# OPTIMIZATION OF PHENOLIC COMPOUNDS EXTRACTION CONDITIONS FROM ARTICHOKE (*Cynara scolymus* L.), ANTIOXIDANT ACTIVITY AND COMPARISON BETWEEN FOLIN-CIOCALTEU AND UV METHODS FOR TOTAL PHENOLIC CONTENT QUANTIFICATION

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**Abstract.** Artichoke (*Cynara scolymus* L.) is well known for its various health benefits, most of which are related to the phenolic composition. The present study concerns the optimization of phenolic compounds extraction conditions from edible part of artichoke (*Cynara scolymus* L.), the assessment of antioxidant activity and the comparison of two methods for total phenolic content quantification by sperctrophotometry, Folin Ciocalteu and UV methods. The investigated parameters included methanol concentration, solvent to solid ratio and extraction time. It was found that methanol concentration and solvent to solid ratio are the most significant factor that influences the TPC. The optimal extraction conditions were found to be: 24 hours extraction time, 10 ml for solvent volume and 100 % for methanol concentration. The second variable, the DPPH scavenging capacity was strongly affected by all the studied factors. The best experimental conditions are different from those of TPC. They were found to be 1 hour extraction time, 10 ml for solvent volume and 80 % for methanol concentration. The experimental results obtained revealed a poor correlation between TPC and DPPH scavenging capacity. The last studied response is the TPC based on UV method to TPC based on FC method ratio. We had recorded a significant effect of extraction time and methanol concentration; we had recorded also a positive and significant correlation between the phenolics content determined by using the two different analytical methods. The results suggest that the UV method can be employed as an efficient and fast tool for the determination of total phenolic compounds in artichoke samples.

**Keywords:** *Cynara scolymus* L.; antioxidant activity; phenolic compounds; Folin-Ciocalteu; UV method.

## INTRODUCTION

Phenolic compounds are the most commonly used and important class of natural antioxidants [33, 60]. These compounds are the most widely distributed group of secondary metabolites in the plant kingdom and are ubiquitous in all plant organs. These bioactive substances are usually produced as a response to defend plants against pathogens and stress, and they are responsible in part for the organoleptic properties of plant foods. More than 8000 phenolic structures are currently identified and these ranged from simple molecules such as phenolic acids to highly polymerized substances such as tannins [15].These secondary metabolites have received increasing interest in recent years from consumers and the food industry due to their flavor, color, biological properties and preventive effect against diseases associated with oxidative stress [9, 26]. Extraction of bioactive compounds from plant material is the initial and the most important step in both the analysis and exploitation of phenolic compounds [15, 65]. Several novel extraction techniques have been investigated for the extraction of phenolic compounds from plant material with the aim of improving the efficiency, extract quality, extraction time and solvent consumption [8]. However, conventional methods (maceration, decoction and Soxhlet) are still hugely preferred over newer techniques (ultrasound assisted extraction, microwave assisted extraction and supercritical fluid extraction). Indeed, a total of 889 publications were recorded for phenolics based on conventional methods in comparison to 521 publications recorded for extraction of phenolic with newer technique [27]. Maceration is a traditional method which presents the advantage to be simpler, more suitable and economical in terms of instrumentation [46, 74]. The optimal extraction method should be simple and rapid for analytical and industrial applications [12, 52, 55]. The efficiency of an extraction method is influenced by several parameters, such as the chemical nature of the sample, the solvent used, agitation, extraction time, solute/solvent ratio and temperature [17, 22]. It must be noted, however, that many phenolic compounds are easily hydrolyzed and oxidized. The use of long extraction times and high temperatures increases the chance of oxidation of phenolics and this decreases the yield in the extracts [63]. Therefore, maceration methods must be carefully developed and evaluated.

A number of spectrophotometric methods have been developed for quantification of plant phenolics. These assays are based on different principles and are used to determine different structural groups present in phenolic compounds [48]. The most abundant used method in the assessment of total phenolic content of plant extracts is the Folin-Ciocalteu method (FC method). It's well known for its simplicity, easiness and availability of the chemical reagent [41]. A disadvantage of the FC method is that it is nonspecific and can be affected by other non-phenolic reducing molecules as organic acids (ascorbic, citric and tartric acid), carbohydrates (fructose, glucose, saccharose)

[64]. Furthermore, another faster procedure based on the absorbance of the aromatic ring is employed by some authors in the order to determinate the TPC. It is the direct reading of the absorbance of the sample at 280 nm (UV method) [30, 32, 60, 68]. According to [60], measuring absorption at 280 nm seems preferable to the Folin-Ciocalteu test as it presents a number of advantages such as speed, reproducibility and assessment without reagent. However, some molecules, including cinnamic acids and chalcones, have no absorption maximum at this wavelength.

Artichoke, scientifically called (*Cynara scolymus* L.) is a diploid (2n=34), belonging of the Asteraceae family. It is a perennial herbaceous plant with typically Mediterranean cycle. It is a highly appreciated vegetable in the Mediterranean diet due to its high nutritional and pharmacological values that are related to its composition which includes high mineral content (sodium, calcium, potassium, iron, magnesium and phosphorus), vitamins (mainly vitamin C), and high levels of inulin, which has an interesting prebiotic action on intestinal bifidobacteria [21, 53].

The edible part of the plant is the enlarged receptacle and the tender bases of bracts, which is the immature Compositae (Asteraceae) inflorescence, used worldwide as both a fresh and canned delicacy product [37, 51]. The edible portion represents 30-40 % of the plant fresh weight, depending on the variety and the harvesting time [37]. Not only is artichoke a tasty food in the Mediterranean diet but also known since ancient times in folk medicine for its choleretic and diuretic effects [38]. Indeed, previous studies had confirmed that artichoke is a very good source of bioactive phytochemicals. It is known that it has a marked health protective capacity: *in vivo* and *in vitro* studies have shown its hepatoprotective functions and the inhibition of cholesterol biosynthesis in hepatocytes [20]. The healthy properties of artichoke extracts have always been related to presence of secondary metabolites such as saponins, alkaloids, tannins, steroids and in particular to the polyphenolic fraction [51].

There has been a renewed interest in this crop as a promising source of polyphenols; it represents a good source of phenolic compounds belonging to different classes: benzoic and cinnamic derivatives, flavonoids and tannins well-documented in literature. Hydroxycinnamoyl derivatives and caffeic acid derivatives are the main phenolic compounds in artichoke, with a wide range of caffeoylquinic acid derivatives with chlorogenic acid (5-O-caffeoylquinic acid) as the most important of these derivatives. Other phenolics such as the flavonoids apigenin and luteolin (both present as glucosides and rutinosides) as well as different cyanidincaffeoyl glucoside derivatives have been identified in artichoke tissues [20, 33, 51, 54]. Artichoke phenolics, separately or in a synergistic way, are effective antioxidants enable to scavenging peroxyl radicals and, in some conditions, hydroxyl radicals *in vitro* [36].

The aim of the work described here was to use maceration as an efficient method for the extraction of phenolic compounds from artichoke. The present study Concerns the optimization of phenolic compounds extraction conditions from edible part of artichoke (*Cynara scolymus* L.), the assessment of antioxidant, activity and the comparison of two methods for total phenolic content quantification by sperctrophotometry, Folin-Ciocalteu and UV methods.

## MATERIALS AND METHODS

### Plant material and preparation of extract

The artichokes used in experiments were purchased from the local market, Algiers, Algeria. Leaves stem and bracts were manually removed, then the edible parts were recovered and ground using a coffee blender and immediately used for the extraction to avoid the oxidation of phenolic compound.

### Standards and reagents

All chemicals used are of analytical grade. Folin-Ciocalteu's phenol reagent, Gallic acid, DPPH (1,1-Diphenyl-2-picrylhydrazyl), Sodium carbonates and pure methanol, BHT and Ascorbic acid were purchased from Sigma-Aldrich. All aqueous solutions were prepared using bi-distilled water.

### Extraction of phenolic compounds

For the extraction of phenolic compounds, 1 g of sample was extracted with different volumes of methanol at different concentration ranging from 0%, 30%, 50%, 80%, and 100%. Then the mixtures were incubated at different time at ambient temperature. The extraction was carried out in an orbital shaker at 150 rpm. At the end of the extraction, the mixtures were centrifuged at 6000 g for 20 min. The supernatants were recovered and filtered through Whatman paper and the filtrates were stored at 4° C prior to analysis.

### Total phenolic content TPC

The phenolic content of extract was determined by use of two different methods.

*Method 1* (Folin-Ciocalteu method): in this method total phenolic content (TPC) of samples was colorimetrically measured according to previous work, with some modifications. This method is based on the reduction of a phosphowolframate–phosphomolybdate complex to blue products by phenolic compounds. An aliquot (200 µl) of appropriately diluted extract was combined with 1 ml of Folin-Ciocalteu's reagent, and the mixture were allowed to react. After 5 min, 1 ml of $Na_2CO_3$ (7.5% w/v) solution was added and shaken. The solution was kept in the dark under ambient conditions for 30 min to complete reaction. After that the absorbance was measured at 750 nm using a UV-VIB spectrophotometer (Shimadzu UV-1605).

*Method 2* (UV method): Based on direct reading of absorbance of samples and standard at 280 nm [59]. This is a faster procedure based on the absorbance of the aromatic ring [68]. In both cases, values of TPC were estimated by comparing the absorbance of each

sample with a standard response curve generated using Gallic acid.

In both methods, results are expressed as mg Gallic acid equivalents (GAE) on a dry mass basis (mg GAE/100 g dry mass).

**Antioxydant activity by DPPH method**

Free-radical scavenging capacity of extract was assessed *in vitro* by DPPH assay according to standard method with some adaptations [66]. This assay is based on the principle that DPPH* on accepting a hydrogen (H) atom from the scavenger molecule i.e. antioxidant (extract), resulting into reduction of DPPH* to $DPPH_2$, the purple color changes to yellow with concomitant decrease in absorbance at 517 nm. In brief, 25 µl of extract were added to 975 µl of DPPH solution (60 µM). The reaction mixture was left in the dark under ambient conditions for 30 min. After incubation the absorbance of the solutions was measured at 517 nm. The radical scavenging capacity (RSC) expressed as a percentage was calculated by the following equation:

$RSC\ (\%) = (Abs_{control} - Abs_{sample}) / Abs_{control}$

Where, $Abs_{control}$ is the absorbance of DPPH in methanol; $Abs_{sample}$ is the absorbance of DPPH solution mixed with sample. All measurements were performed in triplicate (n = 3).

**Statistical analysis**

The data reported in this paper are the averages of three replicates. The experimental data were analyzed using the statistical software Minitab 17. The significance of the difference between all the terms was determined by evaluation of variance (ANOVA). Not significant ($p$> 5 %), *: significant ($p$< 5 %)

## RESULTS

**Effect of experimental conditions on the total phenolic compounds content**

In the present study, the impacts of three parameters were investigated (Table 1). The significance of each effect was determined by ANOVA. It was checked using the $p$- values of the F test. Variables or combinations of variables had a significant effect on the response if $p$<0.05 (Table 2).

The result listed in table 2, revealed that the $p$-values were highly significant only for the solvent volume ($p$ =0.000), significant for the methanol concentration with the $p$- value of 0.035. The $p$- values of the other parameters and interactions between parameters were higher than 0.05, it indicated that they were not significant.

**Table 1.** Factor Information

| Factor | Levels | Values |
|---|---|---|
| **Time (h)** | 3 | 1; 12; 24 |
| **Methanol concentration (%)** | 5 | 0; 30; 50; 80; 100 |
| **Solvent volume (ml)** | 4 | 10; 20; 30; 40 |

**Table 2.** Analysis of Variance

| Source | DF | *F*-Value | *P*-Value |
|---|---|---|---|
| **Time (h)** | 2 | 2.42 | 0.111 |
| **Methanol concentration** | 4 | 3.08 | 0.035* |
| **Solvent volume** | 3 | 60.70 | 0.000* |
| **Time*Methanol** | 8 | 2.34 | 0.051 |
| **Methanol*Solvent volume** | 12 | 1.54 | 0.177 |
| **Time *Solvent volume** | 6 | 0.71 | 0.648 |
| **Error** | 24 | N/A | N/A |
| **Total** | 59 | N/A | N/A |

Note: DF: degree of freedom; N/A: not available; $R^2$ (determination coefficient) = 90.93%; R (correlation coefficient) = 0.95

**Effect of the extraction solvent**

The effects of the methanol concentration in extracting polyphenols were studied by testing 5 levels of methanol concentration (0 %, 30 %, 50 %, 80 %, and 100 %). These results showed that the total phenolic compounds content (TPC) varied greatly among different concentrations of methanol with a $p$-value of 0.035. This indicated the possible influence of methanol concentration on total phenolic contents. An increase in the TPC was recorded with the increase of methanol concentration as illustrated in Figure 1.

Among all the extracts, 100 % methanol was found to be the most efficient solvent for extracting maximum of phenolic compounds from artichoke samples, when compared with all other solvent systems used. The level of these compounds ranged from 2.8 to 171.9 mg GAE * 100 $g^{-1}$ dry weight with 100 % methanol.

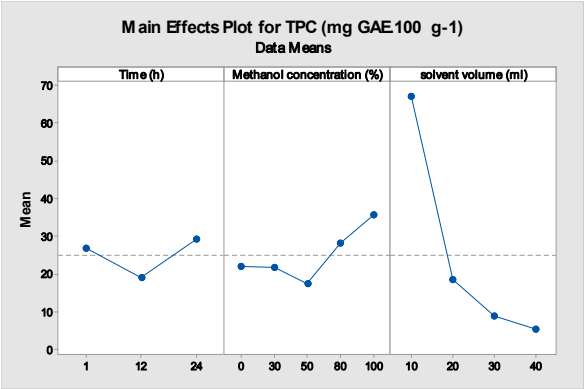


**Figure 1.** Main effect plots for the TPC (mg GAE * 100 $g^{-1}$ dry mass)

**Effect of solvent volume**

The influence of solvent to solid ratio was studied by carrying out extraction of the same quantities of artichoke samples (1 g) in different volumes of solvent. Various solvent volumes were tested (10 ml, 20 ml, 30 ml, 40 ml). It can be seen from the results in Table 2 that the TPC was strongly affected by the volume of solvent with $p$- value of 0.000. The effect of this parameter was illustrated with the two other variables shown in Figure 1. The highest values of TPC were recorded by 10 ml of solvent.

**Effect of extraction time**

The results suggested that the extraction time had a negligible effect on the recovery of total phenols with a *p*- value of 0.111 > 0.05.

**Optimization of the response**

The optimization of extraction conditions were carried out using Minitab statistical software based on the experimental results in order to maximize the recovery of total polyphenols extracted from the vegetative parts of artichoke samples. The final results suggested that the extraction of 1 g of artichoke sample in 10 ml of methanol (100 %) for 24 hours was the best combination which allows obtaining the highest recovery of total polyphenols (Table 3). As represented in Figure 2, the extraction time of 24 h allowed recovering the highest amount of phenolic compounds for all the solvent volumes.

**Table 3.** TPC values under optimal conditions of methanol concentration (100 %), extraction time (24 h) and solvent volume (10 ml)

| Response | Predicted value | Experimental value |
|---|---|---|
| TPC (mg GAE.100g$^{-1}$ Dry mass) | 142.08 (SD: 64.78) | 171.9 (SD: 20) |

Note: SE: standard deviation

**Effect of the experimental conditions on the DPPH scavenging activity**

Overall, the results obtained revealed that the $R^2$ value is closer to one (0.9457); it implied a good correlation between observed and predicted values, and suggested that the model as fitting could explain 94.57 % of the results. According to the statistical results, the developed model can be applied efficiently in the prediction of the responses.

The significance of each parameter was determined using *F* Tests and *p*-values (Table 5).

**Table 4.** Factor Information

| Factor | Levels | Values |
|---|---|---|
| Time (h) | 3 | 1; 12; 24 |
| Methanol concentration (%) | 5 | 0; 30; 50; 80; 100 |
| Solvent volume (ml) | 4 | 10; 20; 30; 40 |

**Table 5.** Analysis of the variance

| Source | DF | F-Value | P-Value |
|---|---|---|---|
| Time (h) | 2 | 16.58 | 0.000 |
| Methanol concentration | 4 | 55.69 | 0.000* |
| Solvent volume | 3 | 10.16 | 0.000* |
| Time*Methanol | 8 | 8.95 | 0.000* |
| Time *Solvent volume | 6 | 2.08 | 0.093 |
| Methanol*Solvent volume | 12 | 3.94 | 0.000* |
| Error | 24 | N/A | N/A |
| Total | 59 | N/A | N/A |

Note: DF: degree of freedom; N/A: not available; $R^2$(determination coefficient) = 94.57 %; R (correlation coefficient) = 0.972

The result listed in table 5, revealed that the *p*-values were highly significant for the solvent volume, methanol concentration, extraction time, interaction between extraction time and methanol concentration, and the interaction between methanol concentration and solvent volume with the *p*-values lower than 0.05. Only the interaction between extraction time and solvent volume did not have an impact on the antioxidant capacity with the *p*- values of 0.093 (>0.05).

**Effect of methanol concentration**

The percentage of inhibition of the DPPH radical was ranging between 4 and 70.9 %. Despite some of our extract showed a good antioxidant capacity, it can be seen that the capacity of inhibition of the DPPH radical by BHT and Ascorbic acid was better.

In our study, methanol concentration was the main factor influencing the antioxidant capacity with the highest *F* value (55.69). Indeed, it can be clearly seen that the percentage of DPPH inhibition increase with the increase of methanol concentration. An increase in antioxidant capacity was observed with increasing of solvent concentration. The maximum yield was registered by 80 % methanol, there after it decreased at the higher concentrations as shown in Figure 2.

The evaluation of antioxidant capacity by determination of the efficiency concentration inhibiting 50 % of DPPH molecules ($IC_{50}$) supported the first results. Only the extracts obtained by 80 % methanol have reached 50 % of inhibition. The $IC_{50}$ is also lower than the $IC_{50}$ recorded for BHT and Ascorbic acid.

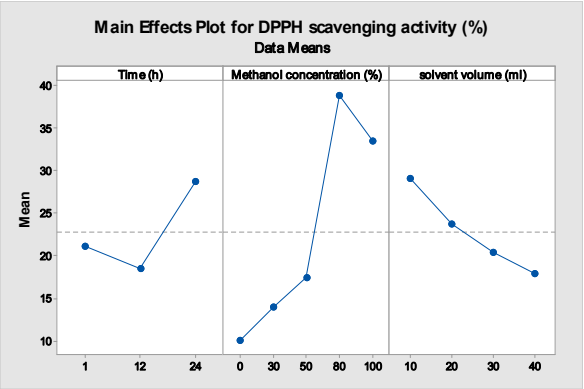


**Figure 2.** Main effect plots for the DPPH scavenging capacity of the phenolic compounds

**Effect of solvent volume**

It can be observed that solid to solvent ratio exert significant effect on the DPPH scavenging capacity (*p*-value = 0.000<0.05). 1:10 appeared to be the best solid- solvent ratio for extracting antioxidants and antioxidant activity in artichoke samples. The lower solvent volume (10 ml) showed the best TFC and DPPH scavenging activity as shown in figure 2.

**Effect of extraction time**

According to results listed in Table 5, the extraction time had significant effect (*p*<0.05) on DPPH scavenging capacity.

The maximum concentration of phenolic compounds was achieved at 24 hours extraction time. In term of antioxidant capacity, it was observed that the DPPH scavenging activity decreased after reaching a maximum value at 1 hour (Figure 2).

**Optimization of the response**

The optimization of extraction conditions were carried out using Minitab statistical software based on the experimental results in order to maximize the DPPH scavenging capacity of total polyphenols extracted from the vegetative parts of artichoke. The final results suggested that the extraction of 1 g of artichoke sample in 10 ml of 80 % methanol for 1 hour was the best combination which allows obtaining the highest antioxidant capacity (Table 6).

**Table 6**. Antioxidant capacity under optimal conditions (methanol concentration (80 %), extraction time (1 h) and solvent volume (10 ml)

| Response | Predicted value | Experimental value |
|---|---|---|
| **DPPH scavenging capacity (%)** | 66.19 (SD: 30.6) | 70.90 (SD: 5) |

Note: SD: standard deviation

The results listed in the table 6revealeda value of 70.90 % for the observed antioxidant capacity which is in close agreement with the predicted value (66.19 % ± 30.6) and the relative error was found to be very low (4.8 < 5). Because of the lower relative error obtained by comparison of the predicted and observed values the adequacy of the model in predicting the response was confirmed. Based on the results listed in Table 3 and Table 6, the found best conditions for maximize TPC are different from those of DPPH scavenging capacity.

**Comparison between the UV method and the Folin-Ciocalteu method (FC method)**

The results of statistical analysis of the impact of the three studied parameters on the ratio TPC (280)/ TPC (FC) are summarized in Table 7. The ratio was mainly influenced by the methanol concentration, extraction time and the interaction between them. The *p*- values were lower than 0.05, with a high $R^2$ value (96.05%).

**Table 7.** Analysis of the variance

| Source | DF | F-Value | P-Value |
|---|---|---|---|
| **Time (h)** | 2 | 14.94 | 0.000* |
| **Methanol concentration** | 4 | 106.93 | 0.000* |
| **Solvent volume** | 3 | 0.84 | 0.487 |
| **Time*Methanol** | 8 | 12.25 | 0.000* |
| **Time *Solvent volume** | 6 | 0.74 | 0.622 |
| **Methanol*Solvent volume** | 12 | 1.79 | 0.109 |
| **Error** | 24 | N/A | N/A |
| **Total** | 59 | N/A | N/A |

Note: DF: degree of freedom; N/A: not available $R^2$ (determination coefficient) = 96.05%; R (correlation coefficient) = 0.98

Solvent system without methanol (0 % methanol): The TPC determined by direct absorbance at 280 nm is three times higher than the value of TPC measured by the FC. It means that the UV method overestimated the TPC.

Solvent system with methanol: We have noticed that the ratio (UV / FC) decreased proportionally with the increase of the methanol concentration as illustrated in Figure 3.

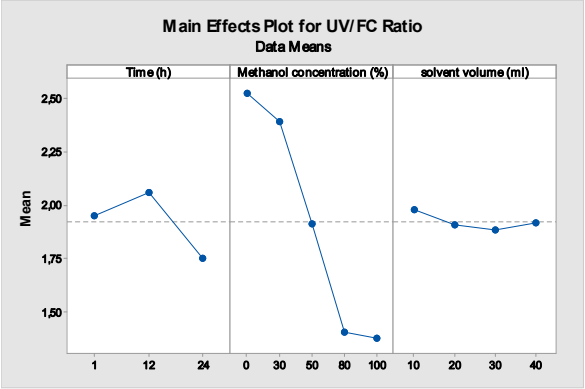


**Figure 3.** Main effect plots for the TPC based on UV / TPC based on FC ratio

Based on statistical analysis; a very high correlations were revealed between TPC based on UV method and TPC based on FC method. The calculated correlation coefficients (R) are between 0.988 and 0.996 for the methanol concentrations (Fig. 4). These results are in agreement with those reported by [76]. They confirmed the existence of positive and significant correlation between the two analytical methods with a correlation coefficient (R) of 0.94.

## DISCUSSION

**Effect of experimental conditions on the total phenolic compounds content**

Several factors such as type and concentration of solvent, temperature, time and pH significantly affect the quantity of polyphenols [69, 70].

**Effect of the extraction solvent**

Amongst the extraction parameters, solvent extraction plays a pivotal role in almost of extraction methods. The recovery of phenolic contents is strongly influenced by the polarity of extracting solvents and the solubility of the studied compound in the solvent used for the extraction process [3]. The mainly used solvents are alcohols: the methanol represents the most employed one followed by the ethanol [59, 68, 43]. Methanol is an organic solvent well known for its efficiency in the extraction of phenolic compound comparatively to the other solvent [16, 18, 28, 56]. Several earlier studies have measured the effect of different solvents in total phenolic compounds and mentioned that methanol is the solvent more efficient in the extraction of phenolic compound from different spices of vegetables [35].

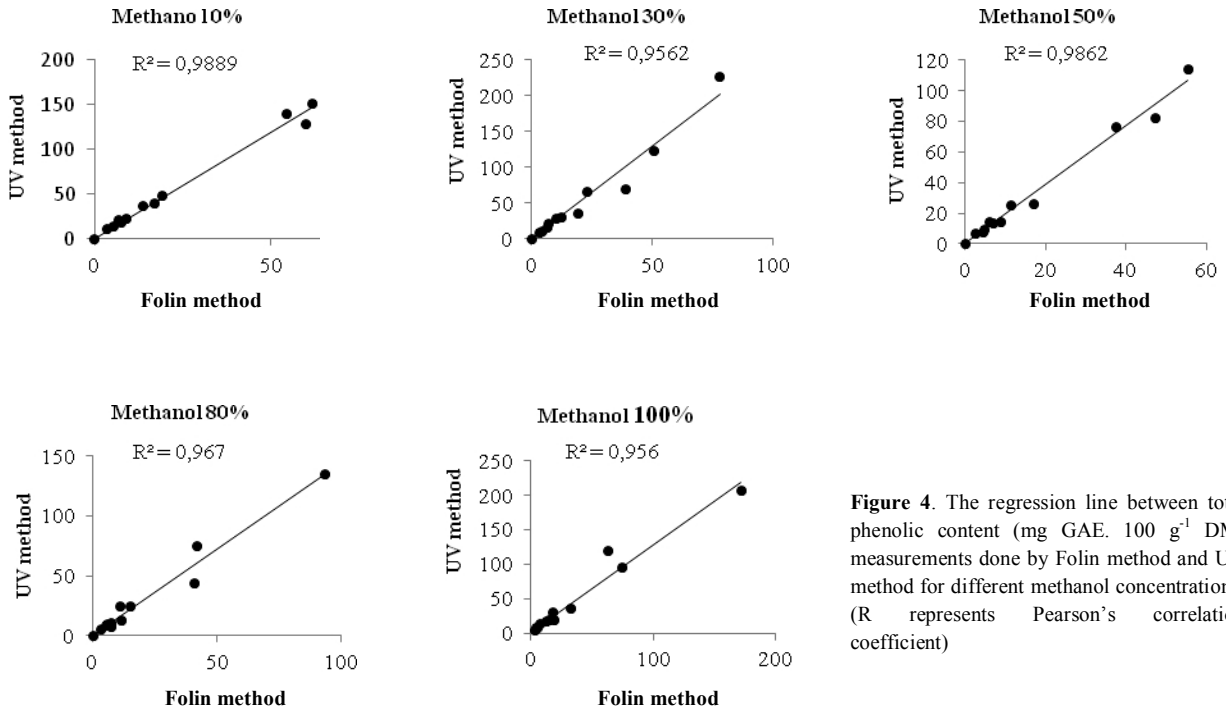


**Figure 4**. The regression line between total phenolic content (mg GAE. 100 $g^{-1}$ DM) measurements done by Folin method and UV method for different methanol concentrations. (R represents Pearson's correlation coefficient)

Methanol (100 %) is more appropriate to be used for extraction of phenolic compounds from different samples of artichoke than the mixture with water. It means that the major part of the phenolic compounds contained by the studied vegetable is not soluble in water. Our results are in agreement with previous works relating to the extraction of phenolic compounds from artichoke [42]. In some case the use of mono-solvent system is recommended. As published by Chew *et al*. [13]; the highest recovery of tannins was exhibited at 100% ethanol concentration (mono-solvent). This result could be due to most of tannins presented in bounded or polymerized forms which were more soluble in moderate polar/ week polar extraction medium. In addition the permeability of solvent into the solid matrix may be achieved by higher concentration of solvent

Our results are in disagreement with the finding of other previous works. They documented that some classes of phenolic compound like phenolic acids present a high polarity and their recovery decreases when the extraction was carried out with pure solvents [69]. In fact only 91.1% of the total phenolic acids were recovered with 100% methanol [39]. Since polyphenols have a wide range of solubility, a mixture of solvent and water may be desirable and effective for separation than single solvents [47, 68]. According to Luthria *et al*. [40], the addition of water to organic solvent induces a swelling of plant material thereby facilitating the penetration of solvent into the solid matrix and improves the extraction of the phenolic compounds. In addition, some phenolic compounds are present in the solid matrix as glycosides, and the sugar makes them more water soluble [2]. Although the addition of water to the solvent system is essential, its quantity must be controlled to avoid the extraction of non-phenolic compounds [25].

**Effect of solvent volume**

The extraction of bioactive compounds from solid matrix to the solvent is related to the solid - solvent ratio. The Total phenolic content is better when a higher solid-solvent ration is used. It may be explained by the principles of mass transfer; the driving force during mass transfer is the concentration gradient between the solid and the bulk of the liquid, the mass transfer of The transfer is better when a higher solid - solvent ratio is used [39, 45]. When the solvent reach his saturation with the extracted phenolic compounds, the gradient becomes null and the phenomena stops [62]. This is not the case of our results.

Similar results regarding the effect of the solvent-to-solid ratio on the extraction of phenolic compounds have been reported by [45]. Some authors reported a great influence of solid to solvent ratio on the TPC in parsley extracts [39]. However in other published studies; the level of solvent to solid ratio was ineffective in the extraction process. It did not contribute to the release of phenolic compounds from plant material cells [25, 44]. This difference can be attributed to the type and the complexity of plant materials studied and also the phenolic compound considered [76].

**Effect of extraction time**

Some studies confirmed the non-significant effect of time extraction on the recovery of phenolic compounds [2]. Nevertheless, other authors reported that extraction time had a pivotal role in the extraction process of phenolic compounds [8]. Based on their

results, some authors have documented that maceration time of 1 h was determined to be sufficient and time efficient [46], in contrary to our results which revealed that the efficient time to recover a maximum of plant phenolic is 24 h.

The mass transfer from plant material to solvent was related to time and temperature. The mass transfer increase with time until the maximum of extraction was achieved [6]. 24 hours represented the necessary incubation duration for solvent to reach the plant matrix and extract a maximum of phenolic compounds. Using a long extraction time generally had only a small positive effect on the extraction yield, while increasing the possibility of degradation of phytochemicals. As stated by [48] and [13], prolonged extraction time would increase the chance for occurrence of oxidation of phenolic compounds.

**Effect of the experimental conditions on the DPPH scavenging activity**

Antioxidants are important substances which possess the ability to protect the cell from damage caused by free radical induced oxidative stress [5]. The antioxidant potential of different plant extracts and pure compounds can be measured using several *in vitro* assays. Each of these tests is based on one feature of the antioxidant activity, such as the ability to scavenge free radicals or the inhibition of lipid peroxidation [10]. In the DPPH test, the ability of the investigated artichoke extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH_-H was investigated. Several extraction procedures using different sequences of solvents at different solid to solvent ratio and times were used in this work to investigate their impact on the DPPH scavenging capacity (Table 4).

DPPH (1,1- diphenyl-2-picrylhydrazyl) is a very stable organic free radical and presents the ability of accepting an electron or hydrogen radical. We have chosen this colorimetric method taking into consideration on its low cost, easiness, its efficiency and fast evaluation of antioxidant activity [1]. The amount of crude extract required for a 50% inhibition of the DPPH radical presents in the reaction mixture (efficient concentration EC 50 % otherwise called the IC50 value) was used as a measure for the radical scavenging activity. This parameter is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) [58]. Strong radical scavenging activity is associated with low EC 50 %values [4].

**Effect of methanol concentration**

In our study, methanol concentration is the main factor influencing the antioxidant capacity with the highest *F* value (55.68). The same result was reported by [13], but the considered solvent was ethanol. It means that the concentration of solvent influence greatly the antioxidant capacity of plant extracts. According to the *p*- values and *F* values, the capacity of inhibition of DPPH radical was significantly affected by the variation of methanol concentrations. It can be clearly seen that the percentage of DPPH inhibition increases with the increase of methanol concentration. This was probably due to the increase in solubility of phenolic compounds.

80 % methanol has a greater effectiveness in extracting the substances which possessed antioxidant properties. Their content decreased at the higher concentrations of methanol, consequently the DPPH activity of extracts decreased as shown in Figure 2.This finding was probably due to the influence exerted by the solvent on the cellular structures [56]. Previous work reported that methanol increased the extraction of flavan-3-ols, it would be expected to also improve extraction of proanthocyanidins, which are oligomers and polymers of flavan-3-ols [46]. These components are known for their antioxidant properties.

Our results are in disagreement with the previous results. It was mentioned that the highest scavenging capacity of phenolic extracted from apple samples are obtained by the methanol without water [45]. Another study reported that 60 % methanol led to obtain the highest antioxidant capacity [4]. It may be due to the difference in the plant materials.

**Effect of solid- solvent ratio**

The concentration of total phenolics and the antioxidant activity of the extracts depend on the solid-solvent ratio. The increase of solvent volume increases both the extraction efficiency and the price of the extract, and decreasing the solvent volume lowers the extraction efficiency due to saturation effects, but decreases the cost [57]. However, this phenomenon was not observed in this study as low solvent volume (10 ml) showed best TFC and DPPH scavenging activity as shown in figure 2. This may be explained by the fact that the interaction among bioactive compounds coming into contact with extracting solvent expands as a result of high extraction solvent and this led to increase the extraction of phenolic components [67, 79].

A similar study was reported by [65] on the effect of solid-to-solvent ratio on the antioxidant properties of phenolics extracted from *Guiera senegalensis*. In that study, the strongest DPPH scavenging capacity was obtained for the same solid–solvent ratio (1: 10). In contrast, [71] documented that the DPPH scavenging capacity increased gradually with the increase of solvent volume from 10 to 60 ml.

**Effect of extraction time**

Extraction time is crucial in minimizing energy and cost of the extraction process [13]. The maximum concentration of phenolic compounds was achieved at extraction time of 24 hours. In term of antioxidant capacity, it was observed that the DPPH scavenging activity was decreased after reaching a maximum value at 1 hour (Figure 2). It is suggested that prolonged extraction time would lead to exposure of more oxygen, light and temperature, and thus increase the chances for occurrence of oxidation on phenolic compounds [48, 15].

In according to other literature results [14, 23, 24, 72, 79], the antioxidant capacity is closely dependent

on the choice of an appropriate extraction time. The extraction time had a remarkable impact on the DPPH scavenging capacity ($p < 0.05$). However the results of the study carried out on 6 varieties of lentil suggested that extraction time had a negligible effect on antioxidant properties of phenolic extracts [2, 46]. This difference can be attributed to the type and the complexity of plant materials studied and also the phenolic compound considered [5, 67, 75]. Also, the contradictory results are most probably due to differences in the experimental conditions used in different assays [10].

**Comparison between the UV method and the FC method**

According to [48], the traditional spectroscopic assays may lead to overestimation of polyphenol contents of crude extracts from plant materials due to the overlapping of spectral responses, and this may be the explication to the overestimation of the TPC by the UV method in the absence of methanol.

We have noticed that the ratio (UV / FC) decreased proportionally with the decrease of the water concentration in the solvent system and, then, inversely with the methanol concentration.

An opposite finding was reported by [68]. They documented that an increase of water content of ethanol was statistically influent in improving extraction yield for UV method. The reduction of water concentration in the solvent system allowed decreasing the extraction of hydro-soluble compounds such as protein which are non-phenolic components and have an absorption maximum at 280 nm. In this way, we will be sure that the measured absorbance at 280 nm did not include non-phenolic compounds. In fact, for the less water concentrations (80%, and 100 % methanol), the ratio is closer to 1. This result signified that both FC method and the UV method gave the same evaluation of phenolic compounds. Previous study supported our result and they reported that the UV method is affected by the nature of solvent employed and the pH of the solution [48]. Moreover, the possibility of interference by UV-absorbing substances such as proteins, nucleic acids and amino acids should be considered.

Contrary to our results, [68] found that the TPC based on UV method is lower to than those based on FC method and they attributed this difference to the different principle of the two analytical methods: reaction with an oxidizing reagent in the Folin–Ciocalteu analysis and absorption of the aromatic ring in direct reading at 280 nm. These contradictory results can be due to the difference in the plant material and in the chemical composition of the studied extracts. Our results are in agreement with those reported by [77]. They confirmed the existence of positive and significant correlation between the two analytical methods with a correlation coefficient (R) of 0.94.

In conclusion, the results showed statistically significant impact of these factors on the total phenolic compounds content. The best extraction yield of phenolic compounds was obtained when extraction was carried out for 24 hours with 100 % methanol and for solid: solvent ration of 1 : 10 (g : ml). Interestingly, these optimal extraction conditions did not match the highest antioxidant activity whose extraction conditions corresponded to 1 h, 80 % methanol and solid: solvent ratio of 1 : 10. These results suggest that when long extraction time is used, phenolic compounds are easily hydrolyzed and oxidized. Moreover, our study showed a strong positive correlation between TPC evaluated by the UV and the FC methods whatever used methanol concentration. As the UV:FC ratio was closer to one, mainly for 80 % and 100 % methanol extraction, that means that the TPC gave by the two analytic methods is similar. Then the UV method can be used to evaluate the total phenolic content since it is direct, faster and easier when compared with FC method.

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