

## Evaluation of Phytochemical Properties and Biological Activities of Leaf Extracts and Oil of *Petroselinum sativum* Collected from Algeria

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**Abstract:** Indigenous medical professionals prescribe plant-based therapeutics for the handling of a range of maladies and use typical medical care at the preventive level. Oxidative stress, obesity, and multidrug resistance to pathogenic microorganisms are major challenges in the healthcare systems and pharmaceutical industries. This study aimed to screen phytoconstituents, estimate total phenol and flavonoid contents, and evaluate the antioxidant, and antimicrobial activities of *Petroselinum sativum* extracts and essential oil growing in Algeria. The antioxidant capacity of n-butanol, dichloromethane extracts and essential oil was determined by using DPPH radical scavenging method. All extracts were also evaluated against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) bacterial species using the disk diffusion method. The leaves are pharmacologically active and related to the detected phytochemical components. In the proximate analysis, plant nutrients that are important for diverse pharmacological activities are found in the leaves. The ethyl acetate extract of *P. sativum* showed the highest phenol content while the n-butanol and ethyl acetate extracts gave high flavonoid contents at  $144.44 \pm 0.07$  and  $100.97 \pm 0.04$  mg/g, respectively. The antioxidant results demonstrate powerful free radical scavenging activity for three extracts. The activity reduced in the following order as concentration increased n-BuOH > AE > DCM.

**Keywords:** *Petroselinum sativum*; phytochemical screening; antimicrobial properties; antifungal activity

### ■ INTRODUCTION

Aromatic and medicinal plants (AMPs) have a therapeutic history and they are essential to the medical industry. Throughout history, every civilization has employed them [1] and their use is still commonplace around the world, particularly among Africans [2]. The modern pharmaceutical business itself heavily relies on a variety of secondary plant metabolites to identify novel compounds with biological and pharmacological features, from aspirin to taxol [3]. According to various researches,

people on all continents have used native plant poultices and infusions for a very long time. Currently, 80% of the populations medicinal plants are still used in developing nations to cure a variety of infections [4].

The Apiaceae family includes the plant *Petroselinum sativum*, also known as "parsley" in English and "maadnous" in Arabic [5]. The *P. sativum* Hoffm is synonymous with *Apium Petroselinum* L, *P. crispum* (Mill) Fuss, and *P. Hortense* Hoffm [6]. Though it is now grown all throughout the world, parsley has its origins in

the Mediterranean region [7]. It shows many advantageous properties such as antioxidant, analgesic and spasmolytic, antidiabetic, immunomodulating, and gastrointestinal actions of *Petroselinum sativum* Hoffm [8]. The primary components of the plant, including the flavonoids (Apigenin, Apiin, Quercetin, Luteolin, and Kaempferol), carotenoids, vitamins, and coumarin, may be responsible for these various benefits [9].

Thus, this study aimed to screen phytochemical constituents of the medicinal plants (*P. sativum*), to estimate their potential total phenol and flavonoid contents as well as to assess their antioxidant, and antimicrobial properties of extracts and oil.

## ■ EXPERIMENTAL SECTION

### Materials

The leaves of *P. sativum* were collected from Hamma Bouziane Constantine east of Algeria in 2018. The plant was identified by Pr. Rebas Khelaf, Faculty of Sciences University of M'sila. The chemicals used were dichloromethane (DCM) p.a. (Merck), *n*-butanol (*n*-BuOH) p.a. (Merck), ethyl acetate (AE) p.a. (Merck), nutrient agar (Merck). All other reagents were of analytical grade and were purchased from Sigma Aldrich (Germany).

### Instrumentation

The primary tools employed in the study were the essential oil determination apparatus, (Clevenger apparatus), evaporator (Buchi R-124), balance, UV-Vis Spectrophotometer (Shimadzu UV-1800), and Spectrophotometer Jenway (6405 UV/Vis, England).

### Procedure

#### Extract preparation

The plant leaves powder (1000 g) was washed three times using a mixture of (methanol:water) (70:30) at room temperature. After filtering, the extracts under reduced pressure were concentrated at 45 °C. The aqueous extracts dichloromethane (DCM), ethyl acetate (AE), and *n*-butanol (*n*-BuOH) were partitioned in order to remove chlorophylls. The mass of 5.88 g (DCM), 8.58 g (AE), and 16.94 g (*n*-BuOH) extracts were obtained by evaporating the extractive solutions to dryness under vacuum.

For the extraction of essential oils In Clevenger-type equipment, plant samples (300 g) were hydro-distilled [10]. The essential oil samples were kept in the dark at 4 °C. The amount was calculated as:

$$\text{Oil (\% v / w)} = \frac{\text{Observed volume of oil (mL)}}{\text{Weight of sample (g)}} \times 100 \quad (1)$$

#### Preliminary phytochemical screening

The samples were screened for the following components as described by Harborne [11] and Savithramma et al. [12].

**Test for alkaloids.** A quantity of 2 mL hydrochloric acid (1 M) was added to 2 mL of plant extract. Mayer's reagent was then added in a few drops. Alkaloids can be detected by the presence of white or green precipitate.

**Test for anthraquinones.** To 1 mL of the extract, a few droplets of a 10% ammonia solution were added, and the precipitate's pink hue indicated the presence of anthraquinones.

**Test for carbohydrates.** A quantity of 1 mL of Molisch's reagent and a few drops of concentrated sulfuric acid were added to 2 mL of plant extract, the presence of carbohydrates was indicated by the Presence of purple or reddish color.

**Test for glycosides.** A mixture of 0.4 mL of glacial acetic acid and a few drops of ferric chloride is added to 1 cc of the extract. It is then placed into a test tube containing concentrated H<sub>2</sub>SO<sub>4</sub> after some time has passed. Positive results for glycoside are indicated by the color change from reddish brown to blue at the intersection of the two stages.

**Test for flavonoids.** The availability of flavonoids is indicated by the appearance of yellow color and by the addition of 2 mL sodium hydroxide to 2 mL of plant extract.

**Test for saponins.** In a graduated cylinder, 2 mL of plant extract and 2 mL of distilled water were combined and agitated for 15 min lengthwise. The presence of saponins was indicated by the formation of a 1 cm layer of foam indicates.

**Test for polyphenols.** The existence of phenols or polyphenols causes the formation of blue or green tint, when added by 1 mL of the extract, 2 mL of distilled water then little droplets of 10% ferric chloride.

**Test for triterpenoids.** The extract (10) mg was dissolved in 1 mL of chloroform and followed by the addition of 2 mL of conc. H<sub>2</sub>SO<sub>4</sub> and 1 mL of acetic anhydride. The presence of triterpenoids is indicated by the development of a reddish-violet hue.

**Test for carotenoids.** Dark-blue coloration is indicative of carotenoids after the addition of 3 mL of antimony trichloride to about 2 mL of the extract.

**Test for coumarins.** In a test tube, a small amount of plant extract was placed. Then it was placed in a hot water bath and covered with a piece of filter paper that had been dampened with dil. NaOH solution. The paper was removed and exposed to UV light after about 15 min. The presence of coumarins is indicated by yellow-green fluorescence.

**Test for steroid.** In a dry test tube, 1 mL of floral extract and 2 mL of chloroform were combined. Following this procedure, the test tube was filled with 10 drops of acetic anhydride and 2 drops of concentrated H<sub>2</sub>SO<sub>4</sub>. The color has changed from red to blue to bluish-green, giving off a pleasant steroid effect.

**Test for proteins.** The ninhydrin test was used to determine if proteins were present. Boiling crude extract with 2 mL of 0.2% solution of ninhydrin, a violet hue developed, indicating the presence of proteins and amino acids.

#### **Measurement of total phenolics (TPC)**

The technique of Folin-Ciocalteu [13] was utilized to calculate the proportion of phenol in total. In the wells of a 96-well microplate, 20 µL of each extract was combined with 100 µL (1:10) diluted Folin-Ciocalteu reagent and 75 µL sodium carbonate solution (7.5%). The microplate reader measured the absorbance at 740 nm after 2 h of darkness at room temperature [14]. The number of phenolics collectively in the extract was measured in mg gallic acid equivalent (mg GAE) per gram of extract based on the calibration curve, the Eq. (2) was used:

$$y = 0.0034x + 0.1044; R^2 = 0.9972 \quad (2)$$

where, y is the gallic acid equivalent (mg/g) and x is the absorbance. The measurements were carried out three times.

#### **Estimating the total amount of flavonoids (TFC)**

The total flavonoid concentration was determined using the aluminum chloride colorimetric method, as described by Ghout et al. [15]. A 96-well plate was filled with 50 µL of extract, 130 µL of methanol, 10 µL of 1 M potassium acetate, and 10 µL of 10% AlCl<sub>3</sub>. After being homogenized, the mixture was allowed to rest for 40 min. In a microplate plate reader, at 415 nm, the solution's absorbance was measured [14]. A quercetin calibration curve was done from 0.1 to 1 mg/mL; to calculate total flavonoid content Eq. (3):

$$y = 0.0048x; R^2 = 0.997 \quad (3)$$

where x is the absorbance and y is the quercetin equivalent in a gram of dry powder (mg QE/g).

#### **Antioxidant capacity (DPPH free radical)**

Extracts' radical scavenging capacity was assessed utilizing 2,2-diphenyl-1-picrylhydrazyl (DPPH) [16-17]. The sample (0.4 mL) was mixed with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL methanol). The absorbance was determined using the spectrophotometer Jenway at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (10–100 mg/L; R<sup>2</sup> = 0.989) was used as the standard and the results were expressed in mg/g Trolox equivalents.

#### **Antimicrobial activity**

The disc diffusion method was used to test the antimicrobial activity of extracts and oil. The new media (10 mL) were used to cultivate 100 µL of the test bacteria until they had a count of nearly 10E5 colony-forming units (cfu)/mL. Then the microbial suspension was injected in a volume of 100 µL on Mueller Hinton agar plates [17-18]. Discs made of 6 mm sterile filter paper were used to evaluate the extracts. The inhibitory zones' sizes were measured in millimeters. Every measurement was made to the nearest full millimeter and was performed in at least triplicate. As a negative control, filter discs soaked in 10 µL of distilled water were used.

#### **Anti-fungal screening**

The extracts' antifungal potency was evaluated against *Aspergillus niger* by disc diffusion method. The standard used was ketoconazole (10 mcg disc<sup>-1</sup>) [19-20].

The ketoconazole disc and the extract-impregnated discs (20 mg mL<sup>-1</sup> each) were placed aseptically into the seeded sabouraud dextrose agar medium and swabbed with the test organism then incubated at 37 °C for 48 h. The inhibition zone was quantified and reported (in mm).

## ■ RESULTS AND DISCUSSION

The yields of the extracts (Yield) are established as being the mass to volume ratio of extract obtained after extraction ( $M_{\text{extract}}$ ) and the mass of the plant material used ( $M_{\text{sample}}$ ). It is expressed as a percentage and calculated by Eq. (4):

$$\text{Yield\%} = \frac{M_{\text{extract}}}{M_{\text{sample}}} \times 100 \quad (4)$$

where,  $M_{\text{extract}}$  = mass of the extract (g),  $M_{\text{sample}}$  = mass of the sample (g).

The yields were shown in Table 1.

### Phytochemical Screening

Polyphenols, flavonoids, saponin, terpenes, anthraquinones, coumarins, alkaloids, and carbohydrates were found in the early phytochemical screening of several secondary products in different leaf extracts (DCM, AE, and *n*-BuOH) of *P. sativum* [21]. However, none of these chemicals could be extracted in a single solvent.

Alkaloids, triterpenes, coumarins, and flavonoids appeared in high concentrations in DCM and *n*-BuOH extracts. A minimal presence of glycosides was detected in the DCM and *n*-BuOH extracts. However, polyphenols were identified practically in each extract. Anthraquinones were present in the AE and *n*-BuOH extracts. Saponins were not present in any of the extracts. The powerless presence of steroids was seen in the AE. Proteins were present in high concentration in *n*-BuOH with a weak presence in DCM extracts. The data are shown in Table 2.

### Chemical Composition

#### Calculation of the total phenolics and flavonoids

Table 3 demonstrates that the extract of ethyl acetate has significant phenol content (TPC) of (290.55 ± 0.07 mg/g), while *n*-BuOH and DCM extracts had lower phenolic content with 264.98 ± 0.08 and 113.8 ± 0.06 mg/g,

respectively. The TPC of *P. sativum* with various solvents was seen. However, more phenol is present in ethyl acetate extract than in other extracts.

Total flavonoid (TFC) differed from 1.08 ± 0.005 to 164.44 ± 0.07 mg/g in the extracts of *P. sativum*. Herein, the highest TFC can be seen in *n*-BuOH and AE extracts at 144.44 ± 0.07 and 100.97 ± 0.04 mg/g, respectively. The DCM extract gave the lowest concentration of TFC (1.18 ± 0.005 mg/g).

### Antiradical and Antioxidant Activity

Due to its stability, dependability, and ease of use in the assay, DPPH is a free radical that is frequently employed to quickly and easily estimate the antiradical capacity. Table 3 displays the ability of extracts to scavenge DPPH radicals.

**Table 1.** The yields of the various extracts

Extract	The yields (% (w/w))
DCM	0.58%
AE	0.85%
<i>n</i> -BuOH	1.69%

**Table 2.** Phytochemical screening of various leaf extracts of the *Petroselinum sativum*

Test	DCM	AE	<i>n</i> -BuOH
Polyphenols	++	+++	++
Flavonoids	+++	+++	+++
Saponin	---	---	---
Glycosides	+	--	+
Anthraquinones	--	++	+
Alkaloids	+++	+++	+++
Carbohydrates	+++	+	+
Triterpenoids	+++	+++	+++
Coumarins	+++	++	+++
Carotenoids	---	----	---
Steroid	---	+	---
Proteins	++	---	+++

**Table 3.** TPC (mg GAE.g<sup>-1</sup>) and TFC (mg QE.g<sup>-1</sup>) of various extracts of *Petroselinum sativum*

Extract	TPC (mg GAE/g)	TFC (mg QE/g)
DCM	113.8 ± 0.06	1.08 ± 0.005
AE	290.55 ± 0.07	100.97 ± 0.04
<i>n</i> -BuOH	264.98 ± 0.08	144.44 ± 0.07

The reduction of phosphate-molybdenum(VI) to phosphate-molybdenum(V) in the total antioxidant assay predicts the antioxidant activity of crude extracts overall and provides information about the sample's inclusion of antioxidant components. The evaluation of extracts' overall antioxidant activity was done, and the results were indicated as mg Trolox equivalents/g (Table 4).

The total flavonoid and polyphenol content correlated with the phosphomolybdenum assay ( $R^2 = 0.86$  and  $0.92$ , respectively). The results in Table 5 revealed that a significant portion of the extracts' antioxidant power is derived from phenolic components. However, the linear regression of a direct relationship between radical scavenging capacity and total flavonoid and polyphenolic contents was not successful.

#### Correlation between Phenolics, Flavonoids and Antioxidant Activity

The antioxidant activity of DPPH, TPC, and TFC of *P. sativum* extracts is affected by the extraction solvent. Due to differences in solubility and polarity, different solvents can be used to extract different molecules. There is a link between antioxidant activity, TPC, and TFC. The TPC in the *n*-BuOH extract should be higher than in the other extracts. The leaves should include polyphenol chemicals like quercetin and kaempferol. Among the other solvents, *n*-BuOH has the highest polarity and can extract the most polyphenol chemicals. The earlier investigation found that *n*-BuOH extract of *P. sativum* discovered results of flavonoids including quercetin, apigenin and kaempferol glycosides. They demonstrated to possess strong antioxidant/free radical scavenging effectiveness. More studies on toxicity and *in vivo* testing for clinical uses are needed to determine the safety of the extracts.

According to the data in Tables 4 and 5, *n*-BuOH extracts exhibited better antioxidant activity than other extracts, which completely agrees with the results for TPC and TFC compared to that of Trolox as standard ( $EC_{50} = 59.52 \pm 0.7 \mu\text{g/mL}$ ) antioxidant [22]. The DPPH radicals inhibition activity of *n*-BuOH extracts ( $EC_{50} = 134.49 \mu\text{g/mL}$ ) was better than previous research [23]. These results and those concerning TPC and TFC of extracts from *P. sativum* leaves, obtained in our work for the Algerian plant and comparing them with those in other studies, confirm the previously made assumption that Algerian plant may have better antioxidant and antibacterial activities than the same species from other parts of the world.

#### Antimicrobial and Anti-fungal Activity

We studied *in vitro* the antibacterial effects of oils and extracts of *P. sativum* [24], using the disk diffusion technique on Mueller-Hinton agar media. Antibacterial activity for the extracts and oil was estimated in terms of the diameter of the zone of inhibition around the discs (Table 6).

According to the results of the antibacterial activity of the oil obtained, we note that the bacterial strains used are not resistant to the essential oil and they showed good activity against *S. aureus*. The inhibitory activity is low for *E. coli*.

**Table 4.** Effect of the examined extracts' overall antioxidant capacity in relation to DPPH scavenging

Extracts	Radical scavenging activity (mg TEAC/g)
DCM	$52.71 \pm 4.08$
AE	$63.61 \pm 7.62$
<i>n</i> -BuOH	$134.49 \pm 3.63$

TEAC – Trolox equivalent antioxidant capacity; mean  $\pm$  standard deviation

**Table 5.** The relationship between the examined *n*-BuOH extract's phenolic and flavonoid levels, antioxidant and antiradical activity

<i>n</i> -BuOH extracts	Reducing power (mg TEAC/g)	Radical scavenging activity (mg TEAC/g)
Total phenolic content mg GAE/g	$R^2 = 0.92$	$R^2 = 0.34$
Total flavonoids content mg QE/g	$R^2 = 0.86$	$R^2 = 0.05$

GAE – gallic acid equivalent; QE – quercetin equivalent

**Table 6.** The fungicidal activity of oil *Petroselinum sativum*

Micro organisms	<i>Staphylococcus aureus</i> ATCC 25923	<i>Escherichia coli</i> ATCC 25922	<i>Aspergillus niger</i> 2CA936
Diameters of inhibition of oil (mm)	22	20	6

**Table 7.** Evaluation of antibacterial and antifungal activity of *Petroselinum sativum* extracts

Microorganisms	Zone of inhibition (mm)		
	<i>n</i> -Butanol	Ethyl acetate	Dichloromethane
<i>Staphylococcus aureus</i> ATCC 25923	18	15	16
<i>Escherichia coli</i> ATCC 25922	22	12	15
<i>Pseudomonas arigunosa</i> ATCC 27835	20	10	13
<i>Bacillus subtilis</i> ATCC 21332	22	10	14
<i>Aspergillus niger</i> 2CA936	10	6	10

The fungicidal activity of oil *P. sativum* was shown to have a moderate effect against *A. niger* by diffusion through the disk. Bacterial resistance of *P. sativum* extracts against Gram-positive and Gram-negative bacteria used, discovered that leaf extract exhibited more significant action against Gram-negative (*E. coli*) than against Gram-positive (*S. aureus*).

The *P. arigunosa* showed low activity against ethyl acetate and dichloromethane, but good activity was shown with *B. subtilis* for the *n*-BuOH extract (Table 7). The results of the antifungal activity of *P. sativum* leaf extracts tested against *A. niger* showed (Table 7) moderate inhibition against all test extracts.

Based on these findings, it is possible to draw the conclusion that *P. sativum* aerial portions have potent antibacterial properties against a variety of microorganisms. As a result, like essential oils from seeds, *P. sativum* has good antibacterial properties. The plant also has potential use in phytotherapy (on human pathogenic bacteria).

## ■ CONCLUSION

The aim of this research was to look into the phytochemical characterization and *in vitro* antioxidant action of *P. sativum* leaf extracts using a variety of methodologies. The presence of different substances

(flavonoids and terpenoids) was discovered during the preliminary screening of phytochemicals. Furthermore, when compared to DCM extracts, AE and *n*-BuOH have the highest antioxidant activity. We can assume that essential oils will be more antibacterial than extracts, while flavonoids will be first-class antioxidants, based on our findings. Because these extracts include larger levels of total phenolics and flavonoids, they may have a more pronounced antioxidant function. Finally, the results of this study refer to Algerian *P. sativum* leaves as a potential natural source of antioxidants. Further research is needed to understand the underlying mechanisms of antioxidant action and to pinpoint the compounds that are responsible for such activity.

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