



# Comparative Study of the Chemical Composition and Antioxidant Activity of Aqueous and Ethanolic Extracts of Nigella (Nigella Sativa L.) And Anise (Pimpinella Anisum L.)

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## KEYWORDS

*Nigella sativa L.*,  
*Pimpinella anisum L.*,  
Chemical composition, Secondary metabolites, Antioxidant activity

## ABSTRACT:

**Introduction:** Nigella (black seed) and anise are aromatic medicinal plants that always remain the reliable source of active ingredients known for their therapeutic properties

**Objectives:** The study aims to determine the chemical composition of the primary metabolite from plant powders and to assess secondary metabolite and the antioxidant activity of two different extracts of *Nigella sativa L.* and *Pimpinella anisum L.*

**Methods:** The aqueous and hydroethanolic extraction of the two species was achieved using the reflux heating and maceration methods. The obtained extracts were estimated for phytochemical contents. Total phenolic content was measured by spectrophotometry according to the Folin-Ciocalteu procedure. The flavonoid content was estimated using aluminum chloride methods, the condensed tannins were determined by the vanillin test and the antioxidant ability was assessed by the method of DPPH free radical trapping.

**Results:** The both seeds are rich in organic matter and primary metabolites, the secondary metabolites and antioxidant properties differed significantly among selected species and extracts. The result revealed that the aqueous extraction by reflux heating obtained the highest extraction yield, thus, the concentration of the secondary metabolites compared with the hydroethanolic maceration for the two seeds. However, a high amount of (total phenol and flavonoid contents) was observed in the *Pimpinella anisum* extracts compared with *Nigella sativa* extracts ( $29.74 \pm 0.51$  and  $37.6 \pm 0.7$   $\mu\text{g E /mg extract}$ ). Even though, we recorded a moderate antioxidant activity in all various extracts studied. The *Pimpinella anisum L.* extracts exhibited the highest antioxidant capacities than the *Nigella sativa L.* extracts with average values (2 and 1.71  $\mu\text{g/ml}$  for aqueous and hydroethanolic).

**Conclusions:** Our study provides basic information on chemical composition and the presence of bioactive and antioxidant capacity in the two seeds *nigella* and *anise* extracts which conferring their several biological properties.

## 1. Introduction

Medicinal plants represent the most ancient form of medication, used for thousands of years in traditional medicine in many countries worldwide. The empirical knowledge about their beneficial effects was transmitted over the centuries within

human communities [1]. Natural products play a pivotal role as a source of drug compounds and, currently, several modern drugs which are derived from traditional herbal medicine are used in modern pharmacotherapy [2].



*Nigella sativa* L. (NS) is commonly known as black cumin, a plant that belongs to the Ranunculaceae plant family and has been historically known as a miracle herb. However, it has an extensive history of medicinal use that dates back thousands of years as a spice and food preservative. It was used in traditional medicine by physicians to treat many kinds of illnesses [3]. Many clinical and experimental studies have demonstrated the therapeutic effects of NS extracts, including immunomodulative, anti-inflammatory, anti-tumor, anti-diabetic, and anti-cardiovascular diseases[4], it plays a significant role in gastrointestinal systems[5].

It is well known that the seeds of *Nigella sativa* L. mainly contain essential nutritional substances rich in nutrients including proteins 80%; 38% fat and 30% total carbohydrates. They also contain beneficial compounds such as vitamins such as A, B, C and E [6], and biological substances such as flavonoids, phenolic acids, tannins, terpenes and major substances called thymoquinone and nigericin, which are potential molecules with anti-inflammatory and antihypertensive properties and increase antihypertensive effects [7,8,9].

*Pimpinellaanisum*L. (PA) (Common names aniseed or anise) is an annual herbaceous plant from the Apiacea family. Various scientific reports have reported that PA has multiple therapeutic properties, such as digestive, gynaecological, neurological, and respiratory disorders [10]. Anise seeds provide essential oils (2 to 6%), which have considerable amounts of phenolic compounds, flavonoids and glycosides. We also find fatty acids, in particular petroselinic acid, up to (9.82 to 11.60%) [11], its molecules exhibit antibacterial, anti-inflammatory effects, analgesics, antioxidants and sedatives [12].

## 2. Objectives

The present study aims to determine the chemical compounds of two seeds of medicinal plants (NS and PA), to evaluate the total phenolics, flavonoids and tannins contents in different extracts prepared from these seeds (aqueous and hydroethanolic extracts) and compare the radical scavenging activity using 2,2 diphenyl-1-picrylhydrazyl (DPPH) free radical.

## 3. Methods

### Plants material

The seeds of black cumin and green anise, imported from Turkey and available in the Algerian market throughout the year, the powder was collected in separate glass jars after cleaning and grinding in an electric mill.

### Preparation of Extracts

For water extraction; 10g of seed powder dissolved in 100 ml of distilled water was stirred for 10 minutes using a magnetic stirrer. The mixture was heated under reflux for 1 hour in a flask at 70°C. The flask was fitted to a cooler where the vapours condensed and fell back into the cooler. After cold filtration, this filtrate was evaporated to dryness under reduced pressure at 65°C using a rotary evaporator at 45°C [13].

For ethanol extraction, 10g samples were macerated in a mixture of ethanol and water (7:3 v/v) at a 1:10 (w/v) ratio with gentle stirring for 24 hours at room temperature. The obtained extract was filtered over Whatman (No. 04) paper, and the ethanol was removed by evaporation under reduced pressure in a rotavapor (BÜCHI)[14]. Both extracts were placed in a plastic bottle, and then stored at -20 C until used.

### Physico-chemical Analyses

#### Determination of Moisture Content (water content)

The plant material was dried at  $103 \pm 2^\circ\text{C}$  temperature in an isothermal oven ventilated at atmospheric pressure until a constant measurement is achieved. The moisture content is determined by calculating the difference between the weight of the sample before and after drying to a constant weight [15].

#### Ash content

The mineral content is conventionally the residue of the substance after mineralization of the sample dry matter in a muffle furnace. It is obtained by incineration at 500-550 C for 30 minutes [16].



## Primary metabolites

### Fat content

The determination of the crude lipid content of the sample was performed by the Soxhlet method [17]. Twenty grams of the powder obtained were extracted for two hours in a Soxhlet extractor using hexane (100 ml) at 60°C as the extraction solvent. After evaporation of the solvent under reduced pressure using a rotary evaporator at 40-60°C.

### Determination of total carbohydrates

The determination is conducted according to the method described by [18], using the phenol test. Place 1 ml of the total sugar extract from the seeds in a test tube. Then add 1 ml of 5% phenol solution and 5 ml of sulfuric acid. Stir the reaction medium and allow it to cool to room temperature. Next, place the test tubes in a water bath at 25°C for 15 minutes and measure the absorbance at 485 nm. A calibration curve ( $y=0.003x+1.535$ ) ( $r^2=0.924$ ) was created for different glucose concentrations.

### Determination of proteins

Protein concentration in extracts from NS and PA was determined using the Bradford method [19] and compared to a Bovine Serum Albumin (BSA) calibration range

$$(y = 0.001x - 0.083)(r^2=0.998)$$

## Secondary metabolite

### Total polyphenol content

The total phenolic content (CPT) of NS and PA extracts was determined using the Folin-Ciocalteu method described by [20]. To 200 µL of the diluted sample extract, 1 ml of the Folin-Ciocalteu reagent (diluted 10 times) was mixed. The mixture was incubated for 6 min, then 0.8 ml of a 7.5% aqueous Na<sub>2</sub>CO<sub>3</sub> solution was added. After 90 min incubation, the absorption was measured at 760 nm against water as a blank. The amount of total phenolic was expressed as micrograms of Gallic acid equivalent per milligram of extract (µg EAG/mg) through the calibration curve of Gallic

acid, an equation obtained from the standard Gallic acid graph:

$$(y = 0.023x - 0.041)(r^2=0.999)$$

### Total flavonoid content

The total flavonoid content (FT) of extracts was assessed according to the method described by [21]. Approximately 250 µl of different extracts of NS and PA diluted was mixed with 75 µl of a NaNO<sub>2</sub> solution (5%) and after 5 minutes 75 µl of (AlCl<sub>3</sub>) (10%) was added. After 6 minutes, 500 µl of NaOH (1N) and 2.5 ml of distilled water were mixed. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 510 nm and the total flavonoid content was expressed in micrograms of Catechin equivalent per milligram of extract (µg CE /mg), an equation obtained from the standard Catechin graph:

$$(y = 0.006x - 0.035)(r^2=0.999)$$

### Total condensed tannin

The total proanthocyanidin content (TC) was determined in the extracts assay reported by [22]. A volume of 50 µl of crude extracts was mixed with 1500 µl of 4% (w/v) vanillin/ methanol solution and 750 µL of hydrochloric acid (HCl). The resulting mixture was vortexed and allowed to react for 20 min at room temperature. Absorbance was measured at 550 nm against a blank. A calibration curve was plotted in parallel under the same operating conditions using Catechin as a positive control. The TC in the extract is expressed as micrograms equivalents of Catechin per milligram of extract (µg CE/ mg), an equation obtained from the standard Catechin graph:

$$(y = 0.001x - 0.114)(r^2=0.991)$$

### Total antioxidant activity determination

The free radical scavenging activity of NS and PA extracts was measured by the 1,1-diphenyl-2-picrylhydrazil (DPPH.) method proposed by Blois [23]. Briefly, a volume of 50 µl of each extract at different concentrations is added to 1.950 ml of a freshly prepared DPPH methanolic solution (0.025



g/l). The negative control was prepared in parallel by mixing 50  $\mu$ l of methanol with 1.950 ml of the DPPH methanolic solution at the same concentration used. Following a 30-minute incubation period in the dark at room temperature, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The DPPH concentration in the reaction medium was calculated from the following calibration curve, determined by linear regression of ascorbic acid ( $y = 0.095x + 46.73$ ) ( $r^2=0.994$ ), and the percentage inhibition calculated according to the equation:

$$\%I = [(Abs\ control - Abs\ test) / Abs\ control] \times 100.$$

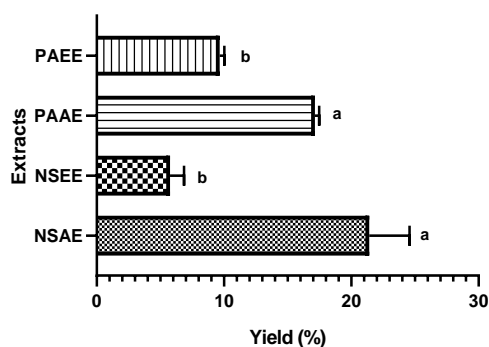
#### Statistical analysis

All results were mentioned as mean  $\pm$  S.D of three replications. The statistical analysis was carried out using the ANOVA test followed by the Tukey post hoc test and the data were analyzed by Graph Pad prisme 8 software.  $P$ -values  $< 0.05$  were regarded as significant and  $P$  values  $< 0.01$  as very significant.

## 4. Results

### Extraction yield

The percentage yield of extracts was expressed as the weight of the extract relative to the weight of the starting plant material. The extraction yield of aqueous and ethanolic extracts of *Nigella sativa* L. (NSAE, NSEE) and *Pimpinella anisum* L. (PAAE, PAEE) is presented in Figure 1.



**Figure 1.** Extraction yield (%) of aqueous and

### ethanolic extracts of *Nigella sativa* L and *Pimpinella anisum* L.

**NSAE:** *Nigella sativa* (Aqueous Extract), **NSEE:** *Nigella sativa* (Ethanolic Extract), **PAAE:** *Pimpinella anisum* (Aqueous Extract), **PAEE:** *Pimpinella anisum* (Ethanolic Extract).

Values are expressed as mean  $\pm$  SD ( $n=3$ ). Columns with different letters indicate a significant difference  $P < 0.05$

Extraction by ethanol and water showed a significant difference  $P < 0.0001$  in extraction yield, aqueous extraction by reflux heating was more significant than the ethanolic maceration for the two seeds, with a rate of ( $21.41\% \pm 3.15$  and  $17.15\% \pm 0.33$ ) for NS and PA seeds respectively. However, hydro-ethanolic extraction for PA seeds represents a higher yield than that for NS seeds.

### Physico-chemical characteristics

The results drafted in Table 1 represent the chemical composition of NS and PA powders.

**Table 1.** Physico-chemical parameters in (%) of NS and PA powders.

Values are expressed as mean  $\pm$  SD of three replicate

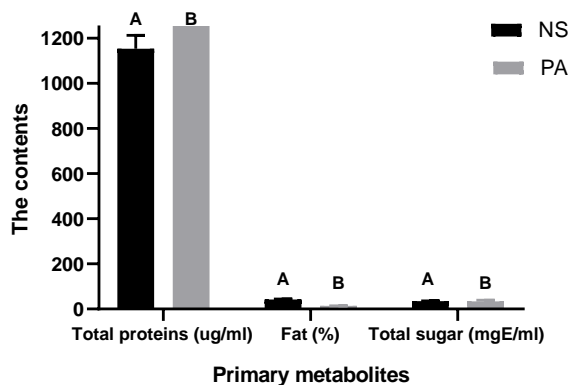
Parameters	<i>Nigella sativa</i> . L(NS)	<i>Pimpinella anisum</i> .L (PA)
Water content	$5.02 \pm 0.04$	$8.41 \pm 0.27$
Ash	$94.97 \pm 0.04$	$91.67 \pm 0.35$
Organic matter	$95.99 \pm 0.12$	$92.42 \pm 0.066$
Inorganic matter	$3.94 \pm 0.17$	$7.58 \pm 0.068$

A comparison of chemical content of NS and PA seed powders showed humidity levels of the order of ( $5.03\% \pm 0.04$  and  $8.42\% \pm 0.27$ ) for NS and PA seeds powders respectively.

We recorded an organic matter level was slightly elevated in NS seeds ( $95.99 \pm 0.16\%$ ) compared to PA seeds ( $92.42 \pm 0.09\%$ ).

### Primary metabolites content

Comparative analysis of primary metabolites profiles in different seeds is represented in Figure 2.



**Figure 2. Primary metabolite content in NS and PA seeds.**

Values are expressed as mean  $\pm$  SD ( $n=3$ ). Columns with different letters indicate a significant difference  $P<0.05$ .

The results showed a significant difference  $P<0.0001$  in the fat percentage between the NS ( $42.64\% \pm 1.6$ ) and the PA seeds ( $13.55\% \pm 0.74$ ). Thus, a significant difference  $P<0.0092$  was found in the total sugar content, we recorded the following values;  $35.02 \pm 0.75$  mgE/ml and  $37.46 \pm 0.48$  mgE/ml for NS and PA seeds respectively. While, a significant difference  $P \leq 0.0034$  was recorded in the total proteins ( $1154 \pm 59.1$  ug/ml and  $1367 \pm 3$  ug/ml) for PA and NS seeds.

### Secondary metabolites (polyphenols, flavonoids and tannins)

The concentration of phenols, flavonoids and condensed tannins in the various extracts of NS and PA seeds are depicted in Table 2.

**Table 2. Total phenolic content (CPT), total flavonoid content (FT), and tannins condensed (TC) in NS and PA extracts.**

NSAE: *Nigella sativa* (aqueous Extract), NSEE: *Nigella sativa* (Ethanolic Extract), PAAE: *Pimpinella anisum* (Aqueous Extract), PAEE: *Pimpinella anisum* (Ethanolic Extract).

Values are expressed as mean  $\pm$  SD of three replicate

The secondary metabolites (CPT, FT and TC) were significantly different  $P<0.0001$  between ethanolic and aqueous extracts in the both seeds. The result revealed that the aqueous extraction by reflux heating obtained the highest concentration

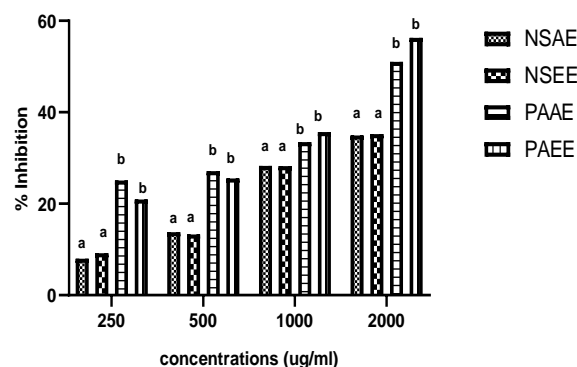
of the secondary metabolite compared with the ethanol maceration for the two seeds, However, a high amount of (CPT and FT) was observed in the PA extracts compared with NS extracts.

Concerning the condensed tannins content, the values recorded show that the aqueous extract of NS was significantly  $P<0.05$  richer on TC ( $36.66 \pm 0.64$   $\mu$ g CE/mg) compared to the PA aqueous extract ( $26.2 \pm 0.947$   $\mu$ g CE/mg).

The results for CPT and FT in the different extracts studied show that the extracts (aqueous and ethanolic) of green anise have high values compared to those of black cumin.

### Antioxidant activity results

The results of the antioxidant effect of PA and NS ethanolic and aqueous extracts values are the mean  $\pm$  SD, columns with different letters indicate a significant difference  $P<0.05$  according to the Tukey test. The results of the antioxidant activity using DPPH free radical test are shown in the Figure 3.



**Figure 3. Inhibition percentage (%) of different extracts of NS and PA seeds.**

NSAE: *Nigella sativa* (Aqueous Extract), NSEE: *Nigella sativa* (Ethanolic Extract), PAAE: *Pimpinella anisum* (Aqueous Extract), PAEE:

	NSAE	NSEE	PAAE	PAEE
CPT ( $\mu$ g EAG/mg)	18,98 $\pm$ 1,84	13,87 $\pm$ 0,59	29,74 $\pm$ 0,51	21,01 $\pm$ 0,81
FT ( $\mu$ g CE /mg)	19,2 $\pm$ 0,86	13,62 $\pm$ 1,34	37,6 $\pm$ 0,7	30,64 $\pm$ 0,09
TC ( $\mu$ g CE /mg)	36,66 $\pm$ 0,58	17,01 $\pm$ 0,34	26,33 $\pm$ 0,47	18,66 $\pm$ 0,07



*Pimpinella anisum* (Ethanloic Extract).

Values are expressed as mean  $\pm$ SD ( $n=3$ ). For each concentration, columns with different letters indicate a significant difference  $P<0.05$ .

According to the ANOVA test, a significant difference was found between all the doses of the two extracts of the two seeds ( $P=0.0007$ ). The inhibition percentage depended the extract type, the seeds and the doses. The PA extracts was found to be more effective than the NS extracts, if we put in order the antioxidant effect, we obtain the following order: PAEE >PAAE >NSEE >NSAE.

Comparing the IC<sub>50</sub> values of aqueous and ethanolic extracts for each plant (Table 3), we found little difference between the results of the two extraction techniques, reflux heating, and the widely used maceration.

**Table 3. The IC<sub>50</sub> of different extracts of NS and PA and Ascorbic Acid.**

	NSAE	NSEE	PAAE	PAEE	AA
<b>IC<sub>50</sub> (mg/ml)</b>	3	2.91	2	1.71	0.034

NSAE: *Nigellasativa* (aqueous Extract), NSEE: *Nigellasativa* (Ethanolic Extract), PAAE: *Pimpinella anisum* (Aqueous Extract), PAEE: *Pimpinella anisum* (Ethanolic Extract), AA: (Ascorbic Acid)

## 5. Discussion

The main objective of this work is to quantify the bioactive compounds in the different powders and extracts of the two seeds *Nigella sativa* L. and *Pimpinella anisum* L.

Our results showed that the highest yield was obtained by aqueous extraction using reflux heating. Comparing with the study of Mezitiet al. [14], who reported a yield of 2.88% obtained by methanolic extraction of NS and which is lower than the one obtained in our study. Regarding the PA extract, our results are lower than that obtained in the work of Bekara et al [24], who found an aqueous extraction yield by a decoction of 20.99%. The yield is the relative parameter and depends on the plant species studied, the drying and

storage conditions, the content of each species in secondary metabolites and the nature of the solvent used in the plant extraction as well as the extraction method itself [10].

On the other hand, our results are concurring with those obtained by Benazzouz-Smail et al [25], who revealed an amount of 4.71% and 4.16% for moisture and Ash in NS seeds, respectively. The results of the ash content in the two seeds studied are approximately lower than of Charles [26] (7%) for PA seed. The relative proportions of chemical content in seeds can widely depending on the species, cultivar and environmental conditions during seed development.

Our finding showed that the both seeds NS and PA are rich in primary metabolites including proteins, fats and carbohydrates, a comparing with the literature result; differences were observed in the measured contents. For NS, the estimated fats content was similar to that presented by [25]. Whereas carbohydrates were higher than the values presented by Kabir et al.[27] and protein content was also relatively inferior to that reported by Nergiz and Ötleş [28]. In contrast, the results for PS are lower than those reported in the literature, which ranges from 25-30% for sugar content [29]. Conversely; they fall within the range of fixed oil and protein content rates estimated by [28]. These discrepancies in nutrient content may be due to variations in environmental factors, such as the geographic and climatic zones where the plants were grown and assay methods employed [30].

The result of secondary metabolites (CPT, FT and TC) was expressed as  $\mu\text{g E/g}$  of extract using the curve standard equation in each parameter for different extract of NS and PA. Our findings revealed that the aqueous extraction yielded the highest concentrations of CPT, FT and TC for both seeds. However, comparing the individual plant extracts, the green anise (PA) extract showed significantly higher concentrations of CPT and FT than the black cumin extract, this is the contrasts with what was found for TC content.

The values for total polyphenols in the aqueous and hydro-ethanolic extracts of PA seeds were (29.01 and 21.74  $\mu\text{g EAG/mg}$ ) respectively. Our findings were in agreement with the result found by



Womeni[31]. However, the content of total polyphenols and flavonoids and condensed tannins in the aqueous extract of this seeds is higher than those found in the literature;  $(2.38 \pm 0.01)$   $(.806 \pm 0.08)$   $(0.40 \pm 0.07)$  ( $\mu\text{g E/mg}$ ) respectively [24]. In addition, the majority of studies conducted on NS have focused on the essential oils of the seeds. Our results revealed a high abundance of secondary metabolites in the aqueous extract, with the following values:  $18.98 \pm 1.84$   $\mu\text{g EAG/mg}$  of total polyphenols,  $19.2 \pm 0.86$   $\mu\text{g EC/mg}$  of flavonoids. These values are lower than those reported by [14] found a total polyphenol content of  $27.07 \pm 0.58$   $\mu\text{g EAG/mg}$  in the aqueous extract of NS. Additionally, Talbi et al., [32] reported flavonoid contents of  $266 \pm 43.3$   $\mu\text{g/mg}$  and  $169 \pm 5.78$   $\mu\text{g/mg}$  in the ethanolic and aqueous extracts, respectively, for the same plant. The condensed tannin contents recorded as Catechin equivalents in  $\mu\text{g per mg}$  extract, show proportions of 17 and  $36.66$   $\mu\text{g/mg}$  for ethanolic and aqueous extracts of NS, respectively. These values are higher compared to those obtained by [25] ( $2.13$   $\mu\text{g/mg}$ ) for the ethanolic extract of NS. The different extraction methods used and the solvents used to affect the phenolic contents and make the data difficult to compare [33]. Our results appear relatively high when compared to those obtained by the authors mentioned above. It is necessary to consider that the secondary metabolite content also varies according to several parameters such as geographical location, soil type and harvest period [34].

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical is a stable radical with a maximum absorbance at 517 nm, that can be readily easily reduced by an antioxidant. Because of the simplicity and convenience of this reaction, it is now widely used in assessing free radical scavenging activity [35].

The antioxidant potential of *Nigella sativa* and *Pimpinella anisum* extracts was evaluated by calculating the inhibition percentages then estimated the inhibitory concentration 50 ( $\text{IC}_{50}$ ). The antioxidant power recorded for the hydro-alcoholic extract of NS ( $2.91$   $\text{mg/ml}$ ) is lower than the results found by (Meziti et al. [14]) for ethyl acetate fractions of NS with  $\text{IC}_{50}$  values equal to  $1.21$   $\text{mg/ml}$  as reported by [32] with  $\text{IC}_{50}$  of  $0.64$   $\text{mg/ml}$  for an ethanolic extract of NS.

Regarding the reducing power of PA seeds, the findings indicate that this plant exhibits a comparatively diminished capacity, particularly in comparison to ascorbic acid (AA). These results are consistent with those previously reported by [10]. The results of Lamiaa et al. [36] on a methanolic extract of fresh green anise indicate an  $\text{IC}_{50}$  value of  $0.648$   $\mu\text{g/ml}$ , which is higher than that observed in the present study on the ethanolic extract of green anise ( $1.71$   $\mu\text{g/ml}$ ).

Several researchers have demonstrated that this activity is predominantly associated with the composition of the extracts and their phenolic and flavonoid content. For instance, the work of [37] reported the anti-free radical activity of green anise at 78.98% at a concentration of 2  $\text{mg/ml}$ , which is higher than that observed in hydroethanolic extracts of green anise (PAEE).

## 6. CONCLUSION

The results of the present study showed the richness of NS and PA seeds by primary metabolites such as proteins, sugar and fats, which have a nutritional values. Thus, the extracts of the two studied seeds obtained by ethanolic maceration and reflux heating using water contained secondary metabolites. We recorded that the reflux heating was the best method of extraction. In addition, in vitro antioxidant ability assessed by DPPH radical scavenging test showed that the all extracts exhibited considerable antioxidant activity. These seeds could have extra nutritional properties and a central role in diet disease relationships. Further studies are required to disclose possible new bioactive constituents and research for their effect on treat digestive disorders.

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