



RESEARCH ARTICLE

Determination of Total Polyphenols and Flavonoids Contents and Iron Reducing Power of Aerial Parts of *Seseli praecox* Guss. from Algerian Flora

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ABSTRACT

In this study, *Seseli praecox* of Algerian flora was investigated regarding their polyphenolic and flavonoids contents using the Folin–Ciocalteu and aluminum chloride colorimetric methods, respectively. TPC of crude aqueous ethanolic extract was $44\text{mg} \pm 0.09\text{GAE/gDw}$, while, TFC have values of $1.53\text{mg} \pm 0.01\text{QuercetinE/gDw}$ and $6.93\text{mg} \pm 0.01\text{RutinE/gDw}$ for ethanolic extract, which mean that this plant is relatively poor source of polyphenols and flavonoids. In the other side, the polyphenolic extract of this plant was evaluated for its reducing power. The capacity of the extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex mention that *Seseli praecox* showed a very high iron reducing power activity with $\text{EC}_{50}=0.25\text{mg/ml}$.

Keywords: *Seseli praecox*, polyphenols, flavonoids, reducing power activity.



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INTRODUCTION

Polyphenols have recently aroused considerable interest because of their potential beneficial biochemical and antioxidant effects on human health. Commonly referred to as antioxidants, they may prevent various diseases associated with oxidative stress, such as cancers, cardiovascular diseases, inflammation and others. Most of the experimental results confirmed that polyphenols have several biological activities including radical scavenging, anti-inflammatory, anti-mutagenic, anti-cancer, anti-HIV, anti-allergic, anti-platelet and antioxidant activities [1]. Aromatic and medicinal plants have attracted attention as a significant source of natural products all over the world [2]. The Apiaceae (or Umbelliferae) is a plant family comprising at the present time 466 genera and about 3800 species. It is distributed nearly worldwide, Africa has about 121 genera, where North Africa encompasses the largest occurrence of 82 genera, 13 of which are endemic [3]. This cosmopolitan family occupying an important area in Algerian flora with 56 genera and 130 species (which 24 are endemic). *Bupleurum* and *Daucus* were more abundant with 14 and 11 species, respectively [4]. The genus *Seseli* is an important genus of the Apiaceae family, with a large number of aromatic species, comprised of 55 species distributed mainly in Europe. *Seseli* is a genus of herbaceous perennial plants. They are sometimes woody at base with a conic tap root. Leaf blades are 1–3-pinnate or pinnately decomposed. Umbels are compound, with bracts few or absent. Petals are white or yellow, and the fruit ovoid or ellipsoid [5]. Species from the genus *Seseli* L. have long been used extensively in traditional medicine in the Mediterranean region, but there is quite limited information on their phytochemicals and biological activities [6]. *Seseli* species are well-known source of the linear or angular pyranocoumarins possessing antiproliferative, antiviral and antibacterial activities [7]. Traditional uses of *Seseli* species as anti-inflammatory agents were supported by the results of Kupeli [8]. *Seseli praecox* is a rare species of Algerian flora [9], herbaceous perennial plant with a thick and branched stems of 10 to 30 cm, subline at the base, bears leaves divided into segments cut into lobes arranged in a fan pattern. The white flowers form umbels with poorly developed involucre. The high demand and curiosity for natural products, especially for the development of new drugs and in the course of our continuing search for bioactive natural plant products, this study is destined to the determination of total polyphenols and flavonoids contents of the plant *Seseli praecox* growing in Algeria, as well as to estimate their reducing power activity, were conducted for the very first time.

MATERIALS AND METHODS

Plant collection and identification

Seseli praecox was collected on April 2017 at Edough peninsula, at Annaba province in the north east of Algeria, and identified by Professor Tarek HAMEL, Laboratory of biodiversity and phylogenetic resources, University of Badji Mokhtar Annaba, Algeria. A voucher specimen is deposited at the herbarium of laboratory of chemistry of university Mohamed Boudiaf of M'sila, Algeria.

Extraction

The fresh, whole plant was collected and shade dried to obtain 100 g dry sample which was later coarsely powdered and used for solvent extraction. The sample was extracted at room temperature for 3 × 24 hours. The extraction was performed with 70% aqueous ethanol solution (70 ethanol /30 water, v/v). The extract were filtered through a Buchner funnel, The aqueous solution was extracted with hexane several times to eliminate lipids and the solvent was removed on rotary evaporator (BÜCHI, 461, Germany) to dryness under reduced pressure at 40° to give crude extract of polyphenols [10].

Determination of total phenolic content

The total phenolic content (TPC) was determined by using the Folin-Ciocalteu assay. This phenol reagent consists of a mixture of the heteropoly acids, phosphomolybdic and phosphotungstic acids in which the molybdenum and the tungsten are in the 6+ oxidation state. On reaction with a reductant, the molybdenum blue and the tungsten blue are

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formed and the mean oxidation state of the metals is between 5 and 6 [11]. In brief, standard curve using gallic acid (7.81 and 250 µg/ml) was made with following steps: 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 04 minutes, 0.8 mL of a sodium carbonate (NaCO₃) solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 765 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of plant extract. The concentration of polyphenols in samples was derived from a standard curve of gallic acid using regression equation. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained [10] (Fig.1).

Determination of total flavonoid content

The total flavonoid content was determined according to the aluminium chloride colorimetric method. The sample contained 1 ml of methanol solution of the extract in the concentration of 1 mg/ml and 1 ml of 2% AlCl₃ solution dissolved in methanol. The samples were incubated for an hour at room temperature. The absorbance was determined using spectrophotometer at λ_{max} = 430 nm. The same procedure was repeated for the standard solution of rutin (2-64 µg/ml) and the calibration line was construed. Based on the measured absorbance, the concentration of flavonoids was read (mg/ml) on the calibration line; then, the content of flavonoids in extracts was expressed in terms of rutin equivalent (mg of RU/g of extract). The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained [11] (Fig.2).

Reducing power

The reducing power of the crude mixture of polyphenols and BHT was determined according to the Topcu et al. method. The capacity of the extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by recording the absorbance at 700 nm after incubation. For this purposes, 250 µl of extract prepared at different concentrations were mixed with 250 µl of phosphate buffer (0.2 M, pH 6.6) and 250 µl of potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes. 250 µl of Trichloro acetic acid (10%) was added. The supernatant was mixed with 1 ml of distilled water and 0.2 ml of ferric chloride was added, and then the solution's absorbance of the incident radiation was determined spectrophotometrically at 700 nm. The absorbance at 700 nm was used as the indicator of the reducing power. Increased absorbance of the reaction mixture indicated increased reduction capability. BHT was used as standard antioxidant [12] (Fig.3).

RESULTS AND DISCUSSIONS

Extraction yield and total polyphenols and flavonoids content

In the aerial parts of *Seseli praecox*, the extraction yield was obtained with 70% aqueous ethanol solvent which gives a brown mixture 10.75% of dry weight of plant. The total phenolic content (TPC) of the plant extract was measured using the Folin-Ciocalteu colorimetric method, based on the principle of reduction of phosphomolybdic acid by phenols in the presence of aqueous alkali [13]. *Seseli praecox* have 44mg±0.09 GAE/g Dw (expressed as gallic acid equivalents (GA) per gram of dry extract). This result is nearly the moiety when we compare it with previous studies concerning *Seseli* species. Data for the extraction yield of phenolic substances from some *Seseli* species have been published by Matejić et al. [14] where specific differences exist because of different methodologies of experimental work as well as different variety of *Seseli* samples. This can be explained probably because of the difference between species and other factors like culture conditions. Extraction time, temperature, solvent-to-feed ratio, the number of repeated extractions of the sample, as well as the choice of extraction solvents are the crucial parameters affecting the extraction yield. The yield and rate of polyphenolic extraction are related to the solvent characteristics. It has been observed that methanol is more efficient in the extraction of lower molecular weight polyphenols while aqueous acetone is a suitable solvent for the extraction of the higher molecular weight flavanols [15]. The total flavonoid content (TFC) of the plant extracts was determined by using the aluminum chloride colorimetric method, based on the reaction of aluminum ion with flavonoids at alkaline medium forming red chelates [13]. For *Seseli praecox*, TFC



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have values of $1.53\text{mg}\pm 0.01$ Quercetin E/gDw and $6.93\text{mg}\pm 0.01$ Rutin E/gDw for ethanolic extract. For best extraction yield of phenolic compounds and flavonoids, we will discuss extraction conditions. In fact, recent studies of different *Seseli* species were focused on essential oil analyses [16, 17 and 18]. This study mentions that the species *Seseli praecox* is a source of polyphenols and flavonoids.

Evaluation of reducing power

Antioxidants are important substances that possess the ability to protect the body from damage caused by free radical-induced oxidative stress. A variety of free-radical scavenging antioxidants exist within the body, many of which are derived from dietary sources such as fruits, vegetables, and teas. Reducing power is associated with antioxidant activity and may serve as a significant reflection of the antioxidant activity [19]. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants [20]. In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound. Presence of reducers causes the conversion of the Fe^{+3} /ferricyanide complex used in this method to the ferrous form.

In the reducing power assay, the more antioxidant compounds convert the oxidation form of iron (Fe^{+3}) in ferric chloride to ferrous (Fe^{+2}) [20]. The results of this research showed that the plant *Seseli praecox* exhibited a significant iron reducing power with $\text{Ec}_{50}=0.25\text{mg/ml}$. In fact, kaempferol, rutin and quercetin (three flavonoids) have an important reducing power with Ec_{50} of 5.70, 6.15 and $1.48\text{ }\mu\text{g/ml}$, respectively [21]. It is widely accepted that some pathological events such as heart disease, chronic renal failure, diabetes mellitus, cancer, immune dysfunction, and aging are closely related to the peroxidation reactions in living organisms. Antioxidants are supposed to protect cell membranes against free-radical oxidative damages. There is growing interest to replace synthetic antioxidants by natural ones mostly found in plants. There is also a worldwide trend toward the use of natural additives in foods and cosmetics. For this reason, an extensive search for different types of antioxidants in various types of plants has been undertaken [19] (Fig.4).

CONCLUSION

A literature survey showed a few phytochemical and biological investigations concerning the plant *Seseli praecox*. Results of our study suggest that the species *Seseli praecox* which screened for the first time, is a source of polyphenols and flavonoids and exhibited a high iron reducing power with $\text{Ec}_{50}=0.25\text{mg/ml}$ which is near to the standard (BHT). We have, also, warranted the concentration dependent reducing ability of the extract. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect. Also a correlation between the reducing power and antioxidant activity can be derived. The extraction of the essential oil from this plant will be carried out.

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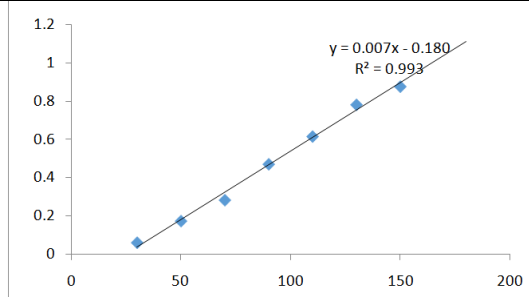


Figure 1: Calibration curve of standard gallic acid for determination of total phenolics content.

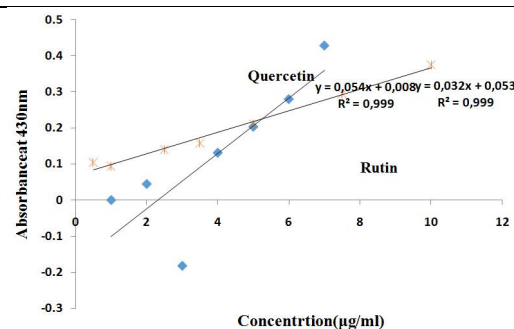


Figure 2: Calibration curves of standards quercetin and rutin for determination of total flavonoids content.

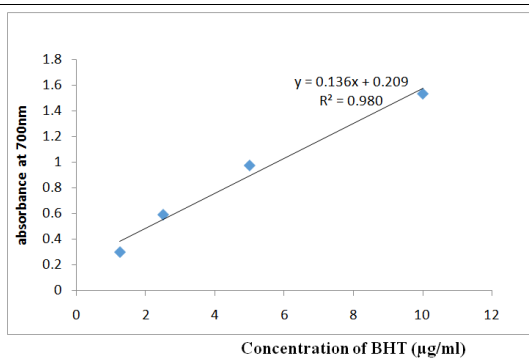


Figure 3: Reducing power of BHT

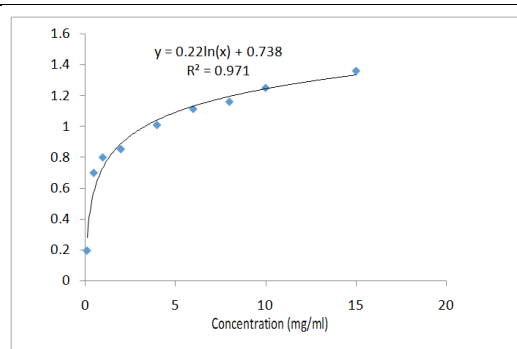


Figure 4: Reducing power of *Seseli praecox* extract

