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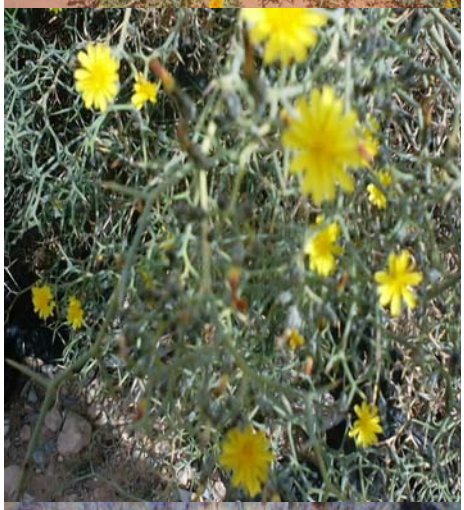

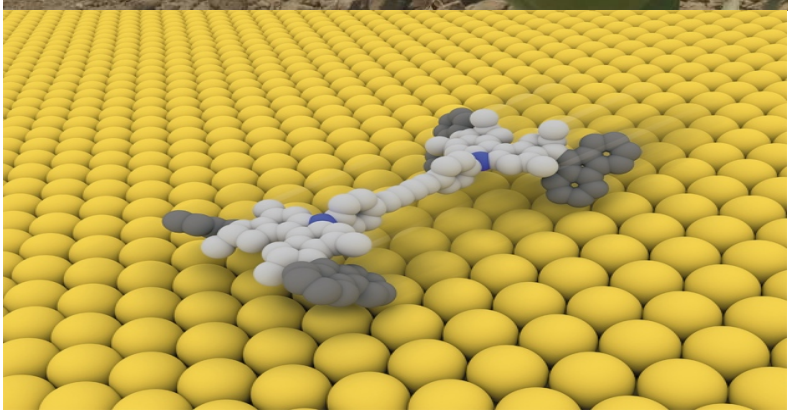

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Investigation of the antioxidant potential and total phenolics of *Bubonium gravelence* aerial parts

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Abstract. *Antioxidant potential of aqueous extract of the aerial parts of Bubonium gravelence was evaluated using superoxide and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging, phosphomolybdenum, reducing power and cyclic voltammetry. In DPPH radical scavenging activity, the IC_{50} value of the aqueous extract was found to be 0.152±0.05 mg/mL. In the phosphomolybdenum and reducing power tests, the extract has an activity in the order of 19 ± 0.45 and 47.42 expressed as µg of ascorbic acid equivalents per mg of extract, respectively. Moreover, the aqueous extract of Bubonium graveolens showed an antioxidant activity of the radical reduction $O_2^{\bullet-}$ in the order of 65.11 ± 1.4 at 1 mg / mL doses, The cyclic voltammetry of the Bubonium graveolens aqueous extract indicates one oxidation irreversible peak at 430 mV/(Ag/AgCl).*

Key Words: *Bubonium graveolens; total phenolics; antioxidant potential; phosphomolybdenum test; cyclic voltammetry*

1. Introduction

Oxidative stress is a major factor in health, aging, and disease and is often defined by the redox balance established by free radicals and antioxidants system defense [1]. A natural product is a substance or a chemical compound produced by a living organism found in nature that usually has a biological or pharmacological activity. Some of these components are derived from desert plants [2-5]. Among these plants in the sahara of Algeria we find *Bubonium graveolens*, belonging to the asteraceae family, this plant as the source of antifungal agents against fusarium oxysporum f. sp. albedinis [6,7], the chemical composition of essential oil from this plant were studied by Cheriti et al [8]. The corrosion inhibition of mild steel by essential oil leaves has been investigating [9]. *Bubonium graveolens* is rich sources of minerals and antioxidants [10]. Currently, several chemicals and electrochemicals technique have been developed for the measurement of the antioxidant activity in the natural extracts [11, 12]. The aim of this research was to investigate the antioxidant potential of *Bubonium graveolens* aqueous extract. For this purpose, different techniques were

applied such as the superoxide and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging, phosphomolybdenum, reducing power and cyclic voltammetry.

2. Plant Material

For the sake of the present investigation, the aerial parts of *Bubonium gravelence* before flowering were collected from Bechar, south-west of Algeria in October 2013. The aerial parts were dried in a dark at the room temperature for 15 days.

3. Extraction procedure

The reflux extraction technique is used in this study; the dried aerial parts (20 g) were cut into small fragments and extracted with a mixture of distilled water (400 mL) for 3h. The solvent (water) was eliminated using a rotary evaporator (at 70 °C) and obtained dried crude extract which was used for this investigation.

4. Total phenol and total flavonoid content determinations

The total phenolic content of plant extracts was determined spectrophotometrically using Folin–Ciocalteu reagent according to the Singleton, V. L., & Rossi, (1965) procedure [13] and the total flavonoid was determined using aluminium chloride colorimetric method according to the Bahorun et al (1996) procedure [14].

5. Tests for antioxidant potential of extract

5.1. Diphenyl Picryl hydrazyl radical scavenging test (DPPH):

The DPPH test was carried out as by described by Brad-Williams et al (1995) [15] with some modification based on the reduction of the DPPH radical on his active form to inactive form and the results of the extract are compared to ascorbic acid.

5.2. Superoxide radical scavenging assay:

The assay of superoxide radical scavenging was carried by the method of E.I. Korotkova et al (2002) [16] based on the inhibition of this radical generated by one-electron reduction of oxygen molecular.

5.3. Reducing power:

The reducing power of the aqueous extract was assayed by the method of Oyaizu (1986) [17] based on the reduction of the ferric ions Fe^{3+} to ferrous ions Fe^{2+}. The reducing power of extract is expressed as the number of equivalents of ascorbic acid

5.4. Phosphomolybdenum test

The phosphomolybdenum assay was performed according to the method described by Prieto et al (1999) [18] based on the reduction of Mo (VI) to Mo (V).

5.5. Cyclic voltammetry

The cyclic voltammetry assay was carried according to the method described by Kilmartin, P. A. (2001) [19] based on the directly determination of the potential reduction of the antioxidant compound by the electroxydation.

6. Results and discussion

Medicinal plants are rich sources for natural antioxidants [20]. In this study, the total phenolics compounds were determined using the Folin–Ciocalteu reagent and the total flavonoid was determined using aluminium chloride method.

The total phenolic and flavonoid contents in the *Bubonium gravelence* aqueous extract was determined and expressed in terms of gallic acid and quercetin equivalents respectively. The

aqueous extract was found to contain average amount of phenols and flavonoids, in the order of 92.12±4.1µg and 14.35 ±1.89µg for on milligram of aqueous extract respectively.
The results obtained in the present study demonstrated clearly that aqueous extract of *Bubonium gravelence* showed variable of antioxidant and antiradical-scavenging activities evaluated by different method chemicals and electrochemicals.
The results of phosphomolybdenum test is expressed as the number of equivalents of ascorbic acid. All concentration of *Bubonium gravelence* aqueous extract demonstrates antioxidant activity with phosphomolybdenum test (figure 1). It showed an activity of the order of 19 ± 0.45 µg of ascorbic acid equivalents for one milligram of aqueous extract.

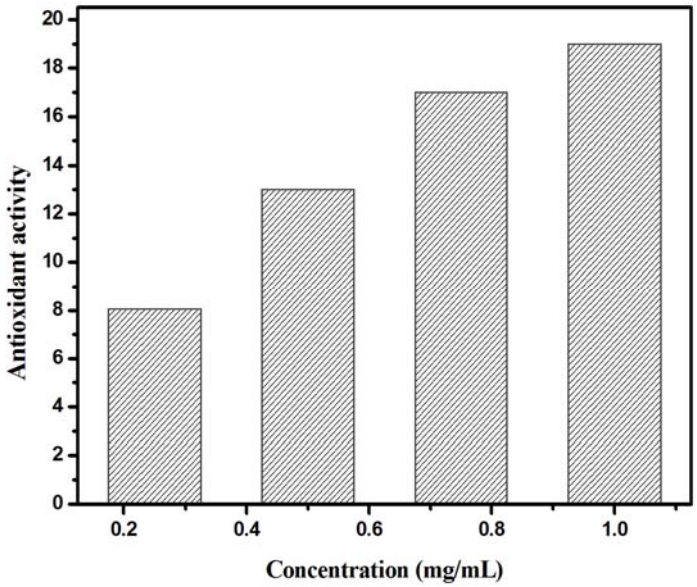

Figure 1. Antioxidant activity of *Bubonium gravelence* aqueous extract in the phospho-molybdenum test expressed as micrograms of ascorbic acid equivalents per milligram extract.

In DPPH radical scavenging activity, the purple colour of DPPH was bleached by yellow spots was the indication of positive antioxidant capacity and the potential of the antioxidants in the aqueous extract was determined by the IC50 values (the concentration with scavenging activity of 50%), a low IC50 value indicates power antioxidant activity in a sample. The figure 2 presented the DPPH• radical elimination activities values of different concentrations of *Bubonium gravelence* compared to ascorbic acid, the aqueous extract an activity of order of 89%±4.01 for one milligram of extract and the IC50 value was found to be 0.152±0.05 mg/mL.
Other methods involving metal ions such as Fe^{3+} have also been used. The ability of the extracts, to effectively on the reduction of the ferric ions Fe3+ to ferrous ions Fe2+., was determined and expressed to that of acid ascorbic equivalents. All concentration of *Bubonium gravelence* aqueous extract showed antioxidant activity with reducing power test. It showed a capacity of the order of 47.42 µg of ascorbic acid equivalents for one milligram of aqueous extract. The reducing power of aqueous extract increases as the concentration of *Bubonium gravelence* increases (Figure 3). The extract show different antioxidative values depending on the concentration.

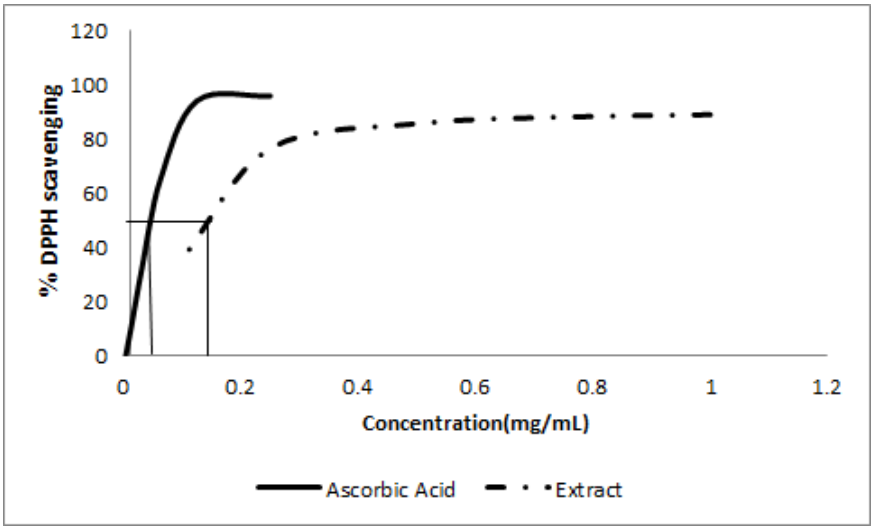

Figure 2. % DPPH• elimination activity of *Bubonium gravelence* aqueous extract compared to ascorbic acid.

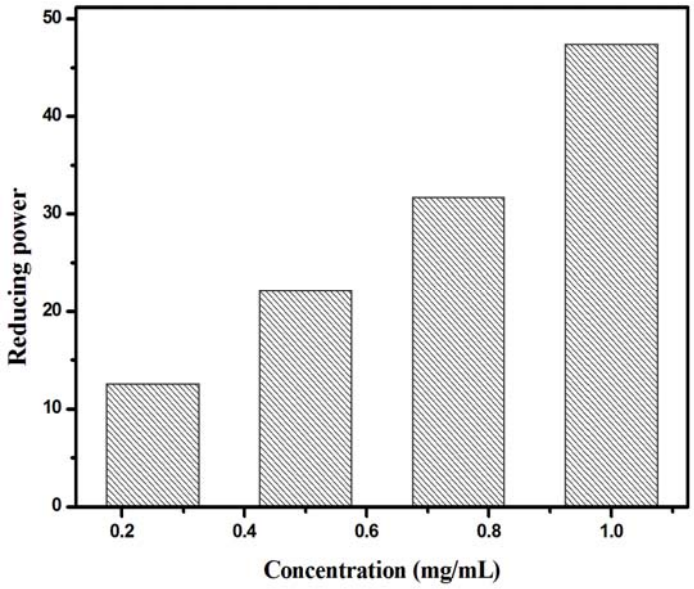

Figure 3. Reducing power from *Bubonium gravelence* aqueous extract expressed in micrograms of ascorbic acid equivalents per milligram extract

Cyclic voltammograms (CV) scanning in the positive potential range 0–1200 mV at a scan rate 100 mV s^{-1} were used to study the electrochemical properties of antioxidants present in the aqueous extract of *Bubonium gravelence*. Three electrodes system: a glassy carbon disc as working electrode, a platinum auxiliary electrode, and a saturated Ag/AgCl in 3 M KCl reference electrode was used. In general, compounds with a lower potential are have power antioxidant activity, the cyclic voltammogram of *Bubonium gravelence* aqueous extract (Figure. 4.(1)) indicate on oxidation peak at 430 mV. The absence of the corresponding reduction peak also points to the irreversibility of oxidation of reaction products produced in this reaction.

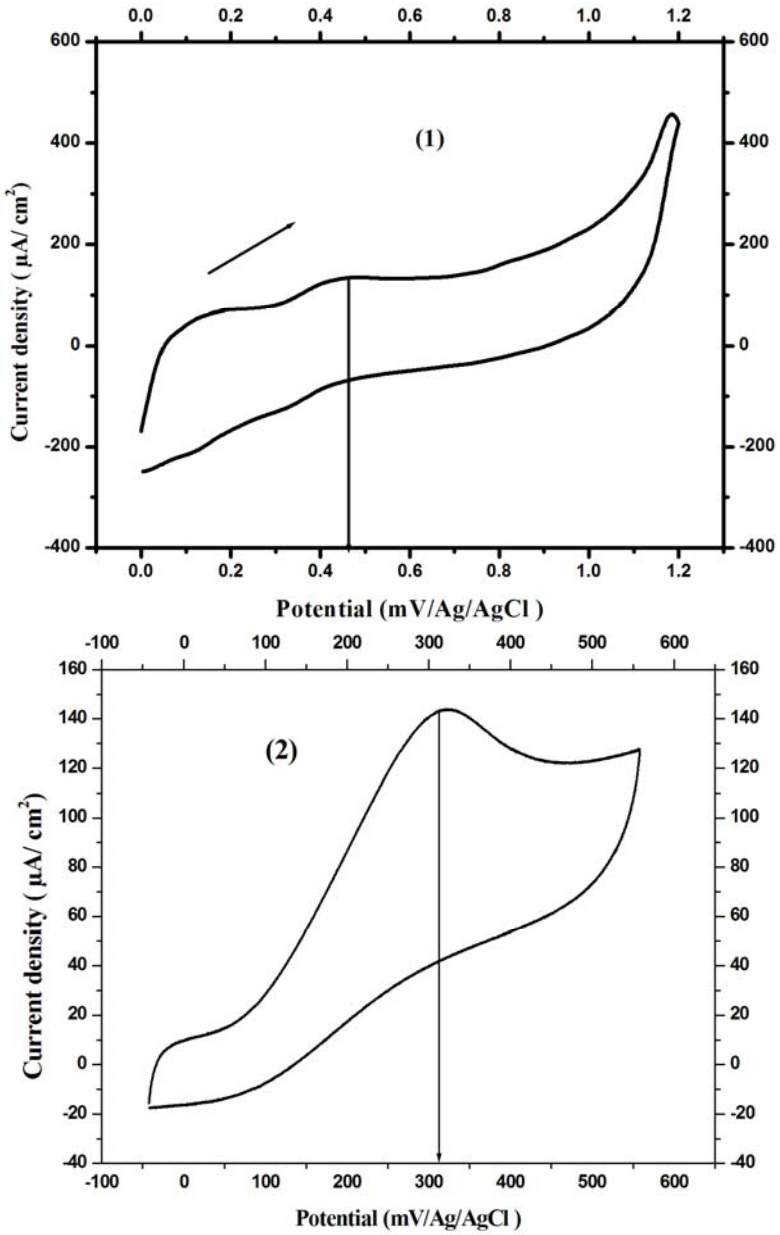

Figure 4: Cyclic Voltammogram of (1) *Bubonium gravelence* aqueous extract (2) ascorbic acid in KCl (0.1 M) as a supporting electrolyte, at scan rate 200 mV s^{-1}

The antioxidant potential assessed by superoxide radical scavenging are presented in figure 5, this assay is based on the inhibition of this radical generated by one-electron reduction of oxygen molecular in DMF media. A conventional three-electrode cell was used with: (a) a saturated calomel electrode reference electrode, (b) a 3-mm diameter glassy carbon working electrode and (c) a platinum wire auxiliary electrode. The aqueous extract of *Bubonium gravelence* showed a percentage inhibition or anti-radical activity of the order of 65.11 ± 1.4 at 1 mg / mL doses.

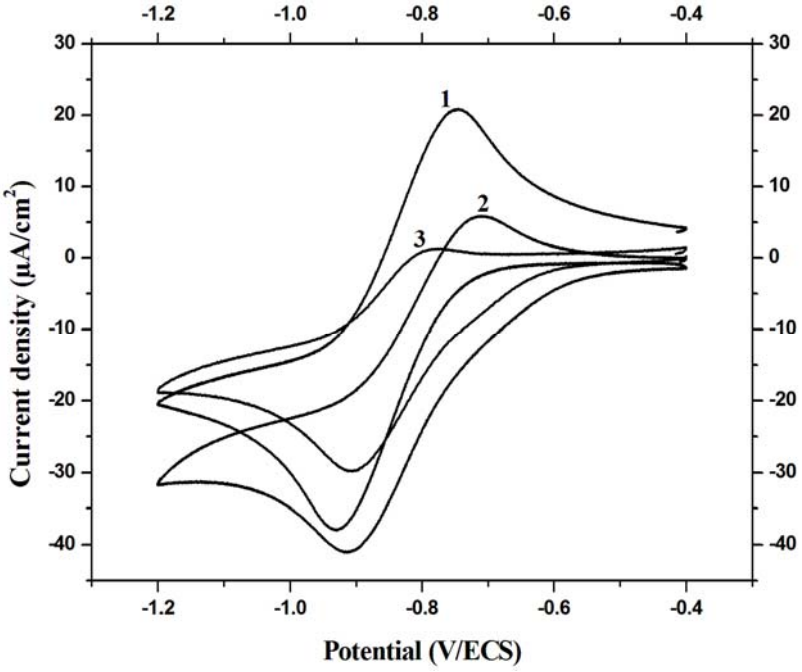

Figure 5. Cyclic voltammograms of O_2 reduction in the absence of antioxidant (1), in the presence of *Bubonium gravelence* aqueous extract (2) and in the presence of ascorbic acid (3) at a scan rate 50 mVs^{-1}, at a steady glassy carbon disk electrode in DMF/0.1 M Bu4NPF6.

7. Conclusion

In this study, the superoxide and 1, 1-diphenyl, 2-picryl hydrazyl (DPPH) radical scavenging, phosphomolybdenum, reducing power and cyclic voltammetry have been tested in parallel for the investigation of the antioxidant potential of *Bubonium graveolens* aqueous extract. It can be concluded that the aqueous extract of *Bubonium gravelence* before flowering possesses remarkable antioxidant potential *in-vitro* models and after these results suggest that the specie of this family could be potential targets for the search for new natural antioxidant compound.

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