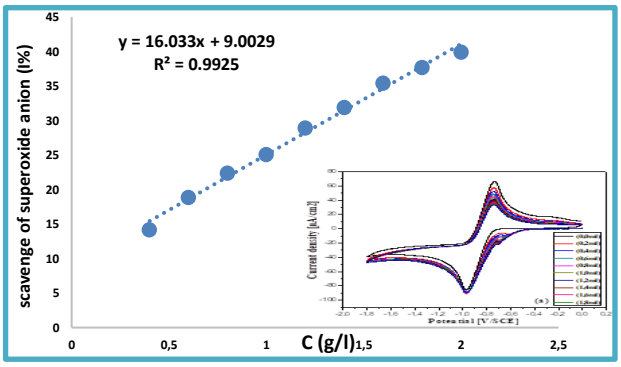
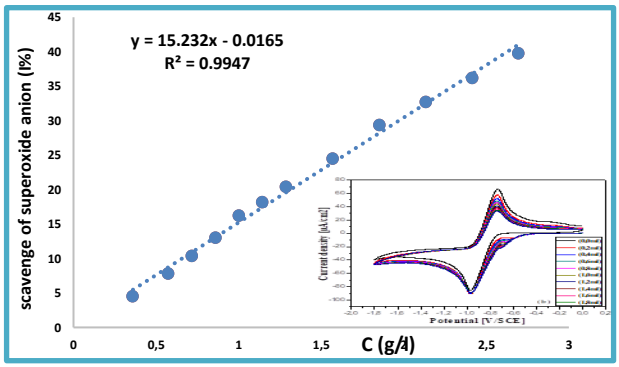


Figure 1: the rate of autoxidation of pyrogallol in the presence of antioxidant

3.2. Cyclic voltammetry assay

The voltammogram were recorded to reduce O_2 with the phenolic extracts of the L. resedifolia to assess their antioxidant capacity for upon the $O_2^{\bullet-}$ reduction. Based on the results, it has been noticed that the excess in the concentration of phenolic extracts leads to a decrease in the anode current density of $O_2^{\bullet-}$, while the cathode current density of O_2 appears to be negligible. As a result, from the voltammogram and applying the above relationship we can draw the curve of rate inhibition superoxide anion of cyclic voltammogram against the corresponding concentration of CE (a), CF (b), AF (c), BF (d).





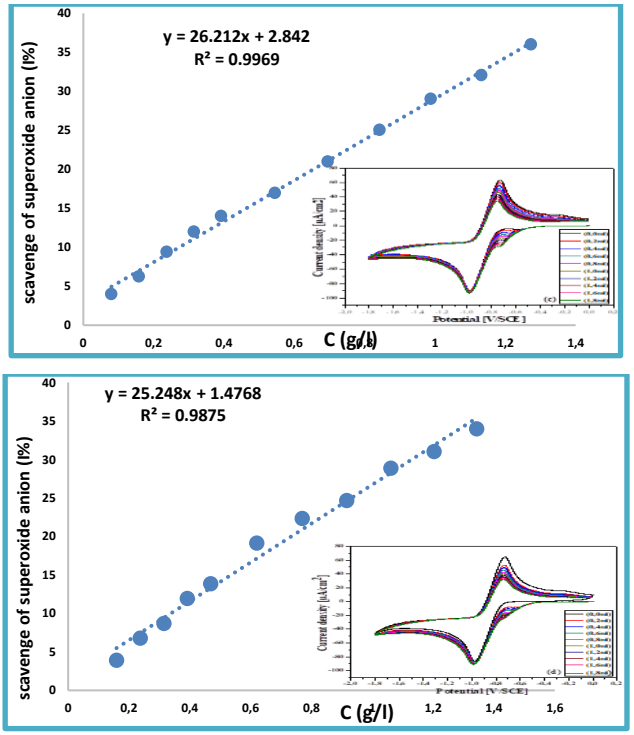


Figure 2: Plotting of scavenging of superoxide anion of cyclic voltammogram against the corresponding concentration of CE (a), CF (b), AF (c), BF (d), in DMF + 0.05 M Bu_4NPF_6 on GC as working electrode vs. SCE at 18°C with scan rate of 0.1 V/s.

The obtained results (figure 2) shows that there is a direct correlation between the rate of inhibition superoxide anion radical ($O_2^{\bullet-}$) I% (rate of the antioxidant activity) and concentration of extracts. In the present acquirement, the gallic acid and ascorbic acid were reliance as standard solutions.

To determine the ability of the extracts to reduce the superoxide anion radical, we calculate the value of the IC_{50} from the linear relation of the antioxidant activity in terms of concentration of the studied extracts. There for the effective scavenging concentration (IC_{50}) on superoxide anion radical decreased in the order of chloroform fraction > crud extract > butanol fraction > ethyl acetate fraction with values 3.2814 g/l > 2.5570 g/l > 1.9218 g/l > 1.7990 g/l, respectively. Where the best effect was recorded in ethyl acetate fraction (1.7990 g/l), whereas, the lowest activity was found in the chloroform fraction (3.2814 g/l) in Table 1. IC_{50} values of all these compounds were higher than the corresponding of gallic acid and ascorbic acid where IC_{50} was achieved at 0.0089 g/l and 0.1166 g/l respectively.

Table 1: Effective scavenging concentration (IC_{50}) and the inhibition rate (I%) values of *L. Resedifolia*'s extracts against superoxide anion radical.

Extracts	**Antioxidant activity**	
	superoxide anion radical scavenging	
	I (%)	**IC_{50} (g/l)**
Crude extract	66.6667	2.5570
Chloroform fraction	75.7576	3.2814
Ethyl acetate fraction	75.7576	1.7990
Butanol fraction	81.8181	1.9218
Gallic acid	40.909	0.0089
Ascorbic acid	-	0.1166

The biological production of reactive oxygen species primarily superoxide anion radical ($O_2^{\bullet-}$) is capable of damaging molecules of biochemical classes including nucleic acids and amino acids. based on the evidence, we hypothesized the extracts from *L. resedifolia* would show antioxidant effects against lipid peroxidation on biomembranes and scavenge the superoxide anion radical at the stage of initiation and termination of peroxy radical [10].

IV. CONCLUSION

The overall results of the evaluation of the antioxidant activity of the extracts of the aerobic parts of *Launaea Resedifolia* growing in the Bashar region (Algeria).

- Chemical and electrochemical methods used to estimate the antioxidant properties of the methanolic extract and their organic fractions of *L. resedifolia*.
- The results showed an inhibition of the superoxide anion radical with an amount of I% varying between (81.8181 ± 0.001) % and (66.6667 ± 5.2486) % in the chemical method and IC50 between 3.2814 g/l, and 1.7990 g/l in the electrochemical method.
- The inhibition rate (I%) in the chemical method was found to be better in the butanol fraction (BF) then the chloroform fraction (CF) and the ethyl acetate fraction (AF) then the extract of (CE).
- The effective entrapment values (IC50) in the electrochemical method were found to be best in the ethyl acetate fraction (AF) followed by the butanol fraction (BF), the cruds extract (CE) and the chloroform fraction (CF).

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DECLARATION OF INTEREST STATEMENT

The author(s) declare(s) that there is no conflict of interest.

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