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Original Article

Comparative and Evaluative Study On Total Phenolic Content and Antioxidant Potential Within Hydromythanol Extracts and Their Fractions from All Parts of *Drimia numidica* (JORD. & FOURR.) J.C. MANNING & GOLDBLATT of Northeastern Algeria

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ABSTRACT

Introduction: *Drimia numidica* is a polyphenol-rich species that has antioxidant capacity, which makes it very important. Nevertheless, *D. numidica* has not been studied in depth. Moreover, its capsules have not been studied so far. This detailed study compared the values of total phenolic content and antioxidant activity of all *D. numidica* parts (scapes, flowers, bulbs, capsules, leaves, roots), from Edough Peninsula, Annaba, Algeria, using hydromethanol extracts and their fractions (cyclohexane, chloroform, ethyl acetate, and n-butanol). **Methods:** The total phenolic content was determined by Folin Ciocalteo assay, and the antioxidant activity by the following methods: DPPH, Reducing Powder, Phenanthroline, Silver Nanoparticles, CUPRAC, and ABTS. Statistical analysis was done using the ANOVA test and a correlation test (between antioxidant activity and total phenolic content). **Results:** The Ethyl acetate fraction was found the sample with the highest phenolic content. The same was true for the antioxidant activity in all tests except for the silver nanoparticles test in which cyclohexane extracts scored the best. Considering the methods used in general, there is a correlation between phenolic content and the highest antioxidant activity, but there are some methods that have not recorded any correlation. **Conclusion:** Choosing the appropriate method or extract/fraction type is extremely important. In addition, extracts of *D. numidica* parts could be an important natural alternative to antioxidants industrial.

Key words: *Drimia numidica,* Total Phenolic content, Antioxidant activity, Hydromythanol extracts and their fractions.

INTRODUCTION

Aerobic organisms need oxygen for oxidative metabolism, which causes the production of ROS (reactive oxygen species). Therefore, makes these organisms susceptible to oxidative stress, which causes many problems, especially those related to humans, such as Alzheimer's disease, premature aging, diabetes, cancer and others. This severe damage has made the study of antioxidants very important owing to their decisive and effective ability to combat oxidative stress.[1,2] Furthermore, antioxidants have the ability to save food from spoilage and rotting, which makes them important in the fight against economic losses, especially since half of the world's fruit and vegetable crops are lost after harvest.[3]

According to their source, antioxidants can be classified into natural and synthetic. The latter are most often used in the food industry and are included in the human diet,[3,4] for their cheap price and easy availability.[5] However, the global trend nowadays tends to abandon them due to information about their toxicity, which causes many diseases, including cancer.[6,7]

The growing demands of consumers for safer products that are free from artificial additives make natural antioxidants the subject of numerous studies.[6,7] In addition, it is important for experts, medical professionals, researchers, and all society layers to know the antioxidants power.[8] For this, a large number of different methods have been developed and attempts have been made to propose unified methods for determining the antioxidant capacity.[1] However, there are no reliable and validated tests to measure the antioxidant capacity of biological and food samples, and relying on a single test to measure the total antioxidant capacity is unrealistic, since no test reflects the total antioxidant capacity of the sample.[8]

Medicinal plants and their extracts are mostly rich in polyphenol contents which are powerful antioxidants. Recently, a large number of pure studies have been interested in its antioxidant properties mainly due to polyphenol compounds.[9]

Drimia term (genus, with bulbous Plants) was first used by Jacquie in 1797,[10,11] derived from the Greek word drimys (acrid, prickly),[12] due to its itching and inflammation caused by potassium oxalate, which is found in the leaves and bulbs of their plants.[13]

According to the classification of APG3, *Drimia* genus (geophytes plants) belongs to the Urgieeae tribe and scilloideae subfamily of the asparagaceae family, whereas it was previously (in APG2) classified in urgieoidacea subfamily of the hiacintaceae family.[14]

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Drimia maritima plant (syn: *scilla maritima*, *urginea scilla*) is one of *Drimia* species.[15] It is perennial, and native to the Mediterranean region[16] and generally grows on hill slopes and sandy lands near the Mediterranean Sea.[17]

Drimia numidica (*Squilla numidica*, *Charybdis numidica*, *Squilla numidica*, *Urginea numidica*, *Urginea maritima var. numidica*) with red bulb color[18,19] is a plant species belonging to the *D. Maritima* aggregate (which includes a minimum six plant species). It can be found in the northern and southern Mediterranean regions.[20] *D. numidica* was described as follows: its leaves are 5-15 cm wide and up to 1 m long lanceolate, multinervis with transverse anastomoses and smooth margins. It has a huge bulb reaching almost 20 cm in diameter (and at least 5 cm). It is characterized by a very robust flowering scape, at least 40 cm long and up to 1.50 m, 4-15 mm in diameter. It has a cluster reaching 60 cm in length, 6-8 mm long white star-shaped flowers, green single-veined tepals, and Stamens with white filament. It is characterized by a green ovary and capsule ovate-obtuse seeds subacute at both ends.[21]

In the process of searching for a natural source of antioxidants and polyphenol compounds as an alternative to synthetic ones, we chose the *D. numidica* species because to the best knowledge of the authors there is a lack of studies that were conducted on the subject. Its large dimensions and common availability[21] makes easy obtaining extracts in very large quantities without significant costs. This paper compared and measured the Antioxidant (AOX) capacity (applying six different methods) and total phenolic content (TPC), based on hydromethanol extracts and their fractions of the six parts of the *D. numidica* plant.

MATERIALS AND METHODS

Plant material

D. numidica grows in Annaba (Algeria) and extends to Tunisia.[22] *D. numidica* parts were collected during two seasons: autumn (aireal parts, October 2021) and winter (underground parts, February 2022) from Edough peninsula, Annaba, Algeria, Alt. 69 m, 36°49'56.065"N, 7°42'2.518"E. The plant was identified and cleaned in the Laboratory of Plant Biology and Environment, BADJI Mokhtar University, Annaba, Algeria. The parts were separated and dried by exposing them to direct air under a shaded place (leaves, scapes and bulbs are sheared before being dried). After that, they have been grinded with a Lab Blender (we used a Lab sieve with a 1,25 mm diameter).

Preparation extracts

Solid-liquid extraction: For each part, by maceration method, the hydromethanol extract was prepared by soaking the powder of *D.numidica* part (about 200g) in a methanol-water solvent mixture (70:30 v/v) during three days at room temperature then filtered the separated residue to get a filtrate. In a same way re-extracted the residue during two and one day, the filtrate of each period was combined. Rotary evaporator was used (40°C temperature) to obtain a pure hydromethanol extract. The latter is divided into two parts, the first one reserved for the preparation the fractions, which is the largest quantity, the second was stored for later use.

Liquid – liquid extraction: In order to prepare the fractions (cyclohexane fractions, chloroform fractions, ethyl acetate fractions, n-butanol fractions), the hydromethanol extract was dissolved in distilled water, was kept for about 24 hours. Thereafter, the liquid-liquid extraction was applied using a separatory funnel, where hydromethanol solution was mixed with one of the following solvents each time: cyclohexane, chloroform, ethyl acetate, n-butanol, from the lowest to highest polarized. Each time the mixture (hydromethanol + fraction solvent) was well shaken until combined, and left in the separatory funnel for a while to separate the organic and aqueous phases. Afterwards, the organic phase was evaporated to obtain the pure fraction (the process repeated three times for each mixture). The procedure was done for every part of the plant.

Determination of TPC

20 µl of extract solution (0.1 % w/v) was placed in a microplate, followed by 100 µl solution of diluted Folin-Ciocalteu reagent (1:10), and 75 µl of sodium carbonate solution (7,5% w/v), keeping the mixture in the dark during two hours, where the microplate reading was at 765 nm. A control was prepared in the same procedure, replacing the extract with the solvent used for dissolving the extract. The Calculation of TPC was based on a gallic acid calibration curve, and the results were expressed as (µg GAE/mg), where GAE is gallic acid equivalents.[23]

Determination of AOX activity

AOX values were determined using six different methods: DPPH and ABTS radical scavenging activities, Cupric reducing antioxidant capacity (CUPRAC), Phenanthroline activity, Reducing power (RP), silver nanoparticles (SNPs). In all tests, a control was prepared in the same way, replacing the extract with the solvent used for dissolving the extract.

A microplate reader was used for reading the results, extract solutions used at different concentrations. The DPPH and ABTS values were calculated using: %Inhibition = [(Control absorbance - Sample absorbance) / Control absorbance] × 100

DPPH test

160 µl of DPPH solution (prepared by dissolving DPPH powder in methanol and mixing them about 20 minutes in a dark room, then the solution must be balanced to be equal to 517 nm before being used) was placed in the microplate, followed by 40 µl of extract solution, read at 517 nm.[24]

ABTS test

40 µl of extract solution was placed in the microplate, followed by 160 ml of a $ABTS^{+}$ solution (prepared by mixing ABTS (0.384 % w/v) and potassium persulfate solutions (0,066 % w/v), mixed well, and let them stay 12 to 16 hours, their absorption value should be 734 nm before using it), wait 10 minutes before measuring the absorption at 734 nm.[25]

CUPRAC test

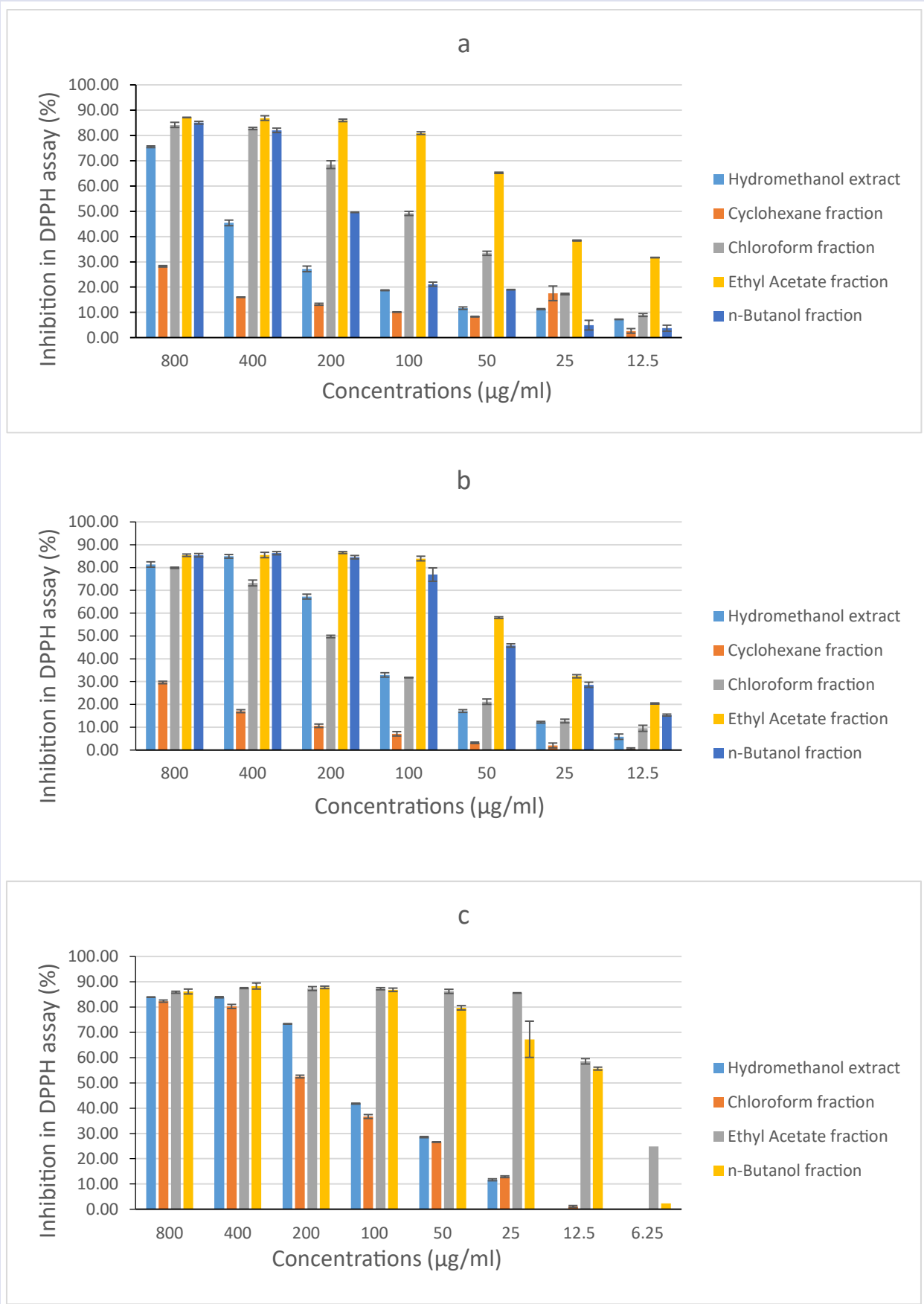
In the microplate, 40 µl of extract solution was putted, followed by 60 µl of S1 (ammonium acetate solution (7,708% w/v), 50 µl of S2 (Neocupronin solved in pure methanol (0.156% w/v)) and 50 µl of S3 (Copper (II) chloride solution (0,1705% w/v) respectively, wait one hour to read a microplate at 450 nm.[26]

Phenanthroline test

In the microplate, 10 µl of the extract solution was placed with 50 ml of Iron (III) chloride ($FeCl_3$) solution (0,2 % w/v), 30 µl of Phenanthroline solution (0,5 % w/v), and 110 µl of pure methanol. They were incubated in obscurity at 30 °C temperature during 20 minutes. The absorbance was measured at 510 nm.[27]

RP test

In the microplate, 10 µl of extraction solution was placed with 40 µl of Phosphate buffer (pH = 6,6), and 50 µl of Potassium ferricyanide solution (1% w/v), then were incubated at 50°C during 20 minutes, then added respectively 50 mL of Trichloroacetic acid solution (10% w/v), finally, putted 40 µl of H_2O and 10 µL of $FeCl_3$ (0.1 % w/v) read in 700 nm.[28]


a
Inhibition in DPPH assay (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
b
Inhibition in DPPH assay (%)
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
c
Inhibition in DPPH assay (%)
800
400
200
100
50
25
12.5
6.25
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

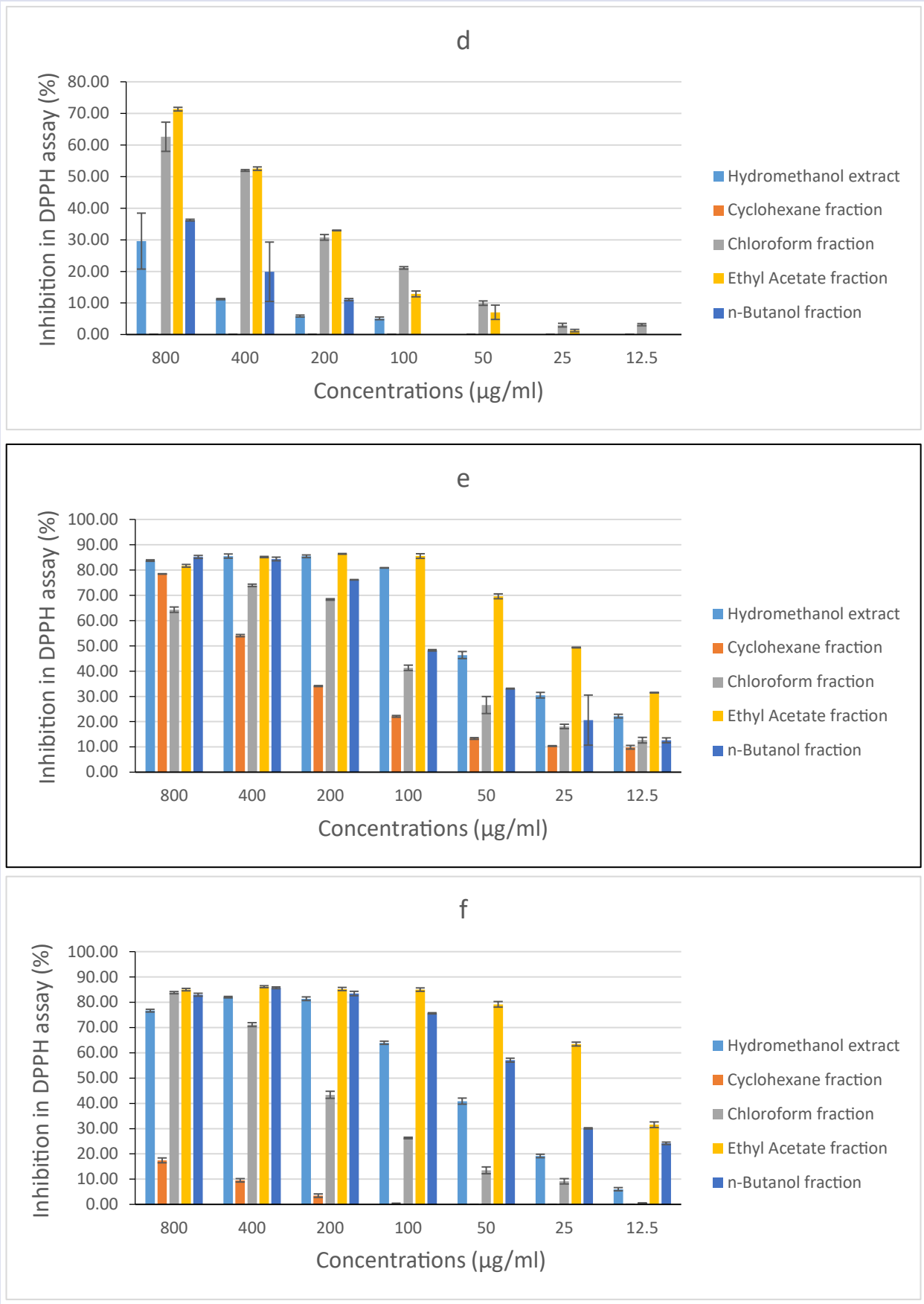
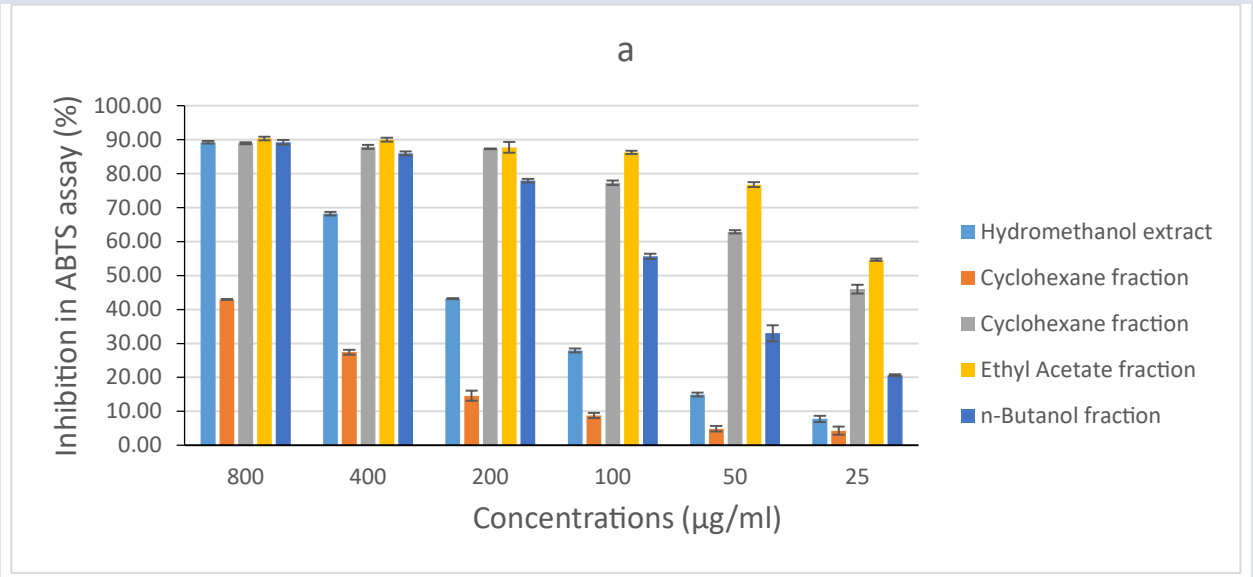
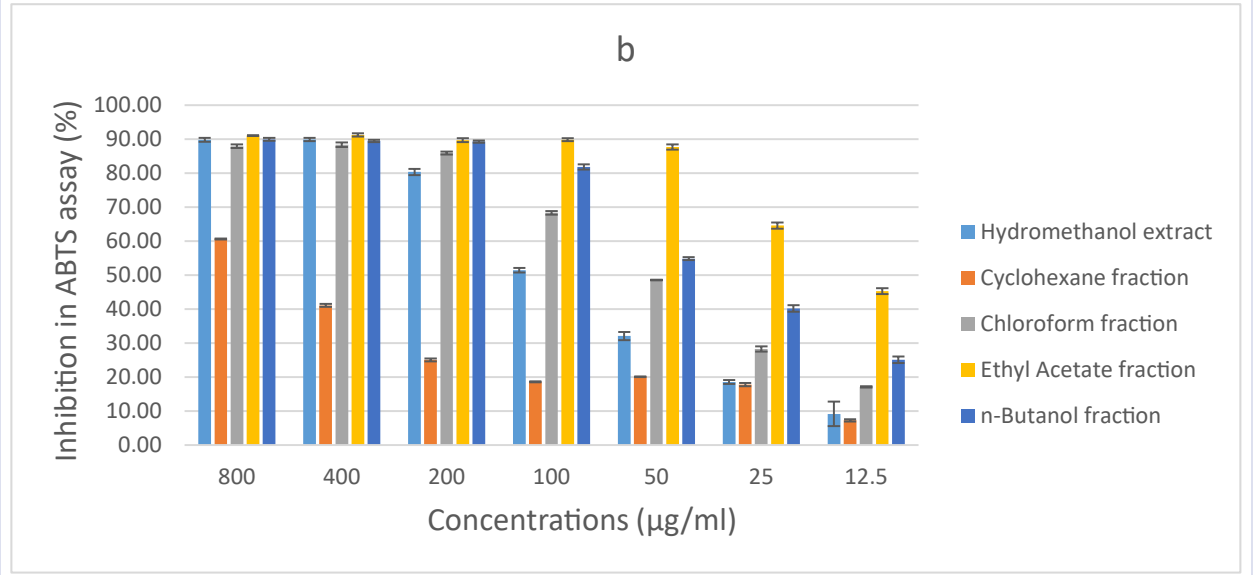
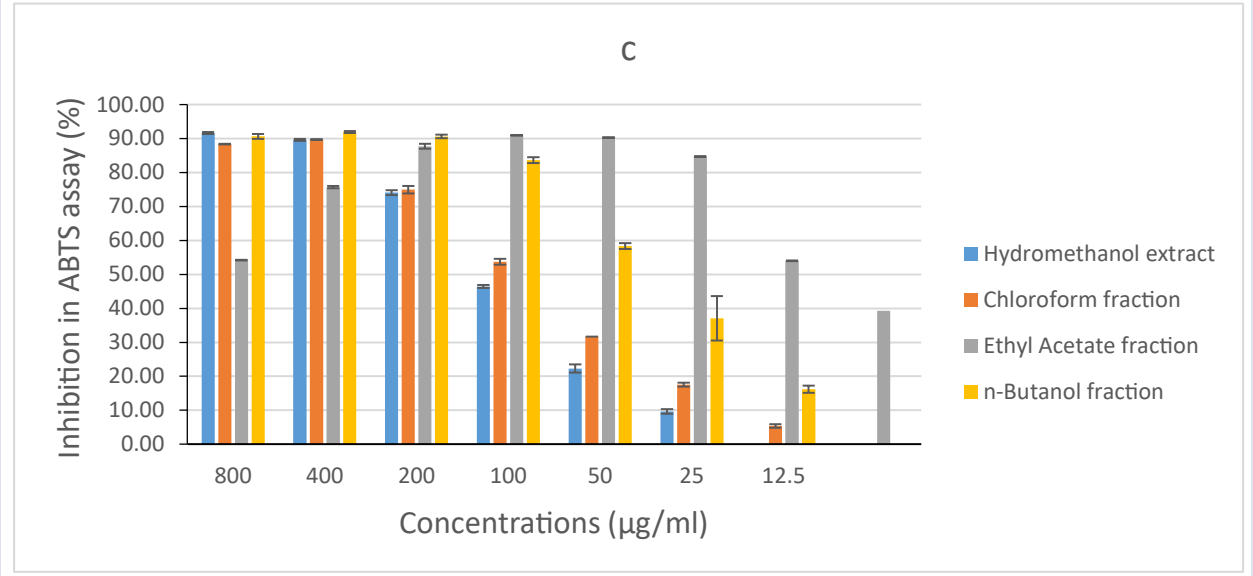


Figure 1: DPPH assay: Histograms, the antioxidant activity of hydromethanol extract and its fractions of six *Drimia numidica* parts, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.


a
Inhibition in ABTS assay (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
800
400
200
100
50
25
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Cyclohexane fraction
Ethyl Acetate fraction
n-Butanol fraction


b
Inhibition in ABTS assay (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction


c
Inhibition in ABTS assay (%)
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

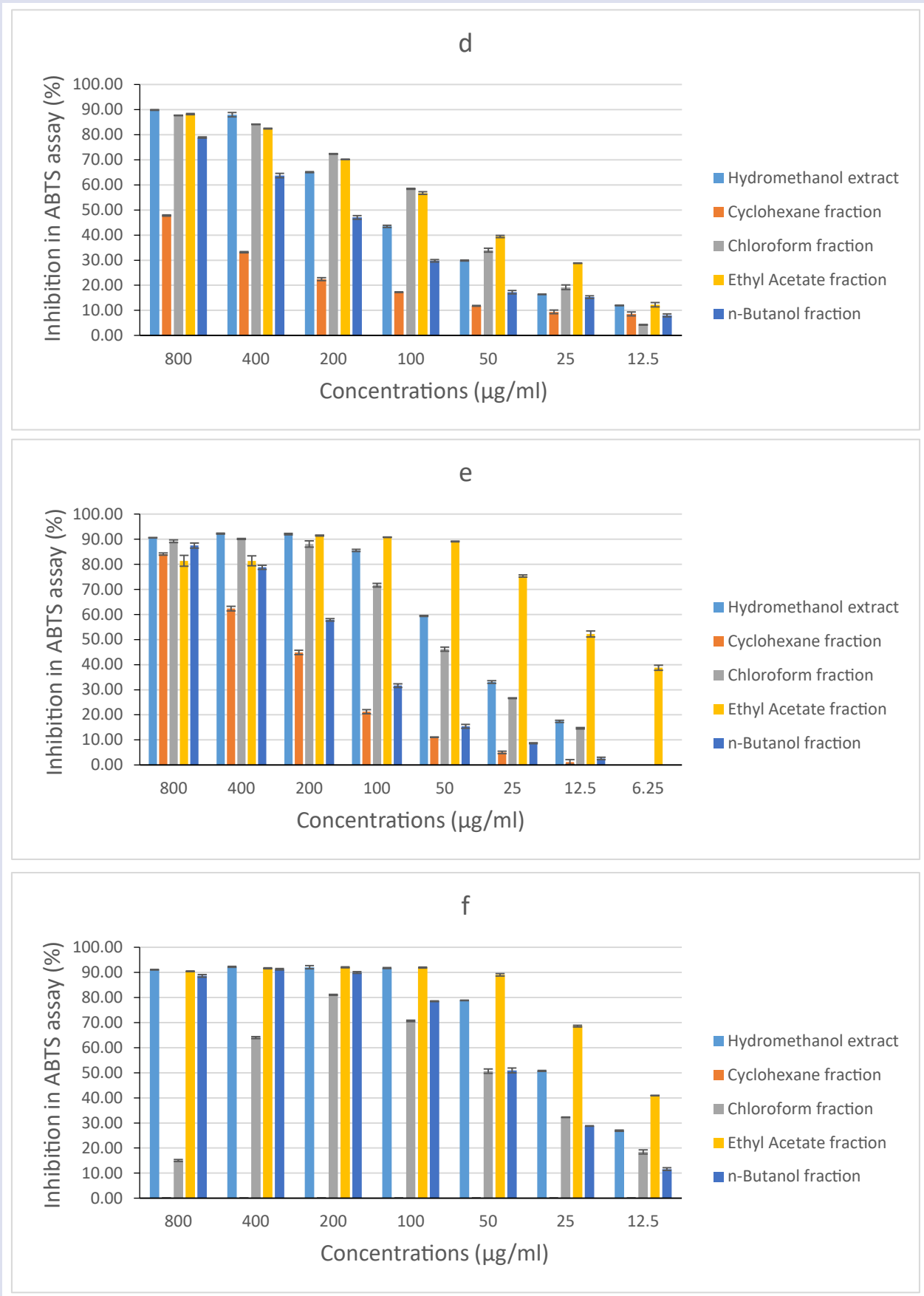
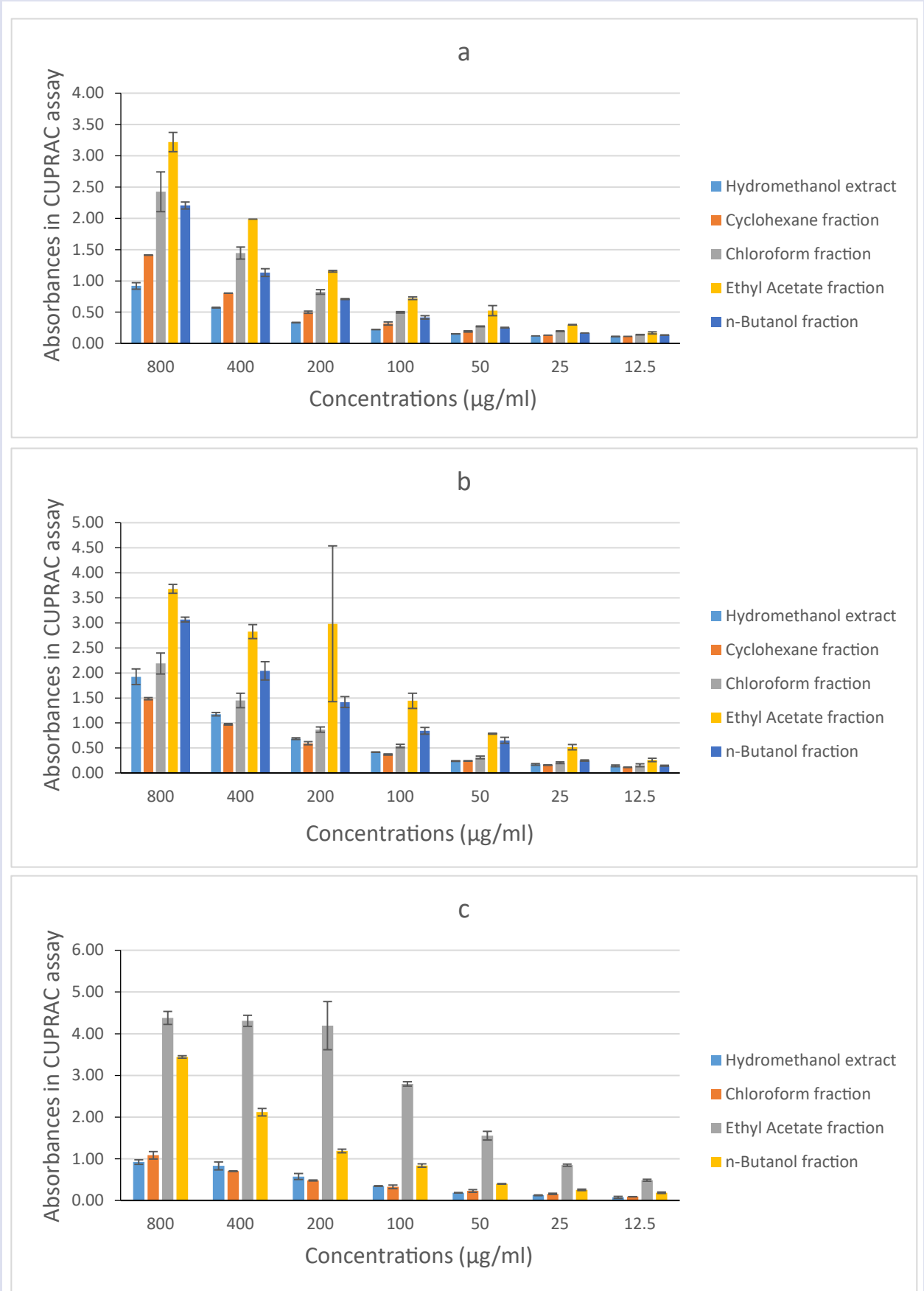


Figure 2: ABTS assay: Histograms, the antioxidant activity of hydromethanol extract and its fractions of six parts of *Drimia numidica* plant, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.


a
Absorbances in CUPRAC assay
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
b
Absorbances in CUPRAC assay
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
c
Absorbances in CUPRAC assay
6.00
5.00
4.00
3.00
2.00
1.00
0.00
800
400
200
100
50
25
12.5
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

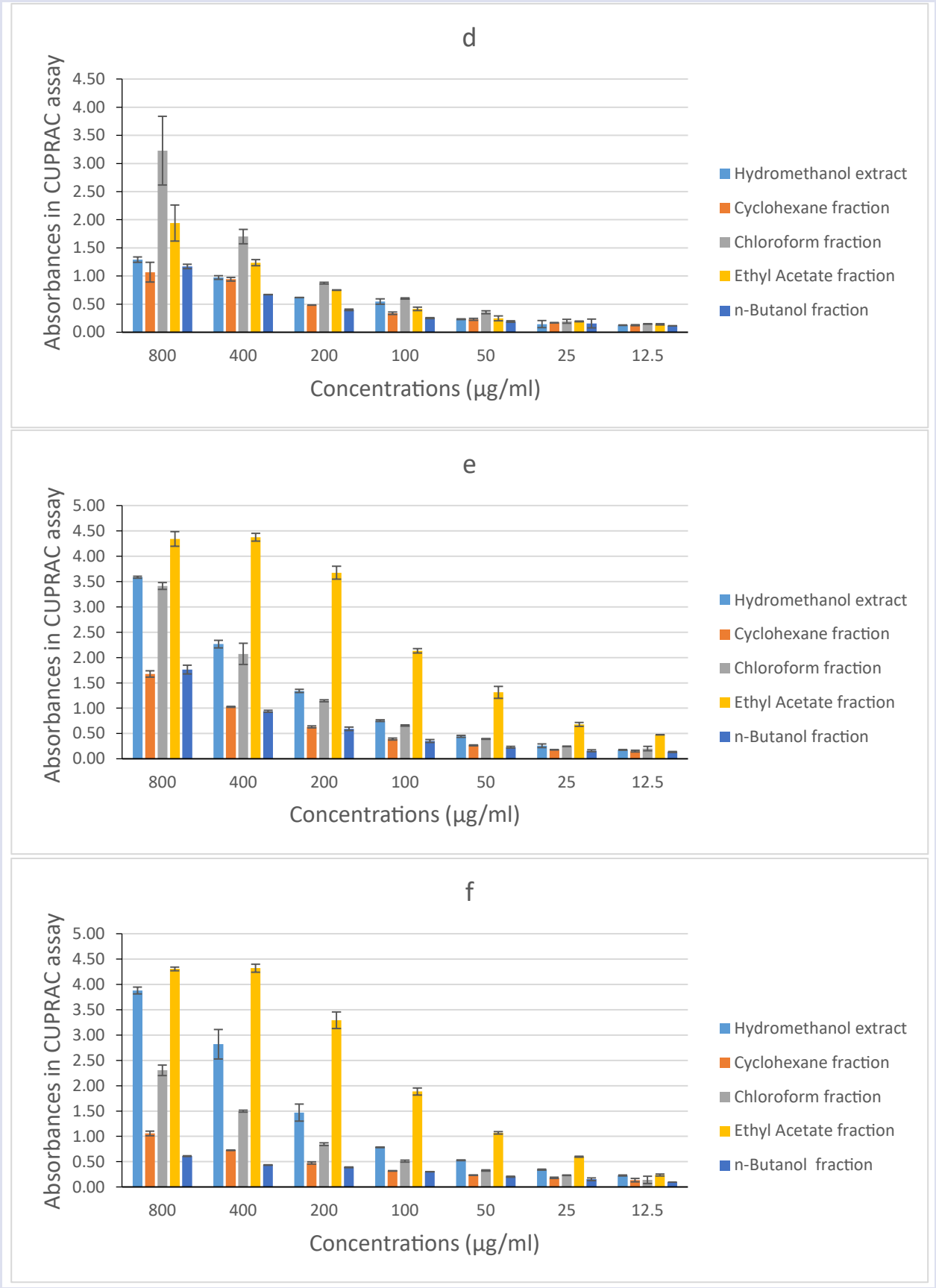
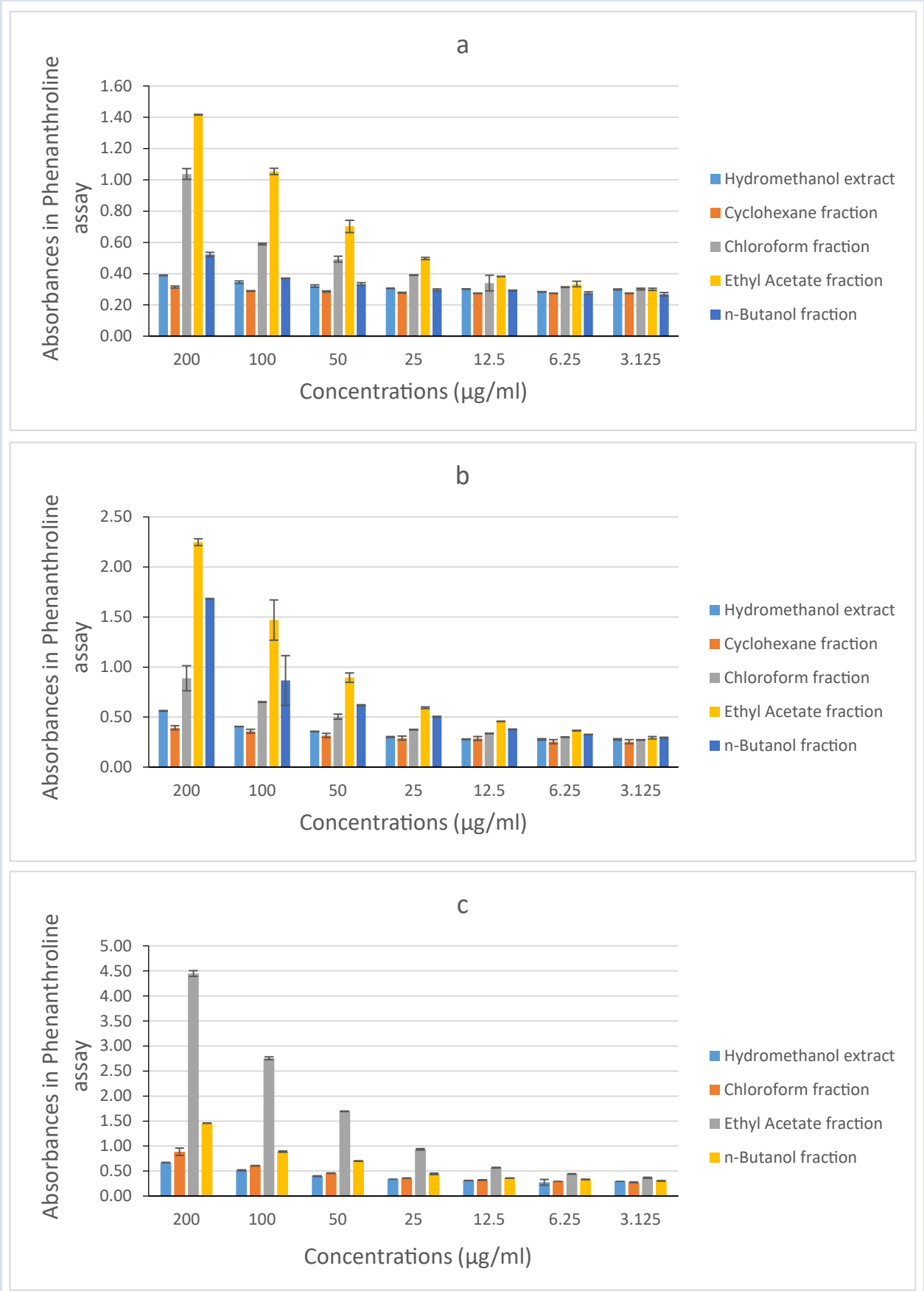


Figure 3: CUPRUC assay: Histograms, the antioxidant activity of hydromethanol extract and its fractions of six *Drimia numidica* parts, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.


a
Absorbances in Phenanthroline assay
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
b
Absorbances in Phenanthroline assay
2.50
2.00
1.50
1.00
0.50
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
c
Absorbances in Phenanthroline assay
5.00
4.50
4.00
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

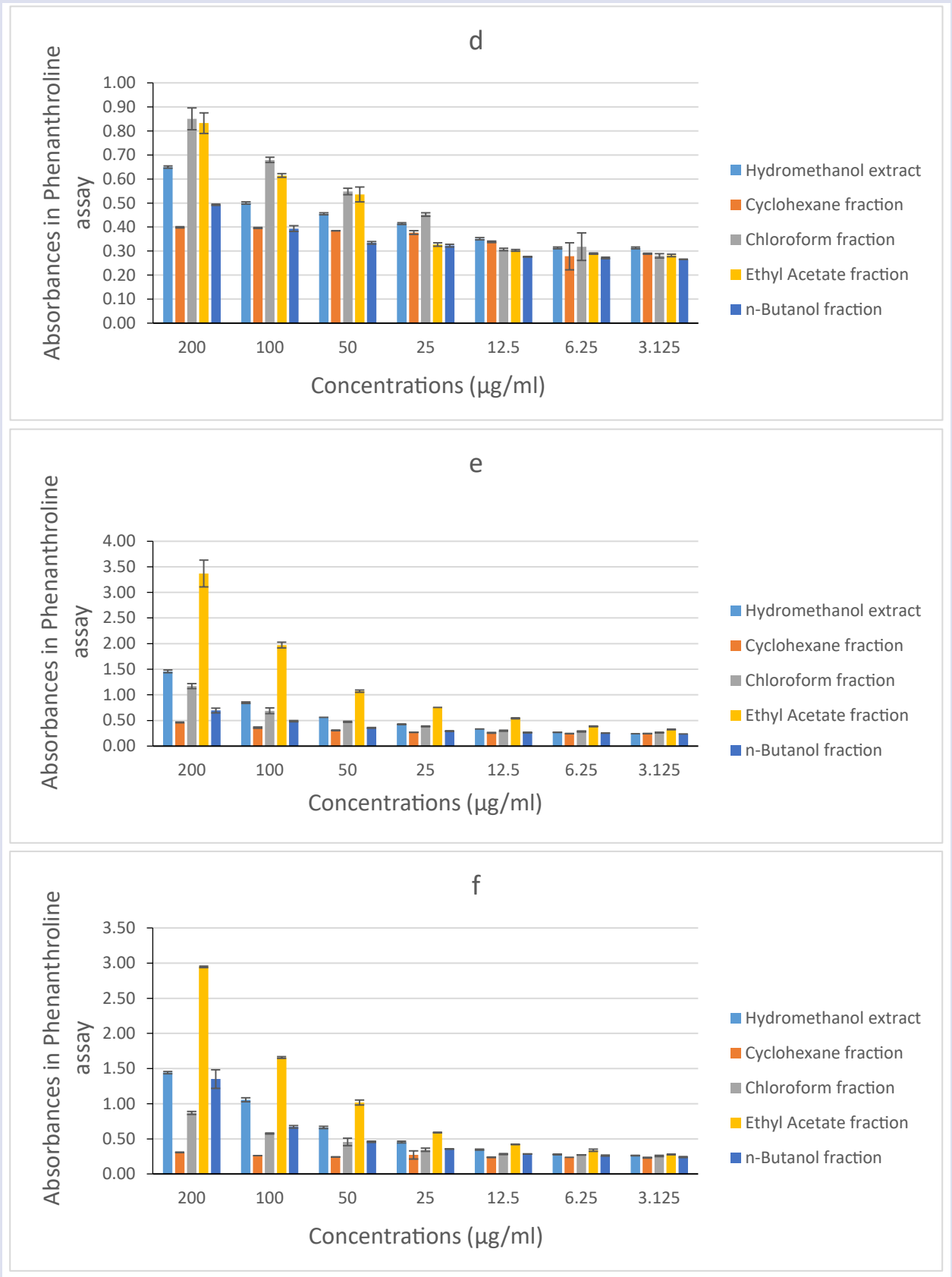
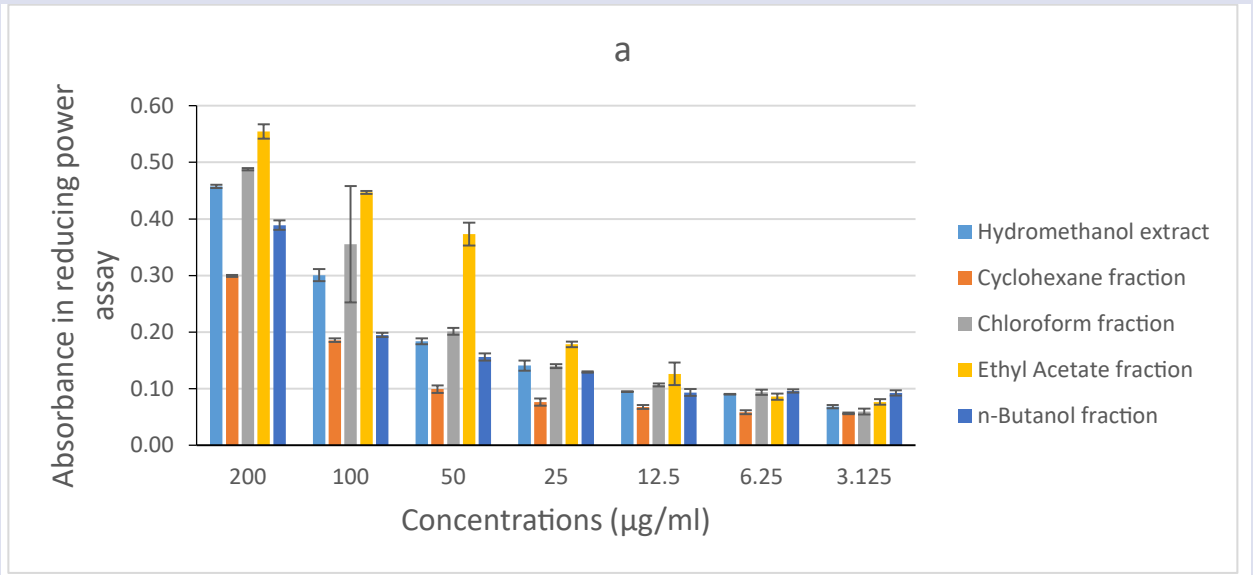
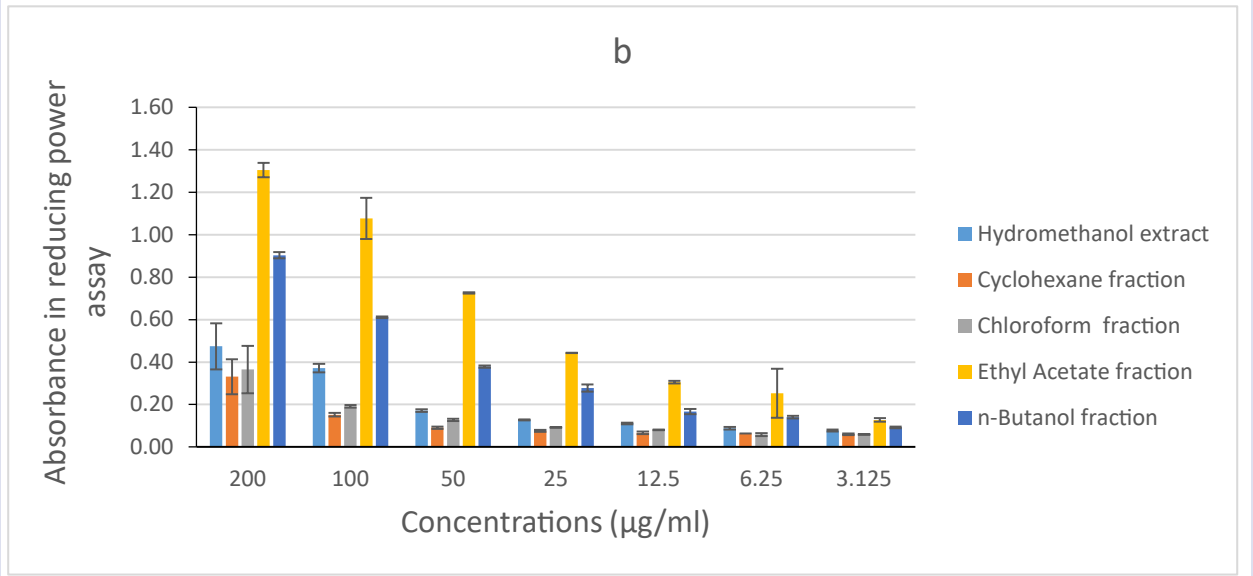
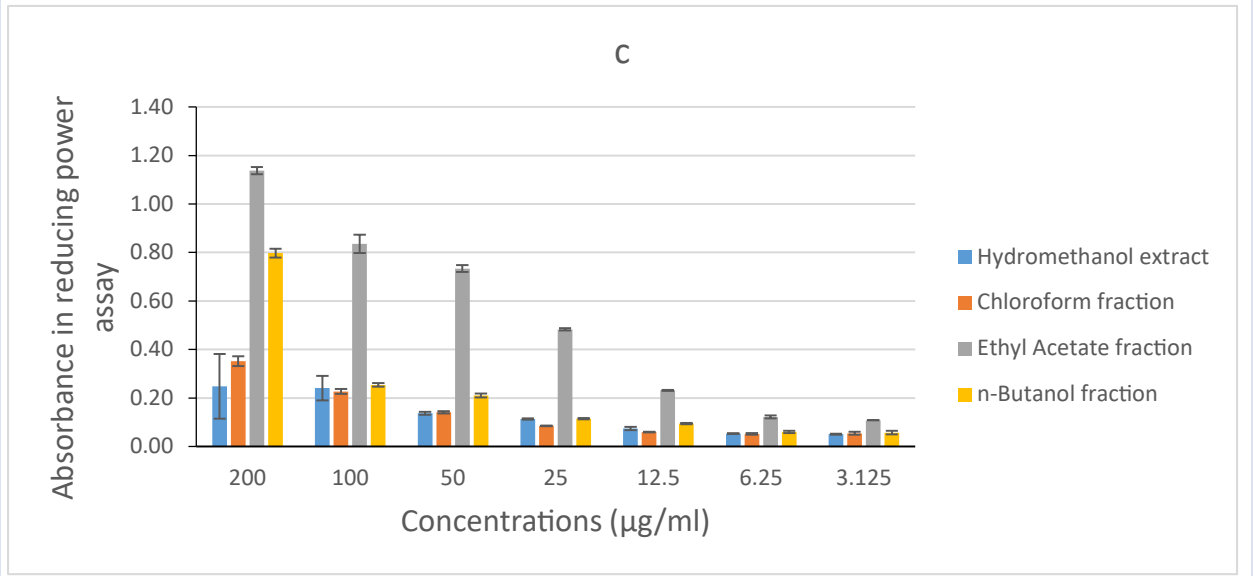


Figure 4: Phenanthroline assay: Histogram, the antioxidant activity of hydromethanol extract and its fractions of six *Drimia numidica* parts, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.a


a
Absorbance in reducing power assay
0.60
0.50
0.40
0.30
0.20
0.10
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction


b
Absorbance in reducing power assay
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction


c
Absorbance in reducing power assay
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
200
100
50
25
12.5
6.25
3.125
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

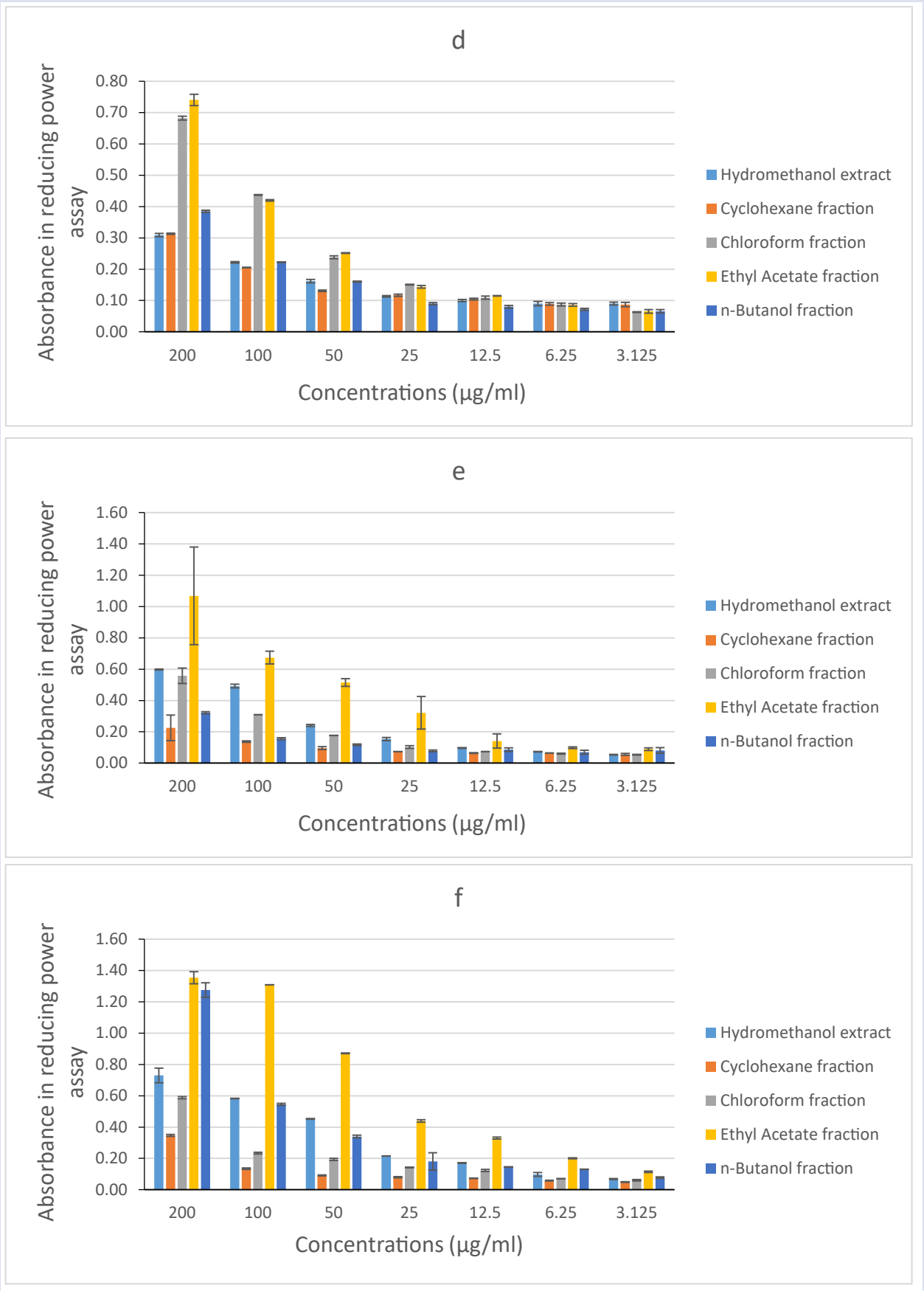
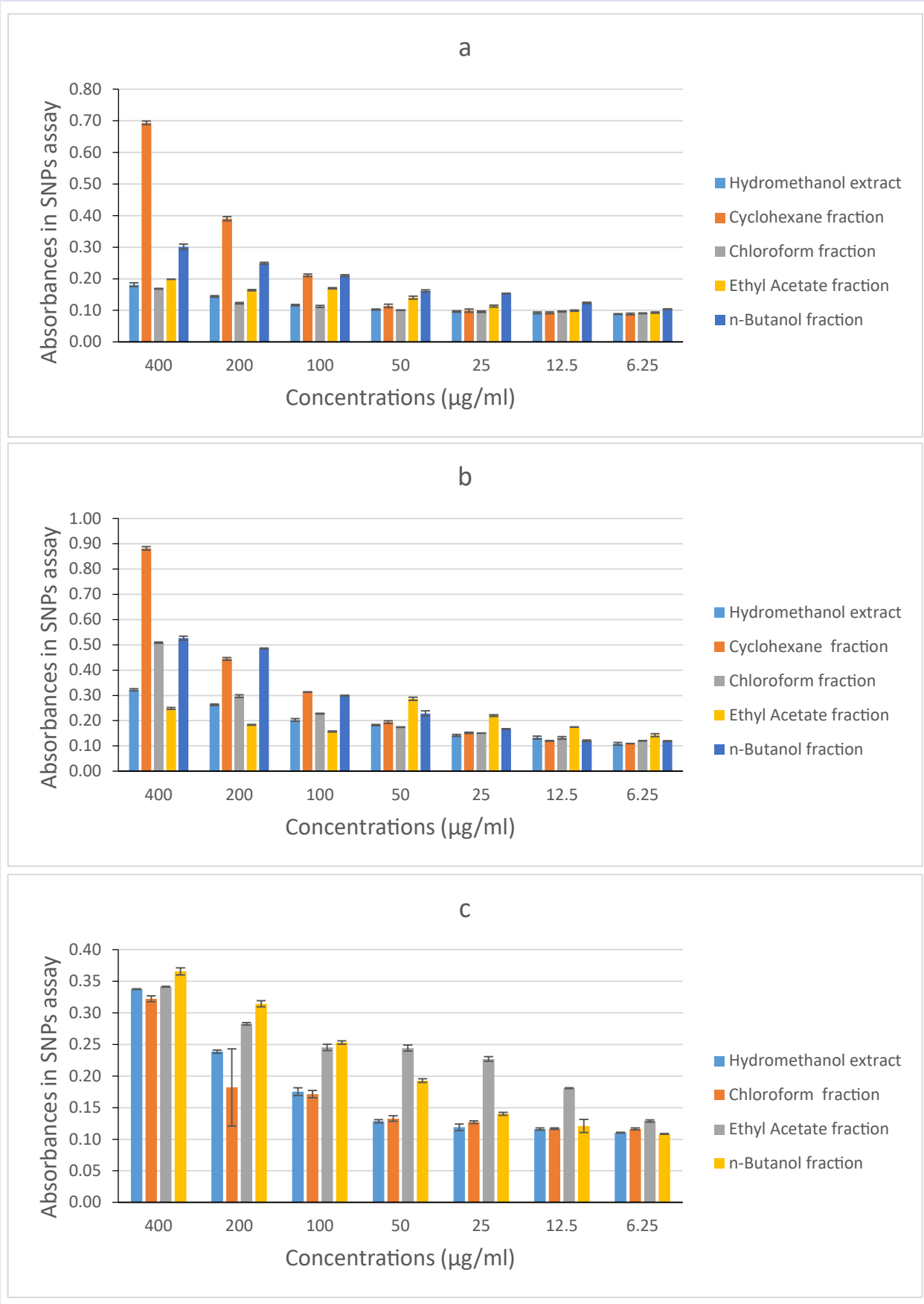


Figure 5: Reducing power assay Histogram, the antioxidant activity of hydromethanol extract and its fractions of six *Drimia numidica* parts, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.


a
Absorbances in SNPs assay
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
400
200
100
50
25
12.5
6.25
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
b
Absorbances in SNPs assay
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
400
200
100
50
25
12.5
6.25
Concentrations (µg/ml)
Hydromethanol extract
Cyclohexane fraction
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction
c
Absorbances in SNPs assay
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
400
200
100
50
25
12.5
6.25
Concentrations (µg/ml)
Hydromethanol extract
Chloroform fraction
Ethyl Acetate fraction
n-Butanol fraction

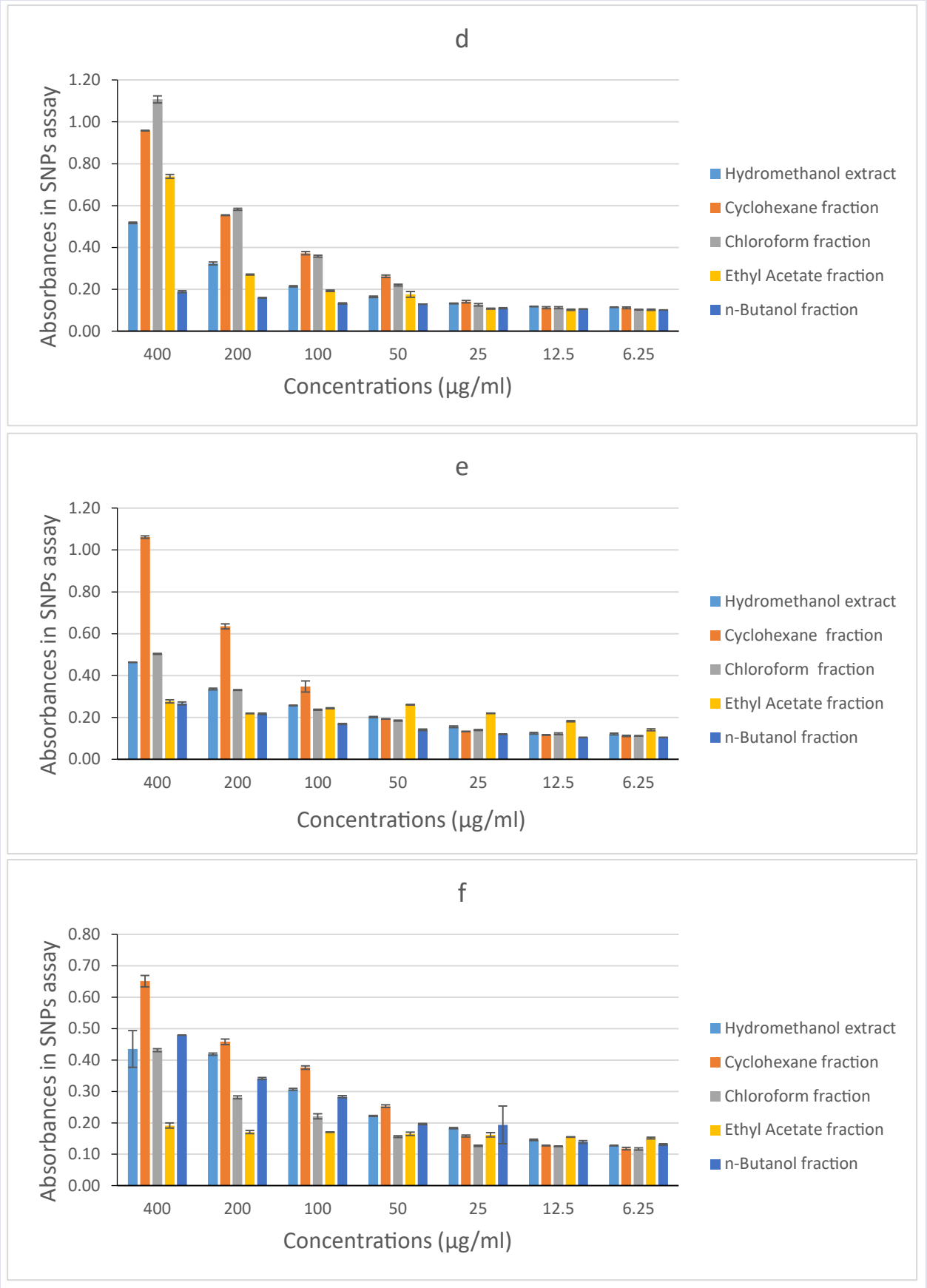


Figure 6: Silver nanoprticles assay Histograms, the antioxidant activity of hydromethanol extract and its fractions of six *Drimia numidica* parts, a) Scapes, b) Flowers, c) Bulbs, d) Capsules, e) Leaves, f) Roots.

SNPs test

20 µL of the extract solution, with 130 µL of SNPs solution (50 ml of silver nitrate solution (0.170 % w/v, solving by distilled water), heated for 10 minutes, combined with 5 ml of Trisodium citrate (1 % w/v), drop by drop until the color changes to a pale yellow, let the solution at the ambient temperature to cool). It was then placed in the microplate, followed by 50 µl of distilled water and incubated at 25 °C during 30 minutes, where the reading was 423 nm.[29]

Statistical analysis

The results were expressed in mean value with a standard deviation (SD) of three replicates. A linear regression analysis was used to determine A0.50 and IC50. r (correlation coefficient, applied between TPC and AOX methods in each plant parts) and ANOVA test (one-way analysis of variance, to detect significant differences: $p < 0.05$) was computed with XLSTAT software.

RESULTS

This comparative study was conducted by preparing hydrometanol extracts and their fractions (derived from five solvents: cyclohexane, chloroform, ethyl acetate, n-butanol according to polarity escalation) for all parts (capsules, flowers, scapes, leaves, bulbs, roots) of *D. numidica* plant. 29 samples were obtained as a total (the treatment of the hydromethanol extract from the bulbs with cyclohexane solvent has not given yield at all).

TPC

The experimental results showed that the ethyl acetate fraction contained the largest amount of TPC in: scapes (115.18±2.65 µgGAE/ml), bulbs (270.96±0.61 µgGAE/ml), leaves (311.84±10.75 µgGAE/ml) and roots (290.18±1.64 µgGAE/ml). Regarding flowers and capsules, the largest amount appeared in n-butanol fraction with 271.65±19.94 µgGAE/ml and 82.82±1.35 µgGAE/ml respectively.

The smallest value was recorded by cyclohexane fraction in all parts (except the bulbs) where: Scapes (24.59±2.04 µgGAE/ml); leaves (20.18±8.25 µgGAE/ml); roots (20.86±13.06 µgGAE/ml); flowers (59±3.85 µgGAE/ml); capsules (3.61±1.19 µgGAE/ml). Concerning the bulbs, hydromethanol extract was the lowest (36.06±0.59 µgGAE/ml). The table 01 shows the results in detail.

Antioxidant activity

The results of the AOX activity in detail are shown in Figures 1-6, Histograms were carried out by Microsoft Excel.

DPPH test

In each part, according to the IC50 (half maximal inhibitory concentration) values, the highest AOX activity was recorded in ethyl acetate fraction, that was different from one part to another: scapes 34.49 ± 0.18 µg/mL; flowers 42.16±0.10 µg/mL; bulbs 12.05 ±0.12 µg/mL; roots 22.32±0.27 µg/mL; leaves 29.01±0.37 µg/mL; and capsules 368.51±3.57 µg/mL.

Values under IC50 were recorded just in: hydrometanol extract, cyclohexane and n-butanol fractions for capsule; cyclohexane fractions of Scapes, Flowers, Roots and Capsules.

ABTS test

For each part of the plant, according to the IC50 values, the highest AOX activity was recorded in ethyl acetate fraction as the following values: scapes 22.57±0.29 µg/mL; flowers 14.78±0.41 µg/mL; bulbs 10.72±0.15 µg/mL; capsules 80.94±.75 µg/mL; leaves 11.77±0.51 µg/mL and roots 15.84±0.15 µg/mL.

The inhibition less than IC50 value Recorded in cyclohexane fraction of scapes and capsules, the cyclohexane fraction of roots has not recorded any inhibition.

CUPRAC test

Results of CUPRAC test showed, all extracts and fractions recorded an inhibition reached to IC50 value. In each part, according to the IC50 values, the highest AOX activity was recorded in: chloroform fraction of capsules 79.34±2.05 µg/mL; ethyl acetate fraction of scapes, flowers, bulbs, leaves, roots by 55.72±6.25 µg/mL, 27.56±2.12 µg/mL, 12.84±0.48 µg/mL; 14.92±0.88 µg/mL and 22.60±0.45 µg/mL successively.

Phenanthroline test

Results showed, only seven extracts or fractions (hydrometanol extract of scapes, n-butanol fraction of capsules, and cyclohexane fraction of: scapes; flowers; leaves; roots; and capsules) didn't reached IC50 value. In each part, according to the IC50 values, the highest AOX activity was recorded at ethyl acetate fraction (scapes 26.03±1.04 µg/mL, flowers 16.95±0.27 µg/mL; bulbs 9.15±0.16 µg/mL; leaves 10.81±0.28 µg/mL and roots18.32±0.35 µg/mL) except for the capsules where it was the chloroform fraction (39.89±1.69 µg/mL).

RP test

The RP test showed, 15 extracts and fractions (hydromethanol extract, and all fractions of scapes except ethyl acetate fraction, chloroform, cyclohexane fractions, and hydromethanol extract for flowers, hydromethanol extract and chloroform fraction for the bulbs, hydromethanol extract, cyclohexane and n-butanol fractions for capsules, cyclohexane, and n-butanol fractions for leaves; and cyclohexane fraction for roots.) did not reached the IC50 value. In every part, according to the IC50 values, the highest AOX activity was recorded in ethyl acetate fraction (scapes 150.34±0.64 µg/mL, flowers 29.94±0.37 µg/mL, bulbs 30.51±0.27 µg/mL, capsules 125.37±3.90 µg/mL, leaves 56.32±11.94 µg/mL and roots 25.99±0.05 µg/mL).

Table 1: Total phenolic compounds content of all samples.

	Total phenolic compounds content (µg GAE/ml)					
Extract/fraction	**Scapes**	**Flowers**	**Bulbs**	**Capsules**	**Leaves**	**Roots**
Hydromethanol extract	29.69±1.5^{d}	94.39±3.41^{c}	36.06±0.59^{c}	68.8±2.38^{b}	156.75±0.45^{b}	167.24±35.37^{b}
Cyclohexan fraction	24.59±2.04^{d}	59±3.85^{d}	/	3.61±1.19^{d}	20.18±8.25^{e}	20.86±13.06^{d}
Chloroform fraction	76.06±4.44^{c}	114.1±3.24^{c}	48.22±0.9^{c}	55.27±4.71^{c}	77.92±6.77^{c}	81.09±22.56^{c}
Ethyl acetate fraction	115.18±2.65^{a}	189.69±0.85^{b}	270.96±0.61^{a}	68.8±1.91^{b}	311.84±10.75^{a}	290.18±1.64^{a}
n-Butanol fraction	102.33±2.54^{b}	271.65±19.94^{a}	135.67±12.14^{b}	82.82±1.35^{a}	52.53±9.43^{d}	108.31±1.51^{b,c}

values: mean ± S.D
n = 3.

Table 2: IC50/A0.50 values of antioxidant capacities of 29 samples from *D. numidica*.

		Antioxidant Methods					
parts of D.numidica	extracts/fractions	DPPH assay IC50 µg/mL	ABTS assay IC50 µg/mL	CUPRAC assay A0.50 µg/mL	Phenanthroline A0.50 µg/mL	Reducing power assay A0.50 µg/mL	SNPs assay A0.50 µg/mL
	hydromethanol extract	474.11±9.39^{a}	260.06±2.07^{a}	331.11±1.05^{a}	>200	>200	>400
	cyclohyxan fraction	>800	>800	208.28±1.50^{b}	>200	>200	275.10±0.95
Scapes	chloroform fraction	115.19±2.04^{c}	33.65± 0.75^{c}	108.60±1.26^{d}	61.72±4.12^{b}	>200	>400
	ethyl acetate fraction	34.49 ± 0.18^{d}	22.57± 0.29^{d}	55.72±6.25^{e}	26.03±1.04^{c}	150.34±0.64	>400
	n-Butanol fraction	228.69±1.85^{b}	87.50 ±1.92^{b}	129.32±2.35^{c}	187.17±13.68^{a}	>200	>400
	hydromethanol extract	147.21±2.43^{b}	95.37±1.50^{b}	135.00±4.75^{b}	157.50±2.34^{a}	>200	>400
	cyclohyxan fraction	>800	600.36±3.07^{a}	158.77±8.49^{a}	>200	>200	209.63±3.21^{c}
Flowers	chloroform fraction	221.62±1.91^{a}	61.51±0.57^{c}	91.65±8.01^{c}	55.63±2.16^{b}	>200	392.19±24.59^{a}
	ethyl acetate fraction	42.16±0.10^{d}	14.78±0.41^{e}	27.56±2.12^{d}	16.95±0.27^{d}	29.94±0.37^{b}	>400
	n-Butanol fraction	57.68 ±1.18^{c}	42.22±0.72^{d}	32.73±6.72^{d}	29.08±0.35^{c}	75.80±1.35^{a}	333.61±23.59^{b}
	hydromethanol extract	123.63 ±0.05^{b}	123.77±0.92^{a}	169.06±18.08^{b}	94.36±4.89^{a}	>200	>400
	Chloroformic fraction	183.62±2.20^{a}	91.26±1.56^{b}	228.85±12.50^{a}	65.78±1.62^{b}	>200	>400
Bulbs	Ethyl acetate fraction	12.05 ±0.12^{c}	10.72±0.15^{d}	12.84±0.48^{d}	9.15±0.16^{d}	30.51±0.27^{b}	>400
	n-Butanol fraction	14.78 ±2.27^{c}	40.93±2.09^{c}	58.77±1.56^{c}	28.86±0.60^{c}	106.48±5.97^{a}	>400
	hydromethanol extract	>800	133.19±0.46^{b}	131.45±6.81^{c}	89.68±3.29^{a}	>200	382.50±2.29^{a}
	cyclohyxan fraction	>800	>800	188.69±4.16^{b}	>200	>200	170.04±1.99^{c}
Capsules	chloroform fraction	382.42±3.10^{a}	82.95±0.29^{c}	79.34±2.05^{d}	39.89±1.69^{b}	132.53±0.53^{a}	163.19±1.54^{c}
	ethyl acetate fraction	368.51±3.57^{b}	80.94±.75^{c}	125.20±5.25^{c}	46.83±4.27^{b}	125.37±3.90^{b}	241.32±4.57^{b}
	n-Butanol fraction	>800	262.26±4.78^{a}	274.88±4.06^{a}	>200	>200	>400
	hydromethanol extract	60.21±10.54^{d}	41.22±0.11^{d}	60.19±0.63^{b}	39.11±0.42^{c}	141.97±4.35^{b}	>400
	cyclohyxan fraction	358.68±3.50^{a}	288.62±4.93^{a}	145.73±6.06^{a}	>200	>200	153.63±1.33^{b}
Leaves	chloroform fraction	133.00±4.00^{b}	61.45±0.12^{c}	70.41±1.61^{b}	55.20±3.09^{b}	179.95±13.84^{a}	387.78±2.55^{a}
	ethyl acetate fraction	29.01±0.37^{e}	11.77±0.51^{e}	14.92±0.88^{c}	10.81±0.28^{d}	56.32±11.94^{c}	>400
	n-Butanol fraction	107.94±0.57^{c}	170.32±1.63^{b}	161.42±11.48^{a}	112.00±4.74^{a}	>200	>400
	hydromethanol extract	73.34±1.12^{b}	27.55±0.10^{c}	46.14±0.99^{d}	30.74±0.98^{c}	76.65±0.50^{c}	>400
	cyclohyxan fraction	>800	0	223.34±2.71^{b}	>200	>200	236.37±2.63
Roots	chloroform fraction	254.07±5.03^{a}	55.96±0.65^{a}	99.40±2.25^{c}	72.26±4.12^{a}	174.59±1.99^{a}	>400
	ethyl acetate fraction	22.32±0.27^{d}	15.84±0.15^{d}	22.60±0.45^{d}	18.32±0.35^{d}	25.99±0.05^{d}	>400
	n-Butanol fraction	43.39±0.67^{c}	48.18±0.89^{b}	492.92±17.56^{a}	59.61±0.65^{b}	88.77±0.76^{b}	>400

IC50: concentration of 50% inhibition percentages, A0.50: concentration at 0.50 absorbance
Values expressed: mean ± S.D, n = 3

Table 3: Correlation between the IC50/A0.5 values of AOX and TPC.

	Correlation coefficient (r)					
D.numidica Parts	DPPH	ABTS	Cuprac	Phenanthroline	Reducing power	Silver nano particles
Scapes	-0.818	-0.827	-0.85	N	/	/
Flowers	-0.769	-0.656	-0.902	-0.674	0.942	N
Bulbs	-0.81	-0.937	-0.905	-0.912	-0.978	N
Capsules	-0.933	0.841	N	N	N	N
Leaves	-0.716	-0.775	-0.869	-0.858	-0.977	0.986
Roots	-0.649	-0.944	N	-0.932	-0.858	/

N: There is no significant correlation because the p-value>0.05.
/: Correlation not calculated because it has only one value of IC50 or A50.

Silver nanoparticles

The results of the SNPs test showed the most extracts (18 extracts) that did not reach IC50. The values achieved for IC50 were: cyclohexane fraction of all part (except bulbes) of *D. numidica*, chloroform and n-butanol fractions of flowers, hydromethanol extract, chloroform, ethyl acetate fractions of capsules, chloroform fraction of leaves. In each part, according to the IC50 values, the highest AOX activity was in cyclohexane fraction except the capsules (with chloroform fraction 163.19±1.54 µg/mL): scapes 275.10±0.95 µg/mL; flowers 209.63±3.21 µg/mL, leaves 153.63±1.33 µg/mL, and roots 236.37±2.63 µg/mL.

Correlation between TPC and AOX activity

The result of correlation between TPC and AOX activity (with the six methods) among *D.numidica* parts was between: -0.649 to -0.933 for DPPH, -0.656 to -0.944 for ABTS except capsules fraction was 0.841, -0.85 to -0.905 for CUPRAC, -0.674 to -0.932 phenanthroline activity, -0.858 to -0.978 RP activity and 0.986 for SNPs activity.

There are few samples that did not show correlation due to a p-value>0.05 as flowers in CUPRAC method. all results showed in Table 3.

DISCUSSION

In recent decades, natural AOXs were considered one of the best alternatives to artificial food additives. Not only is it natural and free of toxins, but it may include new molecules and compounds that have not yet developed resistance.[6] Medicinal plant extracts are rich sources of polyphenols which are considered a strong natural AOX.[30,31]

With regards to *D. numidica* TPC and AOX capacity, none of them are covered extensively in literature. Kakouri *et al*[32], considered the first research work on this plant which covered hydromethanol extracts of all *D. numidica* except capsules, which were collected from Greece using two methods to measure the AOX capacity (DPPH and ABTS tests) and Folin-Ciocalteu assay for determining TPC.

Our research work, carried out a detailed and comparative study of the AOX activity and TPC of all parts of *D. numidica* (six parts) from the north-eastern Algerian region, through the hydromethanol extract and its fractions (29 samples). The difference between the current study and Kakouri et al study is: the method of drying; harvest season in some parts of the plant (bulbs and roots); and the area of collection. These differences may affect the amount of polyphenols and biological activities of the plant.[33] In addition, some research has shown that the chemical composition of *D.numidica* for the southern Mediterranean differs from that of the north, both in terms of quality and quantity.[34]

In the previous study of *D.numidica*, TPC values (expressed by mg GAE/g) were estimated as follows: flowers 16.13 ± 0.02, scapes 7.83 ± 0.01, leaves 17.40 ± 0.01, bulbs 5.95± 0.01, roots 9.38 ± 0.01. Compared to our study, these results show a marked and clear difference, where the values appear much lower than our values (expressed by µg GAE/ml) which are: flowers 94.39±3.41, scapes 29.69±1.5, leaves 156.75±0.45, bulbs 36.06±0.59, roots 167.24±35.37.

Regarding the AOX tests (DPPH and ABTS, expressed by µg/mL), based on a comparison of results (Kakouri et al. and our study), the AOX tests (DPPH and ABTS, expressed by µg/mL) showed that: the number of our extracts that reached a IC50 value were more than those recorded in the previous study. Where in Kakouri *et al*. study, DPPH test showed only flowers extract reached to IC50 value (90.2±3.5). As for the ABTS test, the extracts were: flowers 32.1 ± 1.7, scapes 58.7 ± 2.1, roots 90.0±3.3. In our study, DPPH test values were: Scapes 474.11±9.39, flowers 147.21±2.43, leaves 60.21±10.54, bulbs 123.63±0.05, roots 73.34±1.12. ABTS test values were: Scapes 260.06±2.07, flowers 95.37±1.50, leaves 41.22±0.11, bulbs 123.77±0.92, roots 27.55±0.10.

When comparing TPC values of our study (using Folin Ciocalteo method) and AOXs capacity (using six methods) for hydromethanol extracts and its fractions of the six parts of *D. numidica* (all parts, 29 samples). For TPC results (unit: µg GAE/ml), a comparison between each extract and their fractions reveals the highest TPC content was in the ethyl acetate and n-butanol fractions. The highest ethyl acetate fraction values were recorded in: scapes (115.18±2.65); bulbs (270.96±0.61); leaves (311.84±10.75); roots (290.18±1.64), as for n-butanol fraction was in: flowers (271.65±19.94), capsules (82.82±1.35). Among all samples, leaves have the best TPC value (311.84±10.75).

Concerning the AOX capacity, generally the IC50/A0.5 results showed a significant difference between extracts and their fractions, example: in DPPH method, the five samples of scapes showed a divergence of IC50 values: hydromethanol extract 474.11±9.39, cyclohexane fraction >800, Chloroforme fraction 115.19±2.04, Ethyl acetate fraction 34.49 ± 0.18, n-butanol fraction 228.69±1.85. But there can be noticed no significant difference in some cases between samples of one part, DPPH method example: bulbs ethyl acetate (12.05 ±0.12) and n-butanol fractions (14.78 ±2.27).

The results also differed from one method to another, where an extract/fraction that does not show strong inhibition or low inhibition in one of the tests does not necessarily have a high inhibition capacity when tested using another method. Especially SNPs method shows a large difference in values compared to the other methods, example: the IC50/A0.5 value of ethyl acetate scapes fraction in DPPH (34.49 ± 0.18), SNPs (>400), and RP (150.34±0.64) methods, showed a divergence of values.

Despite the difference in IC50/A0.5 values, the ethyl acetate fractions often showed greater inhibitory capacity in most methods, except for SNPs test where cyclohexane fraction shows the best AOX activity (this result appears to be quite the opposite compared to the other tests).

The data revealed that there is a correlation between TPC and AOX capacity: in the DPPH test, there was strongly correlated in all parts. The higher the amount of TPC, the stronger the antioxidant activity. In the ABTS test, all parts are strongly correlated, the higher the amount of TPC, the stronger the antioxidant activity, except capsules where the higher the amount of TPC, the weaker the antioxidant activity. In the case of CUPRUC test, all parts are strongly correlated except capsules and roots which have no correlation. Phenanthroline test, all parts are strongly correlated except capsules and scapes. RP test, strongly all parts are correlated except capsules (the scapes not calculated). For SNPs activity, low correlation in leaves is noted, no correlation in flowers, bulbs, capsules (the scapes and roots are not calculated).

CONCLUSION

The only study of *D. numidica* focused just on a single extract (hydromethanol), moreover, no studies were conducted on its capsules. The current work offers an in-depth study of antioxidant activity by six different methods and phenolic content of hydromethanol extract and its fractions for all six parts of *D. numidica* (including capsules). Based on the results, the values vary from one extract/fraction to another (for TPC and AOX, and from one AOX method to the other), which makes choosing the appropriate extract/fraction or AOX method very important and sensitive. Additionally, TPC often correlates with AOX activity.

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