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Determination of phenolic content and antioxidant capacity of *Launaea resedifolia* from Algerian Sahara

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ABSTRACT

The present inquiry attempts to determine the antioxidant capacity of *Launaea resedifolia's* hydroalcoholic extract which is obtained from Algerian Sahara. The antioxidant capacities of various extracts of aerial parts of *L. resedifolia* were estimated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity, ferric reducing power (FRP), and total antioxidant capacity. In addition, the total phenols, flavonoids, and tannins contents of various extracts were measured by using colorimetric methods, the greater content of phenols and flavonoids was registered in crude extract (6.642 ± 0.262 mg gallic acid equivalent/g DW and 0.929 ± 0.018 mg quercetin equivalent/g DW, respectively), while the tannins was found in water fraction (1.246 ± 0.153 mg RE/g DW). Both capacities of DPPH scavenging and FRP were found best in ethyl acetate fraction (IC_{50} = 403.551 µg/ml and AEAC = 0.151 mM, respectively). For total antioxidant capacity, the superior capacity was observed in water fraction (EEAC = 0.149 Mm).

1. INTRODUCTION

The genus Launaea (tribe Lactuceae, family Asteraceae) contains about 40 species in the Algerian flora, where nine species of them grow mostly within the Sahara [1,2]. They are the following species: *L. acanthoclada*, *L. angustifolia*, *L. anomala*, *L. arborescens*, *L. cassiniana*, *L. glomerata*, *L. nudicaulis*, *L. quercifolia*, and *L. resedifolia* [3,4]. The regional name of *Launaea resedifolia* is "laadid, Azim" [4], synonym *Scorzonera resedifolia* is a perennial herb, it has length up to 40 cm, exceedingly distributed in the wetland of the arid zones of Mediterranean area, and *as well existing* in many several countries, e.g., Algeria, Libya, Tunisia, Pakistan, and India [5].

Many species of this genus are utilized in alternative medicine in bitter stomach, skin diseases, and reported to have antitumor, insecticide, and cytotoxic activities [6]. The antimicrobial activities of coumarin constituents [7] and the neuropharmacological properties [5] have been investigated as well.

Previous works on parts of this species revealed the presence of compounds from the following families: flavonoids [8], phenolics [9], coumarins [10,11], and terpenoids [12,13]. Therefore, we investigated the antioxidant capacity of *L. resedifolia* from Algeria-Sahara.

2. MATERIALS AND METHODS

2.1. Plant Material

The *L. resedifolia* was collected in the flowering duration (March 2013) from the region of Bashar, which is located in the south-west of Algeria. The aerial part of *L. resedifolia* was identified by Doctor Halis Youcef researcher in Scientific and Technical Research Centre for Arid Areas-Touggourt.

2.2. Chemicals and Reagents

Folin-Ciocalteu's reagent was purchased from Sigma-Aldrich, and sodium carbonate (Na_2CO_3) (99.8%), aluminum chloride (97%), 1,1-diphenyl-2-picrylhydrazyl (DPPH) (99%), potassium

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ferricyanide (99%), ferric chloride (99%), trichloroacetic acid (99%), disodium phosphate (99%), ammonium molybdate (99%), gallic acid (99%), quercetin (99%), catechin (99%), ascorbic acid (99.7%), BHA (98%), and BHT (98%) were obtained from Sigma-Aldrich and Biochem. All chemicals and reagents used are of analytical grades.

2.3. Preparation of Extracts

A 100 g of *L. resedifolia* powder was macerated in MeOH-H_2O (7:3, v/v) and left at room temperature and then was filtered after 48 hours (repeated three times). The filtrates are collected and evaporated in the rotary evaporator under 40°C then recovered with warm distilled water (40–60 ml distilled water per 100 g of plant powder) and kept in the dark for a full night and then filtered. The filtrate was partitioned successively using chloroform, ethyl acetate, and n-butanol. The extracts and also the remaining water fraction are concentrated under reduced pressure and then re-dissolved with a minimum of ethanol or water and kept at 4°C.

2.4. Yield of Extracts

Extraction yield was calculated following the equivalent of %yield = ($m_{extract}/m_{dried\ sample}$) × 100, where $m_{extract}$, $m_{dried\ sample}$ are the weight of extract and weight of the dried sample, respectively

$$\%\text{yield} = \frac{m\,\text{extract}}{m\,\text{dried sample}} \times 100$$

2.5. Phytochemical Investigation

2.5.1. Total phenolic content (TPC)

TPC of *L. resedifolia*'s extracts was determined with the Folin-Ciocalteu reagent [14,15]. Briefly; 0.1 ml of extract sample was jumbled with 0.5 ml of 10% Folin-Ciocalteu reagent. Then 2.0 ml of 20% aqueous Na_2CO_3 solution was added after 5 minutes, the mixture was kept for 30 minutes in the dark at room temperature, and then the absorbance was read at 760 nm *versus* prepared blank solution. Results were expressed as mg gallic acid equivalents/g of the plant (mg GAE/g P).

2.5.2. Total flavonoid content

Total flavonoid content (TFC) of *L. resedifolia*'s extracts was determined following the method described by Kumazawa et al. [14]. Briefly, a mixture of 0.5 ml of extract and 0.5 ml of 2% $AlCl_3$ ethanol solution was prepared and allowed to stand for 30 minutes at room temperature. The absorbance was recorded at 430 nm against a blank solution. Results were presented as mg quercetin equivalent/g of the plant (mg QE/g P).

2.5.3. Total tannin content

Total tannin content (TTC) of *L. resedifolia*'s extracts was determined by a colorimetric method [15,17]. Briefly; the 0.3 ml of extract sample was jumbled with 1.8 ml of 4% ethanol vanillin solution and 0.9 ml of concentrated hydrochloric acid (HCl). The mixture was allowed to stand for 15 minutes in the dark at room temperature. The absorbance was recorded at 500 nm *versus* prepared blank solution. Results were expressed as mg catechin equivalent/g of the plant (mg CE/g P).

2.6. Antioxidant Capacities

2.6.1. DPPH radical scavenging capacity

The DPPH• scavenging capacity of extracts of *L. resedifolia* was assayed by Thaipong et al. [18] with some modifications. A 0.14 ml of diluted plant extract was mixed with 2.66 ml of 0.1 mM DPPH• ethanol solution. The mixture was saved for 30 minutes in the darkish chamber then the absorbance was recorded at 517 nm and ascorbic acid was used as a positive control. The DPPH radical inhibition was calculated as

$$I\% = \frac{\left(A_0 - A_s\right)}{A_0} \times 100$$

Where A_0 and A_s are the absorbance of the control and the sample extracts, respectively.

2.6.2. Ferric reducing power (FRP)

The FRP of the different extracts of *L. resedifolia* was examined following the method of Kumaran et al. [19] with a small modification. Two hundred microliters of the extracts, 0.5 ml of phosphate buffer (0.2 M, pH 6.6), and 0.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%) were prepared and saved at 50°C for 20 minutes. Then 0.5 ml of trichloroacetic acid solution (10%) was added. The resulting mixture (1.7 ml) was blended with 1.7 ml of distilled water and 0.34 ml of ferric chloride (1%). The absorbance was read at 700 nm, using ascorbic acid as a positive control, and the results were expressed as mM equivalents to ascorbic acid.

2.6.3. Total antioxidant capacity

The total antioxidant capacities of the different extracts of *L. resedifolia* were assayed by the method of Prieto et al. S. 0.2 ml of sample extracts was jumbled with 2 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The resulting solutions were saved in a water bath (95°C) for 90 minutes. Then the mixture was cooled to room temperature and the absorbance of the mixture was read at 695 nm, using ascorbic acid as a positive control, and the results were expressed as mM equivalent to ascorbic acid.

2.7. Statistical Analysis

The results were expressed as mean *f* three replicates together with standard deviations. Statistical calculations were done by Microsoft Excel 2010. IC_{50} was calculated from linear regression.

3. RESULTS AND DISCUSSION

3.1. Yield of Extracts

The yield of the crude extract (CE) of *L. resedifolia* was 11.93%. As for the fractions, the elevated yield was in water fraction

Table 1. Yield of extracts and phytochemical contents.

	Yield	TPC	TFC	TTC
	%	mg GAE/g P	µg QE/g P	µg CE/g P
Crude extract	11.930	7.297 ± 1.150	929.698 ± 18.657	457.858 ± 31.282
Chloroform fraction	0.168	0.267 ± 0.125	20.360 ± 1.822	56.96 ± 1.852
Ethyl acetate fraction	0.278	0.480 ± 0.116	51.669 ± 0.511	10.049 ± 0.191
Butanol fraction	2.491	3.402 ± 0.387	287.166 ± 18.461	84.746 ± 1.183
Water fraction	8.172	4.475 ± 0.333	604.674 ± 3.349	1,239.472 ± 152.953

We find that *L. resedifolia* have high contents of phenols as compared to flavonoids and tannins.

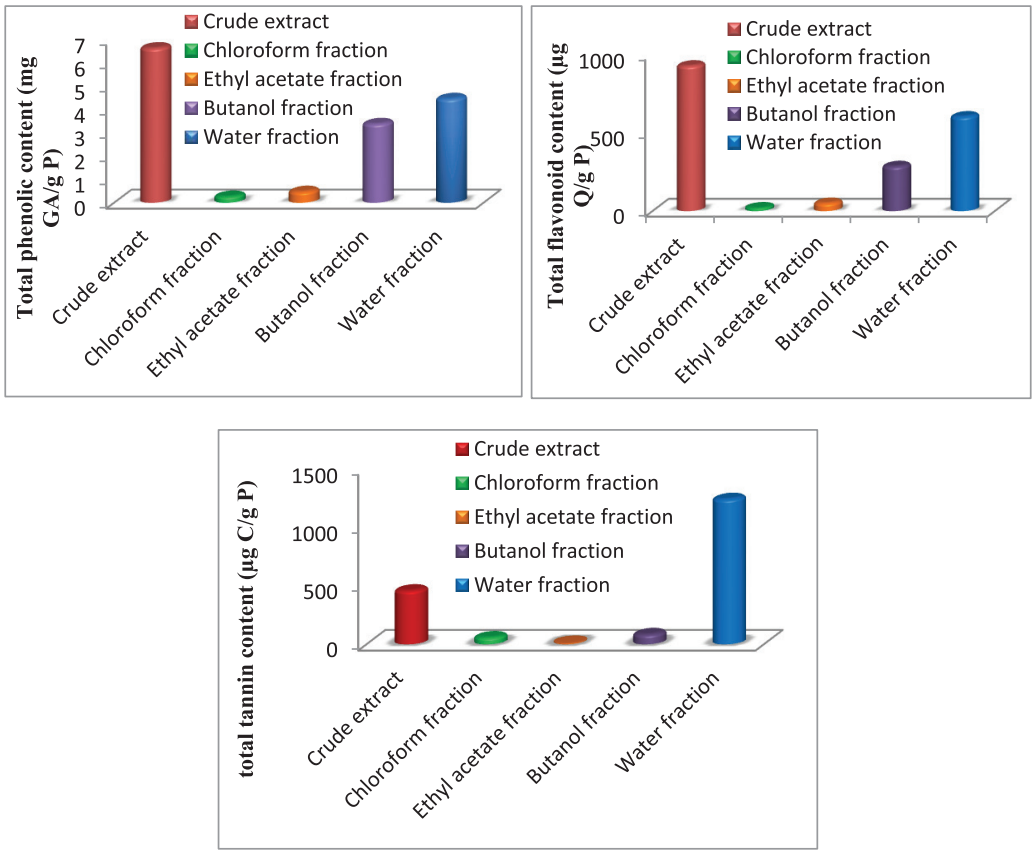

Figure 1: Phytochemical contents of L. resedifolia's extracts .

(WF) (8.17%), followed by butanol fraction (BF) (2.49%) then by ethyl acetate fraction (AF) (0.27%) and the lowest yield of extraction was in chloroform fraction (CF) (0.16%) (Table 1).

3.2. Phytochemical Contents

The amount of TPC from *L. resedifolia's* extracts was ranging from 7.297 ± 1.150 to 0.267 ± 0.125 mg GAE/g of the plant. Where the extracts gave the following order: CF < AF < BF < WF < CE with values 0.267 ± 0.125 mg GAE/g < 0.480 ± 0.116 mg GAE/g < 3.402 ± 0.387 mg GAE/g < 4.475 ± 0.333 mg GAE/g < 7.297 ± 1.150 mg GAE/g of the plant (Table 1).

The amount of TFC from *L. resedifolia's* extracts was ranging from 929.698 ± 18.657 µg QE/g to 20.360 ± 1.822 µg QE/g of the plant. Where the extracts gave the following order: CF < AF < BF < WF < CE with values 20.360 ± 1.822 µg QE/g < 51.669 ± 0.511 µg QE/g < 287.166 ± 18.461 µg QE/g < 604.674 ± 3.349 µg QE/g < 929.698 ± 18.657 µg QE/g of the plant (Table 1).

The amount of TFC from *L. resedifolia's* extracts varied between 1,239.472 ± 152.953 µg CE/g and 10.049 ± 0.191 µg CE/g of the plant. Where the extracts gave the following order: AF < CF < BF < CE < WF with values 10.049 ± 0.191 µg CE/g < 56.96 ± 1.852 µg CE/g < 84.746 ± 1.183 µg CE/g < 457.858 ± 31.282 µg CE/g < 1,239.472 ± 152.953 µg C/g of plant (Table 1).

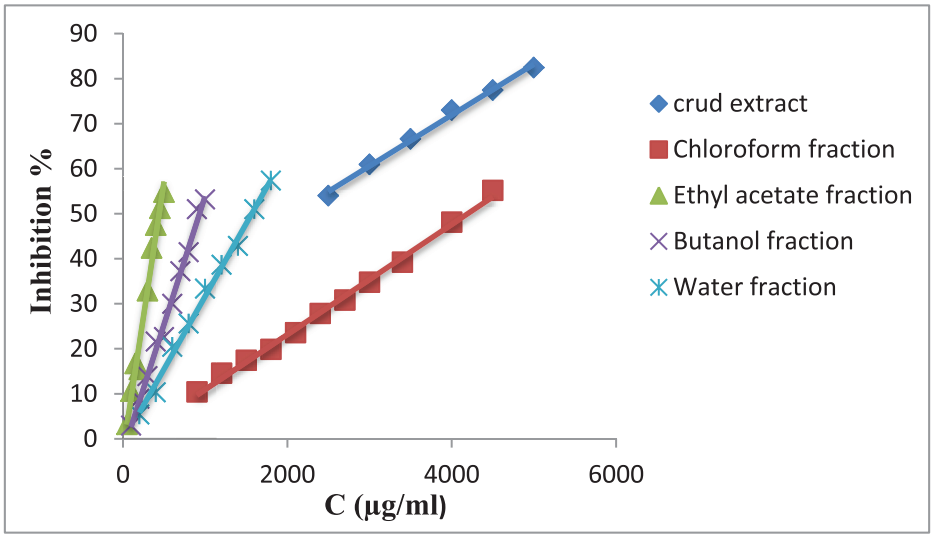

Figure 2: Percentage scavenging of DPPH in L. resedifolia's extracts

Table 2. DPPH scavenging, reducing power, and total antioxidant capacity.

	DPPH IC_{50} (µg/ml)	FRAP (mM)	Molybdate (mM)
Crude extract	2,673.951 ± 24.900	112.126 ± 0.483	125.763 ± 8.119
Chloroform fraction	4,226.413 ± 81.564	17.772 ± 0.722	86.758 ± 7.748
Ethyl acetate fraction	437.999 ± 26.097	156.799 ± 22.795	40.807 ± 0.367
Butanol fraction	976.193 ± 36.191	45.922 ± 2.158	25.778 ± 0.721
Water fraction	1,528.452 ± 46.936	116.701 ± 2.414	157,.928 ± 1.270
BHA	83.350 ± 4.150	1.126 ± 0.041	0.535 ± 0.034
BHT	-	1.960 ± 0.031	1.211 ± 0.171
Ascorbic acid	83.116 ± 3.750	-	-

We have determined the amount of phenolic and flavonoids contents present in different extracts of *L. resedifolia*. However, we have observed that TPC and TFC are greater in the polar fractions (WF and BF), this suggests that these polyphenol compounds are more hydroxylated and/or glycosylated. The phenolic or flavonoid compounds contained in extracts were influenced by their solubility in the solvent used for extraction. Polyphenols are present in polar fractions more than non-polar fractions, Results similar to those found in Nagalapur and Paramjyothi [21] and Belboukhari et al. [22].

3.3. Antioxidant Activities

3.3.1. DPPH radical scavenging capacity

The antiradical capacity of the extracts of *L. resedifolia* was assayed by using the DPPH. This method is based on the granting of hydrogen from phenolic hydroxyl groups to reduction DPPH•. This reduction is accompanied by a color change of DPPH• (violet) to DPPH-H (yellow), where reading was done at 517 nm. Figure 2 shows the percentage of scavenging DPPH in extracts of *L. resedifolia*. The values of IC_{50} ranged from 437.999 ± 26.097 µg/ml to 4,226.41332 ± 81.5639287 µg/ml (Table 2). The highest capacity recorded at AF and BF with an IC_{50} value of 437.999299 ± 26.0969056 µg/ml and 976.193157 ± 36.1905122 µg/ml, respectively, followed by WF and CE with an IC_{50} value of 1,528.45228 ± 46.9357862 µg/ml and 2,673.95055 ± 24.9000979 µg/ml, respectively, and the lowest capacity recorded at a CF with an IC_{50} value of 4,226.41332 ± 81.5639287 µg/ml.

The fractions showed better scavenging capacity than the CE, except for the CF. IC_{50} values of all these compounds were higher than that of BHA and ascorbic acid where IC_{50} was achieved at 83.34988 ± 4.1495129 µg/ml and 83.1162377 ± 3.74982633 µg/ml, respectively..

3.3.2. Ferric reducing power (FRP)

The antioxidant capacity of this method depends on the mechanisms which measure the transformation of a ferricyanide complex (Fe^{3+}) to the ferrous (Fe^{2+}) form by granting an electron according to the chemical reaction (Eq. 1) [23]:

$$\text{Reductive antioxidant} + [Fe(CN)_6]^{3-} \rightarrow \text{oxidation product} + [Fe(CN)_6]^{4-} \quad (1)$$

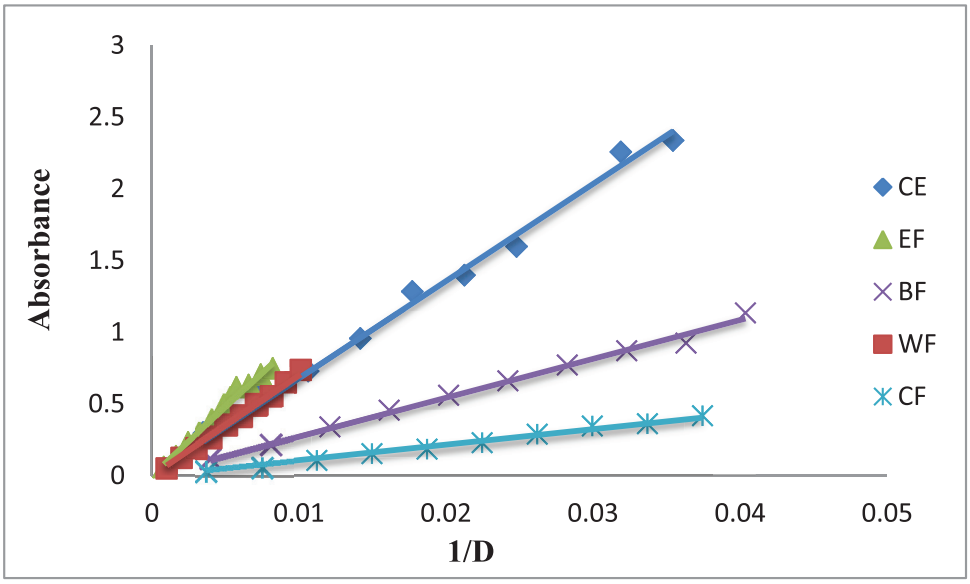

Figure 3: Reducing power capacities of L. resedifolia's extracts.

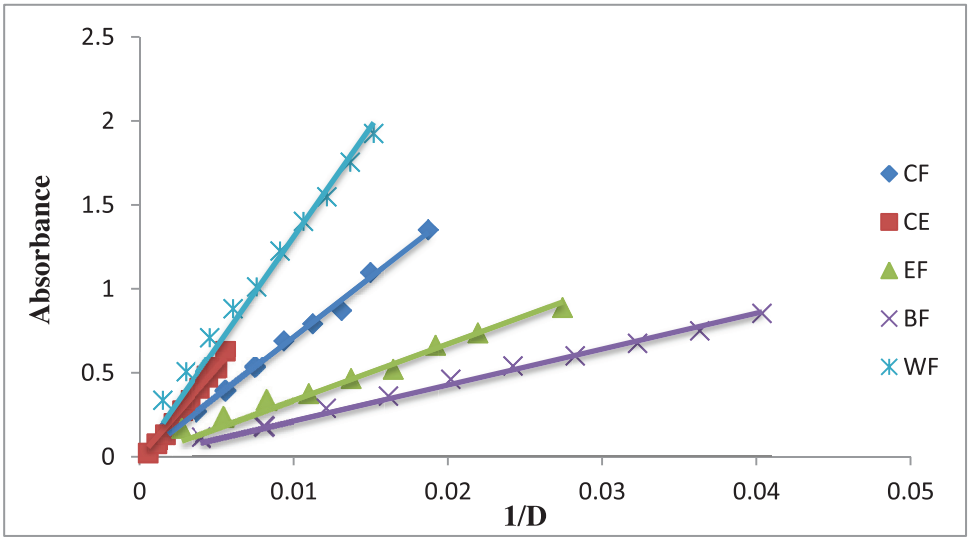

Figure 4: Total antioxidant capacity of L. resedifolia's extracts.

Figure 3 shows the reducing power capacities of extracts of *L. resedifolia* expressed as absorbance in terms of the inverse of the dilution factor.

Ferric reducing capacity of the various extracts of *L. resedifolia* varied between 17.772 ± 0.722 mM and 156.799 ± 22.795 mM. EF had the best-reducing capacity with a value of 156.799 ± 22.795 mM, followed by WF and CE with values of 116.701 ± 2.414 Mm and 112.126 ± 0.483 mM, respectively. The lowest reducing capacity was registered in BF and CF (45.922 ± 2.158 and 17.772 ± 0.722 mM, respectively). All extracts showed a ferric reducing capacity bested than BHT and BHA (1.960 ± 0.031 and 1.126 ± 0.041 mM, respectively).

3.3.3. Total antioxidant capacity

The total antioxidant capacity is based on the reduction of molybdenum hexavalent oxidation state Mo (VI) to molybdenum pentavalent Mo (V) by the effect of the electron donor by the antioxidant and formation of molybdenum complex colored green to acid pH, according to the chemical reaction (Eq. 2) [23]:

Reductive antioxidant + Mo (VI) → oxidation product + Mo (V) (2)

Figure 4 shows the total antioxidant capacity of *L. resedifolia*'s extracts expressed as absorbance in terms of the inverse of the dilution factor.

Total antioxidant capacity of the different extracts of *L. resedifolia* varied between 25.778 ± 0.721 mM and 157.928 ± 1.270 mM. WF and CE had the best-reducing capacity (157.928 ± 1.270 mM and 125.763 ± 8.119 mM, respectively), followed by CF with a value of 86.758 ± 7.748 mM. The lowest reducing capacity was registered in EF and BF (40.807 ± 0.367 mM and 25.778 ± 0.721 mM, respectively). All extracts showed a ferric reducing capacity better than BHT and BHA (1.211 ± 0.171 mM and 0.535 ± 0.034 mM, respectively).

The antioxidant capacity demonstrated by these methods used in this study possibly mainly due to the existence of phenolic compounds in these polar extracts. Thus, in a study conducted earlier, the phytochemical studies of this genus showed that this genus was rich in phenolics, flavonoids, and tannins [24–27]. In addition, BF of the aerial parts of *L. resedifolia* gave four flavonoids [8]. Finally, it is well known that the presence of polyphenolic compounds increases antioxidant activities [22].

4. CONCLUSION

The various extracts of *L. Resedifolia* were examined for their phytochemical contents and antioxidant capacities. The results obtained from this present work showed that the extracts of *L. resedifolia* have high antioxidant capacity. The strong antioxidant capacity of *L. resedifolia* extracts' shown in this study encourages further studies such as anti-corrosion [28] and anti-bacterial activity; isolation and identification of active compounds present in these extracts.

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