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POLYPHENOLS FROM *POLIANTHES TUBEROSA* L. (AMARYLLIDACEAE) LEAVES AND THEIR ANTIOXIDANT PROPERTIES

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Abstract

Description of the subject: Tuberose *Polianthes tuberosa* L. (Amaryllidaceae) is an ornamental plant, cultivated for its cut flowers and perfume industries.
Objective: This study aims to study *P. tuberosa* L. leaves polyphenols through their antioxidant activity.
Methods: *P. tuberosa* L. leaves polyphenols were extracted using solvent maceration, followed by a fractionation by different increasing polarity solvents. To highlight the impact of *P. tuberosa* leaves polyphenols as antioxidant, their antioxidant activity was determined using DPPH, β-carotene and iron chelating tests.
Results: The obtained results show that the crude extract and its fractions are rich in polyphenols, flavonoids and condensed tannins, especially in aqueous phase. The percentage of DPPH inhibition and that of iron chelating varied according to extracts concentrations using generally non-linear mathematical models. The results revealed that the crude extract has the higher effect than the other fractions.
Conclusion: It appears that polyphenols from *P. tuberosa* leaves extract have a moderate antioxidant effect, which could limit the use of chemical antioxidants.
Keywords: *Polianthes tuberosa* L.; polyphenols; flavonoids, condensed tannins; antioxidants.

POLYPHÉNOLS EXTRAITS DES FEUILLES DE *POLIANTHES TUBEROSA* L. (AMARYLLIDACEAE) ET LEUR PROPRIETE ANTIOXYDANTE

Résumé

Description du sujet : La tubéreuse *Polianthes tuberosa* L. (Amaryllidaceae) est une plante ornementale, cultivée pour ses fleurs coupées et pour la parfumerie.
Objectifs : Dans ce présent travail, nous allons étudier les polyphénols des feuilles de *P. tuberosa* L. Pour leur activité antioxydante.
Méthodes : La technique de macération par solvant a été utilisée dans cette étude, suivie d'un fractionnement par différents solvants de polarité croissante. Pour mettre en évidence l'impact des polyphénols des feuilles de *P. tuberosa* comme agent antioxydant, on étudie leur activité antioxydante par le test de DPPH, de β-carotène et du pouvoir chélateur.
Résultats : Les résultats obtenus montrent que l'extrait brut et ses fractions sont riches en polyphénols, en flavonoïdes et en tanins condensés, surtout dans la phase aqueuse. Le pourcentage d'inhibition et celui de chélation varient en fonction des concentrations des extraits selon des modèles mathématiques généralement non-linéaires. Les résultats obtenus révèlent que l'extrait brut des feuilles présente un effet plus important par rapport aux autres fractions.
Conclusion : Il s'avère que les feuilles de *P. tuberosa* ont un effet antioxydant modéré, et qui peuvent contribuer à la diminution de l'utilisation des antioxydants chimiques.
Mots clés: *Polianthes tuberosa* L.; polyphénols; flavonoïdes; tanins condensés; antioxydant.

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INTRODUCTION

Oxidative stress is an imbalance caused by an over production of free radicals or by a decrease in defense systems [1, 2]. Oxygen derived radicals represent the most important class of radical species generated in living organisms, which may be one of the potential factors for multifactorial diseases appearance such as diabetes, cardiovascular and Alzheimer diseases [3]. Phenolic compounds such as flavonoids and catechins, have a potent antioxidant activity. They have been mentioned to prevent or delay numbers of chronic and degenerative diseases such as cancer, cardiovascular disease, Alzheimer's disease and inflammation [4, 5]. They are widely extracted from plants by maceration in polar organic solvents or by mixing them with water, in addition to recent used methods such as ultrasound and supercritical CO_2 [6].

Polianthes tuberosa L. (Amaryllidaceae) is an ornamental plant, cultivated in China, Egypt, South Africa, India and Japan for its fragrant cut flowers and perfume industries [7-10]. In Algeria, *P. tuberosa* was widely cultivated in Mitidja region for its essential oil in the seventies. It is reported that *P. tuberosa* L leaves contain a tuberolactone and three flavonoids (kaempferol, kaempferol-3-O-xyloside and kaempferol-3- 4'-O-dixyloside), 9,10 dehyrohecogenin-3-O-glucose xylose galactoside, kaempferol-3-O-xyloside, α-D-glucoside and polianthosides B and C [11], spirostanols, polianthoside B and C and D-G, furostanols and saponins were also isolated and identified from bulbs [12, 13].

While chloroform and methanol leaves extracts had DPPH scavenging activity [14], polyphenols from this plant leaves showed also anti-inflammatory and antibacterial effect against *Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus, Escherichia coli, Pseudomonas aureginosa* and *Salmonella typhi,* and antifungal effect against *Aspergillus niger* and *Candida albicans* [15]. Moreover, polyphenols from bulbs have been reported to exhibit an antioxidant, antimicrobial, cytotoxic, membrane stabilizing and thrombolytic activities [16]. Polyphenolic compounds from flowers extract are reported to have antioxidant activity [17] and antimicrobial effect against *Proteus mirabilis* and *Escherichia coli* [18].

Considering the importance of this Amaryllidaceae polyphenols plant as antioxydant agents especially in flowers and bulbs according to previous studies [16, 17], and the economic value of flowers, this study aims to determine phenolic compounds (polyphenols, condensed tannins and flavonoids) from leaves and to study their antioxidant activity using the DPPH, β-carotene tests and their chelating power.

MATERIAL AND METHODS

1. Plant material

From Amaryllidaceae plants *Polianthes tuberosa* L. was studied. bulbs were obtained from a nursery situated in Chiffa-Blida region (Central part of Algeria 36° 28′ 12″ N, 2° 49′ 39″ E). They were next cultivated in pot contain sol:loam mixture, under natural climate of Setif region (Eastern part of Algeria 36° 11' 29" N, 5° 24' 34" E) in May 2015. *P. tuberosa* L. leaves were collected during September 2015 at mature stage, and they were air-dried at room temperature for 20 days, and grinded to a fine powder.

2. Chemical products

All chemical products in experiments were of analytical grade. Methanol, hexane, dichloromethane, chloroform, hydrochloric acid (HCl), vanillin, catechin, quercetin, gallic acid, Folin-Ciocalteu reagent, aluminum trichloride ($AlCl_3$), Diphenyl-2-picrylhydrazy (DPPH), sodium carbonate salt, β-carotene, linoleic acid, tween 40, ascorbique acid, ethylene diamine tetraacetic acid (EDTA), butylated hydroxytoluene (BHT), 3-(2-pyridyl)5,6bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), Iron bichloride tetrahydrate ($FeCl_2$) were purchased from Fluka, Prolabo and Sigma-Adrich companies.

3. Extraction of polyphenols

The solid-liquid extraction of polyphenols from *P. tuberosa* leaves was carried out according to the method described by Meziti *et al.* [19]. 100 g of leaf powder was mixed with a 70% aqueous solution of methanol (v:v) in a ratio of (1/10 w/v). The mixture was macerated twice for 48 h with mechanical agitation at room temperature in enclosed flasks, in order to ensure maximum exhaustion of the extract from the powder.

Extract obtained from each maceration was filtered; filtrates were combined and concentrated by a rotary evaporator (BÜCHI) at 40 °C to give crude extract (EC). Liquid-liquid partition of the methanol crude extract was carried out using hexane (defatted agent) and dichloromethane in a ratio of 1:1 (v/v). The organic phase and the remaining aqueous phase were collected and concentrated under reduced pressure at 40°C. Concentrated crude extract (CE), hexane fraction (HxF), dichloromethane fraction (DcMF), aqueous phase (Aq) were dried at 50 °C in a dry-air oven. All extracts were stored at 4 °C until use.

4. Phytochemical analysis of extracts

4.1. Concentration of total polyphenol

Total polyphenols content has been determined using Folin-Ciocalteu reagent [20]. The reducing character of phenolic compounds led to the formation of a blue colored complex that can be measured colorimetrically at 765 nm. A volume of 0.2 mL of the extract (1 $mg.mL^{-1}$) was mixed with 1 mL of the Folin-Ciocalteu reagent diluted to 1/10. Incubation (4 minutes) has been carried out, then, 0.8 mL of sodium carbonate (75 $mg.mL^{-1}$) was added. After 2 hour of incubation at room temperature, the absorbance was measured at 765nm. The calibration curve was obtained in the same conditions using a range of concentrations (10-160 $\mu g.mL^{-1}$) of gallic acid solution. Total phenolic levels have been determined using regression curve y=0.0091x+0.0125, with y: absorbance and x: concentration of gallic acid, R^2=0.9945, and expressed in microgram equivalent of gallic acid per milligram of extract (µg $EAG.mg^{-1}$ E).

4.2. Concentration of flavonoids

Flavonoids quantification was made by the method of the trichloride aluminum [21]. 1 mL of the solution of $AlCl_3$ (20 $mg.mL^{-1}$ of methanol) was added to 1 mL of the extract. After incubation for 15 minutes, the absorbance was determined at 430 nm. The calibration curve has been established using quercetin (1-50 $\mu g.mL^{-1}$). Flavonoids levels were expressed as microgram equivalent quercetin per milligram of extract (µg $EQ.mg^{-1}$ E), using regression curve y=0.036x+0.0257 with y: absorbance, x: concentration of quercétine, R^2=0.9965.

4.3. Concentration of condensed tannin

Condensed tannins quantification has been carried out using the vanillin method reported by Chaouche et *al.* [22]. 50µL of extract (10 $mg.mL^{-1}$ in methanol) was mixed with 3 mL of solution of vanillin 4% (in methanol) and 1.5 mL of concentrated HCl (37%). The mixture was incubated 15 minutes, after that the absorbance was measured at 500 nm against the blank. The calibration curve was established in the same conditions using catechin as a standard (0-300 $\mu g.mL^{-1}$). The content of condensed tannins was expressed in microgram equivalent of catechin per milligram extract (µg $EC.mg^{-1}$ E), using this equation y=0.0014+0.0195 with y: absorbance, x: concentration of catechin, R^2=0.9915.

5. Antioxidant activity

5.1. DPPH scavenging activity

The DPPH scavenging activity was assessed according to the method described by Boumerfeg *al.* [23] with slight modification, 50 µL of each concentrations of the extracts 100-6.25 $mg.mL^{-1}$ (prepared in methanol) and ascorbic acid have been mixed with 2.5 mL of the solution of DPPH (0.004%). After incubation for 30 minutes, the absorbance was measured at 517 nm. The percentage of inhibition (I%) of the radical DPPH was calculated as follows: I%= 100[(AC-AE)/AC]. AC: absorbance in the absence of inhibitor (negative control), AE: absorbance in the presence of the inhibitor (extract).
The free radical scavenger was expressed by IC_{50} (50% of DPPH inhibitory concentration) of each extract and it was calculated from equation that determines the DPPH inhibition percentage depending on the inhibitor concentrations, It was expressed in mg/ml.

5.2. β-carotene/linoleic acid test

β-carotene/linoleic acid test assesses the ability of extracts to trap the free radicals generated during the lipid peroxidation of linoleic acid. The method is based on the spectrophotometric measurement of the discoloration of β-carotene by linoleic acid degradation by-products [24]. A solution of β-carotene /linoleic acid was prepared as follows: 1mg of β-carotene was dissolved in 1 mL of chloroform, 25 µL linoleic acid and 200 mg Tween 40 were added.

Chloroform was completely evaporated using a rotary evaporator, then; 100 mL of saturated oxygen water (100 mL.min^{-1} for 30 min) were added with vigorous agitation. This mixture was aliquoted (2.5 mL) in test tubes and 350 µL of the extract (2 mg.mL^{-1}) were added. The emulsion was then incubated at room temperature and the absorbance at (490 nm) was recorded after 1 hour, 2 h, 4 h, 6 h, 24 h and 48 h [24]. The same procedure was repeated with the synthetic antioxidant BHT and ascorbic acid (2 mg.ml^{-1}), water and methanol as the negative controls. The antioxidant activity percentage (AA%) was calculated as follows: $AA\% = 100(AE/AC)$. AE: absorbance in the presence of the extract after 48 h., AC: absorbance in the presence of positive control BHT after 48 h.

5.3. Metal chelating activity

The iron chelating power of *P. tuberosa* leave extracts has been assessed by the test of Ferrozine, which product when associated with free iron Fe^{2+} the ferrozine- Fe^{2+} complex have a purple color. In the presence of the chelating agents the formation of this complex will be prevent and consequently the intensity of the color decreases. The test of ferrozine was carried out according to the method cited by Li et *al.* [25]. Briefly, 500 µL of different concentrations of extracts and chelating agent standard EDTA (extract 0-35 mg.mL^{-1}, $Na_2EDTA\text{-}2H_2O$ 0-25 µg.mL^{-1}) were mixed with 100 µL $FeCl_2$ (0.6 mM in water) and 900 µL MeOH. The control tubes contain the same reagents except the extract or EDTA. The mixture was well shaken and allowed to react for 5 min, then 100 µL of ferrozine (5 mM in methanol) was added, the mixture was agitated again and incubation at room temperature for 10 minutes. The absorbance was measured at 562 nm against the blank. The chelating activity percentage (%C) of the extract and of the standard has been calculated as follows: $\%C = 100\ [(1\text{-}\ AE)/AC]$. AE: absorbance in the present of sample test, AC: absorbance of control.

The metal chelating activity was expressed by EC_{50} (effective concentration for 50% chelating metal) of each extract and it was calculated from equation that determines the metal chelating percentage depending on the inhibitor concentrations, it was expressed in mg/ml.

6. Statistical analysis

Each test was repeated three times and the results were represented as mean±standard deviation. Mathematic modelisation and graphs were done by Excel 2007. Analysis of variance and Tukey tests were performed, using GraphPad Prism 06 software and p values were significant at $p<0.05$.

RESULTS

1. Extraction Yields

Polyphenols extraction from *P. tuberosa* L. leaves was carried out. The yield of extraction of the crude leaf extract (CE) was 26.98 ± 0.44%, which was fractioned by different solvents in increasing polarity, the dichloromethane fraction (DcMF) represents the highest yield of 24.94 ± 0.45%, and this can be explained by the solubility of the crude extract compounds in this solvent which are generally glycosides polyphenols, followed by the aqueous phase yield (AqP) 0.74 ± 0.03% which represents the remaining phase of liquid-liquid fractionation and the fraction of hexane (HxF) yield 0.52 ± 0.04% which serves as a defatted agent.

2. Concentrations of polyphenols, flavonoids and condensed tannins

Table 1 showed the concentration of polyphenols, flavonoids and condensed tannins contents in different extracts.

Table 1: Contents of polyphenols, flavonoids and condensed tannins in *P. tuberosa* L. leaves extracts.

Extract	Total Polyphenols µg EAG/mg E	Flavonoids µg EQ/mg E	condensed Tanins µg EC/mg E
Crude extract	29.74±0.00	0.38±0.03	0.65±0.01
Dichloromethane Fraction	29.38±0.02	0.45±0.04	0.82±0.01
Aquous phase	29.95±0.01	1.04±0.01	8.23±0.01
Hexane fraction	16.32±0.03	0.61±0.02	3.82±0.00

Each value represent the mean±SD (n=3).

The obtained results showed that leaves extracts of *P. tuberosa* are rich in polyphenols contents where, phenolic acid was higher in the aqueous phase (29.95 µg EAG.mg^{-1} E) followed by the dichloromethane fraction (29.38 µg EAG.mg^{-1} E) and the crude extract (29.74 µg EAG.mg^{-1} E). *P. tuberosa* leaves extracts seem to be poor in flavonoids, whereas, the crude extract and dichloromethane fraction had the lower flavonoids levels by 0.38 and 0.45µg EQ.mg^{-1} E respectively, but the richest fractions were the aqueous phase and the hexane fraction (1.04 µg EQ.mg^{-1} E and 0.61µg EQ.mg^{-1} E respectively). The leaves of *P. tuberosa* L. are found to be poor in hydrophobic tannins, especially in the crude extract and the dichloromethane fraction, but the aqueous phase had a high condensed tannin content 8.23 µg EC.mg^{-1} E.

3. Antioxidant activity

3.1. DPPH scavenging activity

The percentage of inhibition of DPPH is different depending on the extracts used; and chosen concentrations (Fig. 1). Inhibition was translated by discoloration of DPPH solution which was in a dose-dependent manner.

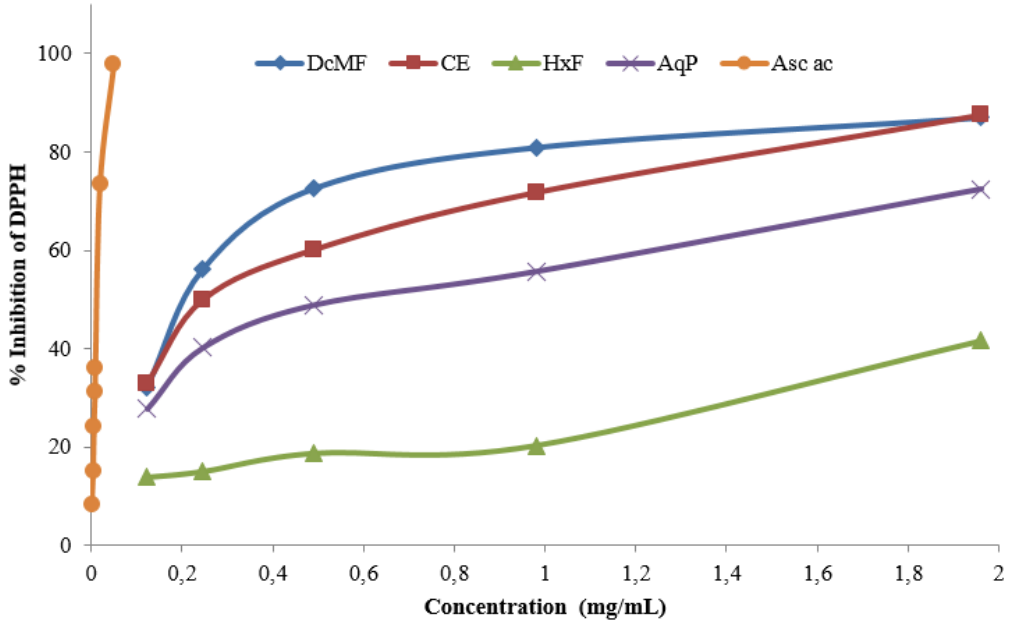

Figure 1: Inhibition percentage of DPPH free radical in the presence of ascorbic acid (Asc ac) and the presence of different extracts of *P. tuberosa* L. leaves

CE: crude extract, DcMF: dichloromethane fraction, HxF: hexane fraction, AqP: aqueous phase.

Mathematical models for antioxidant leaf extracts activities were adopted to determine the IC_{50} of each extract comparing with ascorbic acid control (table2). The different models used for IC_{50} determination are representative, since the coefficients of determination R^2 are approaching to 1; The most effective antioxidant is that having the lowest concentration at 50% inhibition (figure 2).

Table2: Mathematical models expressing the percentage change in DPPH inhibition (I%) versus antioxidant concentration (C on mg/mL)

Antioxidant	Model	R^2
Crude extract	I% = 18.92ln(C) + 73.96	0.993
Dichloromethane fraction	I% = 19.42ln(C) + 79.65	0.928
Hexane fraction	I% = 14.71(C) + 10.73	0.941
Aqueous phase	I% = 15.11ln(C) + 59.78	0.979
Ascorbic acid	I% = 30.69ln(C) + 184.56	0.914

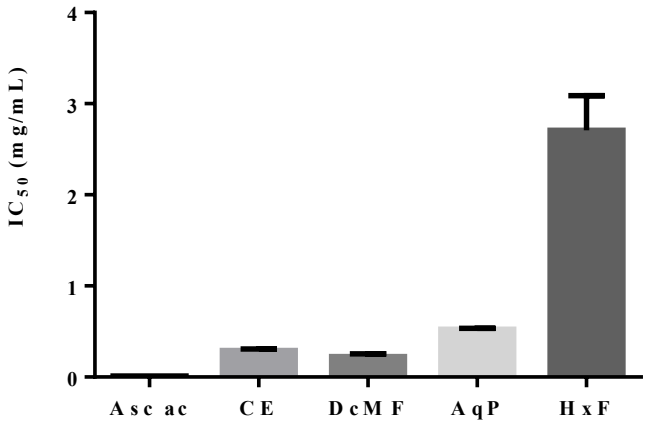

Figure 2: The IC_{50} values of the crude methanol extract of *P. tuberosa* L. leaf, its fractions and ascorbic acid as the reference antioxidant. Each value represent mean±SD (n=3).

The dichloromethane fraction has an important antiradical effect with IC_{50} = 0.22 $mg.ml^{-1}$ followed by the crude extract with IC_{50} = 0.29 $mg.ml^{-1}$, where no significant difference was found with ascorbic acid (IC_{50} = 0.01 $mg.ml^{-1}$) ($p<0.05$), the aqueous phase with IC_{50} = 0.52 $mg.ml^{-1}$ and the hexane fraction has a low antioxidant effect with an IC_{50} = 2.71 $mg.ml^{-1}$ had low antioxidant activity compared to ascorbic acid ($p>0.05$).

3.2. β-carotene / linoleic acid test

The inhibition of linoleic acid/β-carotene oxidation was evaluated by the bleaching test of β-carotene. The percent inhibition of the oxidation of β-carotene is shown in figure 3.

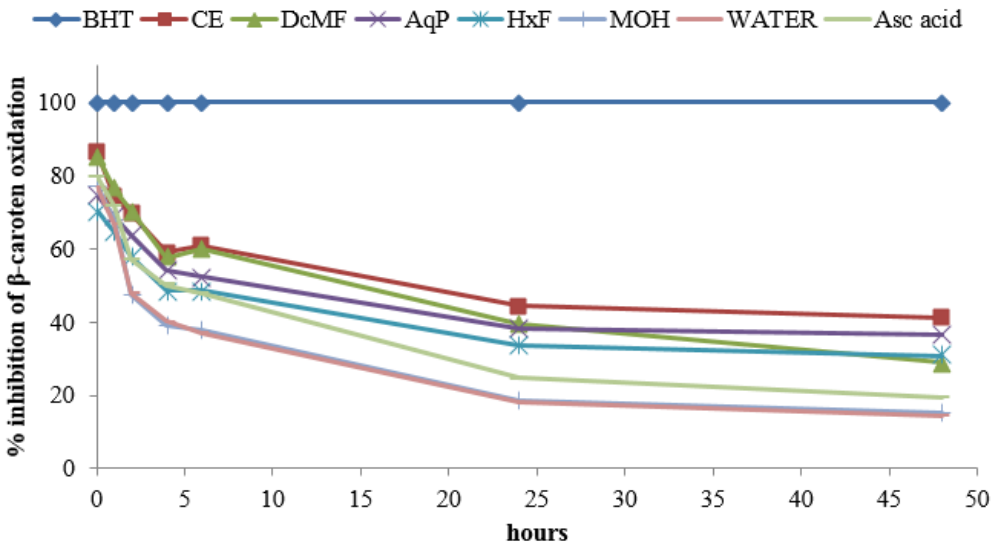

Figure 3: Inhibition of β-carotene oxidation by *P. tuberosa* L leaves extracts
CE: crude extract, DcMF: dichloromethane fraction, HxF: hexane fraction, AqP: aqueous phase, reference antioxidants BHT and ascorbic acid and negatives controls: water and methanol.

P. tuberosa L leaves crude extract; the antioxidant BHT and dichloromethane fraction have a better protective effect against oxidation of β-carotene than the other fractions. The kinetics of the oxidized linoleic acid reducing reaction through β-carotene and/or the extract shows a decrease of β-carotene chromophore during the first incubation hours, until 24h which was stable threshold up to 48h for all extracts.

Thus the controls apart for the dichloromethane extract, where its effect was not stable after 24h but its protective effect against β-carotene oxidation was decreased which may be explained by the stability of the glycosides polyphenols in this extract. The percentage inhibition after 48 h of incubation indicated that the methanol crude extract had a moderate inhibition effect of 41% followed by the aqueous one 37%, whereas the hexane fraction 31% and the dichloromethane fraction 29% had a low protective effect (Fig. 4).

Antioxidant activity of β -carotene is generally due to the polyphenols, flavonoids and tannins contents. Although, the protective effect is stronger than ascorbic acid for all fractions, it remains less than the antioxidant BHT (p>0.05). These results were in agreement with those found for the DPPH test which may still be related to the low polyphenol content, or the possibility of the antagonistic effect of mixed secondary metabolites in the extract.

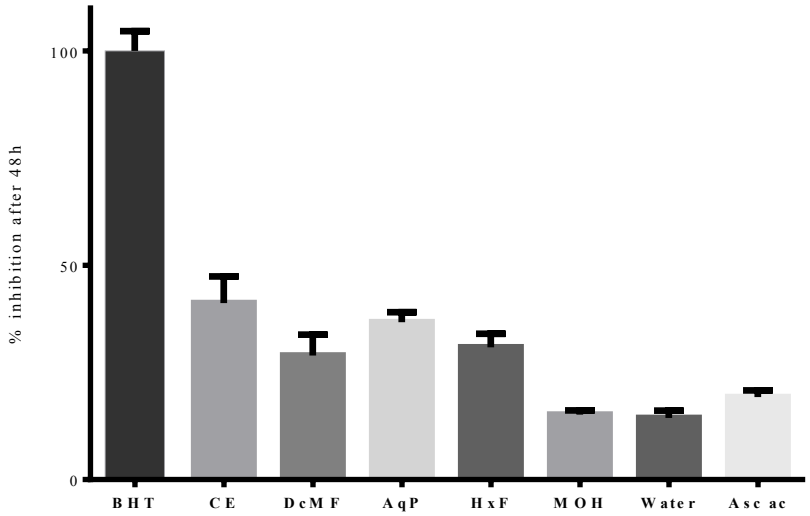

Figure 4: Inhibition percentage of β-carotene after 48 h by *P. tuberosa* L. leaves extracts.
CE: crude extract, DcMF: dichloromethane fraction, AqP: aqueous phase and HxF: hexane fraction, antioxidant references: BHT and ascorbique acid and negatives controls: water and MOH: methanol. Each value represent mean±SD (n=3). Comparisons are made with the positive control.

3.3. *Metal chelating activity*

The presence of traces of transition metals (Fe^{++}, Cu^{+}) leads to the formation of the hydroxyl radical OH•, through Fenton reaction, so to prevent the harmful effects of this toxic radical, the detection of metal chelators is indispensable compared with EDTA (Fig. 5, Fig. 6) (chelating agent).

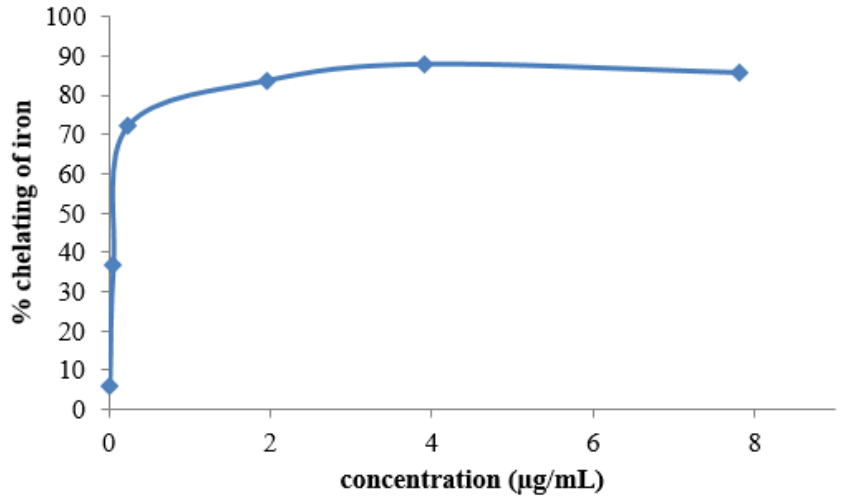

Figure 5 : Percentage of chelating of iron as a function of different concentrations of EDTA

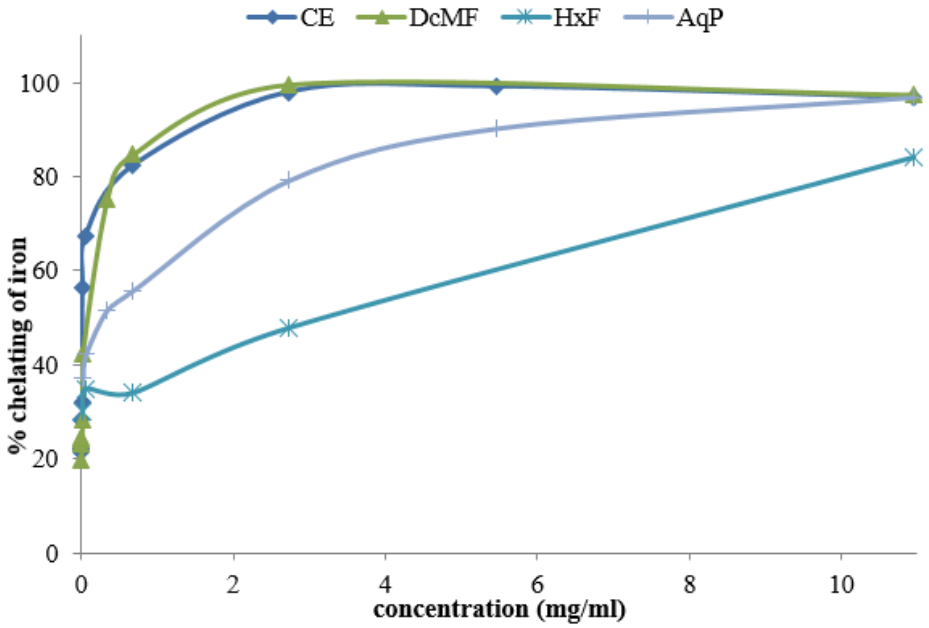

Figure 6: Percentage of chelating of iron as a function of different concentrations of *P. tuberosa* L. leaves extracts.

DcMF: dichloromethane fraction, CE: crude methanol extract, HxF: hexane fraction, AqP: aqueous phase.

The results show that iron chelating was in a dose-dependent manner and the best chelating power was recorded with the methanol crude extract and the dichloromethane fraction at 97% iron chelating in the reaction medium, followed by the aqueous phase by 96% chelating and the hexane fraction by 84% iron chelating. In general, the chelating effect of the extracts is lower than that of standard EDTA, where at low concentration of 7 µg/ml had a chelating effect of 88%. The chelating power was determined by the EC_{50} effective concentration that cause 50% of iron chelating in the reaction medium and which was determined using mathematical models expressing the percentage of chelating as a function of the extract concentrations (Table 4).

Table 4 : Mathematical models expressing the chelating percentage as a function of the concentrations

Chelator	Models	R^2
Crude brut	%Ch = 9.00ln(C) + 84.27	0.945
Dichloromethane fraction	%Ch = 8.61ln(C) + 80.83	0.926
Aqueous phase	%Ch = 10.23ln(C) + 67.37	0.910
Hexan fraction	%Ch = 4.45C + 35.34	0.974
EDTA	%Ch = 13,16ln(C) + 67,68	0,958

The models used to determine the EC_{50} of each chelator are representative, and the coefficients of determinations corresponding to each model R^2 are approaching to 1, the EC_{50} values are presented in figure 7.

It appears that the methanol crude extract has the best chelating effect with an EC_{50} = 0.02 $mg.ml^{-1}$ followed by the dichloromethane fraction with EC_{50} = 0.03 $mg.ml^{-1}$ and the aqueous phase has a weak chelator effect with EC_{50} = 0.15 $mg.ml^{-1}$. Difference was not significant with the reference EDTA with EC_{50} = 0.17 $\mu g.ml^{-1}$, ($p<0.05$), for the hexane fraction whereas the EC_{50}= 3.264 $mg.ml^{-1}$ was significantly different from the reference ($p>0.05$).

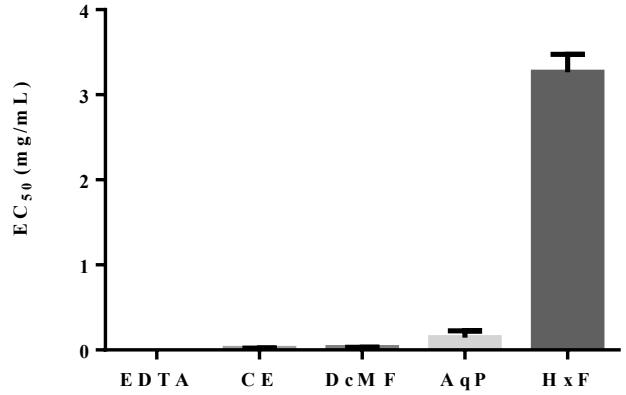

Figure 7. EC_{50} of the methanol crude of *P. tuberosa* L. leaves extract, its fractions and EDTA as reference chelator

Each value represent mean±SD (n=3). Comparisons are made with the positive control.

DISCUSSION

1. Concentrations of polyphenols, flavonoids and condensed tannins

Aqueous phase seems to be the most rich in polyphenols, flavonoids and condensed tannins fraction. The very polar phenolic acids and the addition of water to the extraction solvent increases the polarity and gives a better yield as reported by Salikas *et al.* [26], which according with our obtained results. Rammamurthy *et al.* [11], also mentioned that *Polianthes tuberosa* leaves are rich in glycosides flavonoids, kaempferol and kaempferol- 3-O-xyloside and kaempferol-3-4'-O-dixyloside. Concerning tannins, our results are in agreement with those of Ghedadba *et al.* [20], where the tannin content is low in the crude extract and the dichloromethane fraction of *Marrubium deserti* de Noé whereas this content is high in the aqueous fraction, while flavonoids level is high in the methanolic extract of this plant.

2. Antioxidant activity

Crude and dichloromethane fraction of *Polianthes tuberosa* L. leaves extracts seems to exhibit a good antioxidant activity, where no significant differences were found with controls. Our results for DPPH test are in agreement with those obtained by Moussa *et al.* [14], where the inhibition percentage of DPPH at 50 µg/ml of tuberose methanolic leaves extract was 12% and the IC_{50} of ascorbic acid is 11.5 $\mu g.ml^{-1}$. The antiradical power is related to the content of polyphenols and its nature or is due to the antagonism effect of compounds which can be found in the leaves crude extract and thus its fractions [14]. Comparing our results with those of the other parts of the plant, it is reported that the methanolic flowers extract has a potent antiradical effect by IC_{50} = 0.01 $mg.ml^{-1}$, followed by dichloromethane extract by IC_{50} = 0.02 $mg.ml^{-1}$, and the hexane extract by IC_{50} = 3.42 $mg.ml^{-1}$ [17], these results are consistent with our results.

Concerning the bulbs, they had a powerful scavenger effect compared to leaves where the IC_{50} of the methanol extract of the bulbs is 0.07 $mg.ml^{-1}$, followed by the hexane fraction and the chloroform fraction by IC_{50} = 0.08 and 0.09 $mg.ml^{-1}$ respectively, and the aqueous phase by IC_{50} = 0.11 $mg.ml^{-1}$ [16]. For β-carotene test, according to Belhadj *et al.* [27], the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants. Concerning metal chelating activity, no work has been found about *Polianthes tuberosa* chelating iron activity. The chelating effect of tuberose leaves extracts is due to the polyphenol content for all extracts, and it may also be due to tannins which have the ability to precipitate metals [28].

Our results are in agreement with those of Desta *et al.* [29] where the aqueous phase of the leaves of *Agastache rugosa* (Lamiaceae) has a good iron chelating activity.

CONCLUSION

This study highlighted the possible exploitation of *Polianthes tuberosa* L. for its leaves antioxidant activity. Leaves crude extract and its fractions are rich in flavonoids and condensed tannins, where the aqueous phase has the highest level, and they are poor in polyphenols, where the best content is found in the dichloromethane fraction. Leaf crude extract has a powerful effect compared to the other fractions (IC_{50} = 0.2914 $mg.ml^{-1}$ for DPPH test, 41% inhibition of β-carotene oxidation after 48 hours and EC_{50} = 0.0193 $mg.ml^{-1}$), also extraction with methanol-water gives a better extraction yield and therefore it can be recommended for *Polianthes tuberosa* L. leaves. Further studies are needed to determine the antioxidant effect of this plant leaves *in vivo* and to study their toxicity as well as characterization of its extracts phenolic compounds.

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