IOSR Journal of Applied Chemistry (IOSR-JAC)
e-ISSN: 2278-5736.Volume 16, Issue 4 Ser. I (April 2023), PP 37-43
www.iosrjournals.org

Datura stramonium: Polyphenols, flavonoids, and antioxidant activity of non-defatted seeds

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Abstract:
In this study we are interested in the extracts of non-delipidated seeds of Datura which are not widely studied. This study aims to optimize the extraction conditions in aqueous medium of polyphenols and to compare the extraction rates in organic solvents at room temperature with that of water at the optimal extraction temperature. We show that the temperature, extraction time and water-to-leaf ratio had significant effects on the yield of extracted polyphenols as well as scavenging and total antioxidant activities. The optimal extraction conditions were 80◦C for 40 min, with a non-delipidated seed/water ratio of 1:100 g/mL. Higher levels of polyphenols and flavonoids were extracted from methanol (5.56±0.06 mg GAE/g and 2.03±0.11 mg QE/g) and acetone (5.92±0.03 mg GAE/g and 2.32±0.03 mg QE/g) compared to ethanol (2.91±0.02 mg GAE/g and 1.39±0.11 mg QE/g) and ethyl acetate (2.16±0.04 mg GAE/g and 0.83±0.05 mg QE/g). The TPC extraction rate in water (5.01±0.14 mg GAE/g) is comparable to the values found for acetone and methanol while the TFC extraction rate in water (0.37±0.01 mg QE/g) is lower than the corresponding values in methanol and acetone. The same trend is obtained in the study of the antiradical activity with DPPH• and CUPRAC. For the DPPH• the TAC is higher for acetone (3.58±0.02 mg TE/g) and methanol (3.55±0.13 mg TE/g) than the value obtained for water (1.95±0.19 mg TE/g). For the CUPRAC the TAC is higher for methanol (11.34±0.22 mg TE/g) and acetone (10.89±0.10 mg TE/g) than the value obtained for water (3.38±0.08 mg TE/g).

Keywords*: Datura stramonium, Seed, Polyphenols, Flavonoids, DPPH•, CUPRAC.*

Date of Submission: 09-04-2023 Date of Acceptance: 23-04-2023

I. Introduction

Oxidative stress generated a lot of free radicals or peroxided that are harmful to the living. Acting on macromolecules, the reactive oxygen species (ROS) induce irreversible damages and causes disastrous diseases such as cancer, diabetes, emphysema, etc. [1]–[4]. Even if they are harmful, they can be useful in the protection of cells against bacteria and viruses [5]. That is why it is important to level and regulate the concentrations of ROS in the organisms. This can be achieved by supplementing the organisms with nutrient capable to neutralize the ROS without being detrimental. That can be attain by using antioxidant like polyphenols. Polyphenols are a large families of compounds [6]–[9] (flavonoids, phenolic acids, coumarins, tannin, lignin …) which are widely distributed in the plant kingdom [10]–[13]. This family of compounds are under scrutiny for several decades to understand their actions; they are normally provided by the consumption of fruits and vegetables. But it is proved that other organs of the plants such as leaves, roots, peels and seeds can be a huge source of those compounds [5], [14], [15]. In this context, *Datura stramonium* known for its narcotic effect and treating asthma [16], [17] offers opportunities. The seeds of *Datura stramonium* are used to attain an hallucinogenic state by smoking it [16] and they can also, when boiled, used as an alternative for atropine. *Datura stramonium* seeds have also an analgesic effects for acute and chronic pain [18]. The consumption of all parts of *Datura stramonium* are known to be toxic for humans and livestock [19], [20].

II. Material and Methods

a. Plant

Datura stramonium seeds were harvested at Mbetit Gouye (region of Fatick in Senegal 14°25’59.99” N, 16°31’59.99” O). The seeds were collected, rinsed and air dry for two weeks. They were grounded using a grinder and stored away from light and air to prevent oxidation. The seeds were not defatted prior to the analysis.

Seeds of *Datura stramonium*

Powder of seeds of *Datura stramonium*

b. Parameters optimization process

Optimization parameters (temperature, duration, and ratio) were conducted using water as solvent and following the procedure used by Gaye et *al*. [14] with some modification. Total polyphenol content (TPC) and scavenging activity by DPPH˙ were used as response value.

For the determination of the optimum temperature (OT), 0.5 g of seeds powder were extracted in 50 mL of distillated water at variable temperature (50, 60, 70, 80 and, 90°C) for 20 minutes using a controlled temperature water bath.

Optimum duration (OD) was determined by setting OT. 0.5 g of seeds powder were weighed and mixed with 50 mL of distillated water at different time (10, 20, 30, 40, 50 and, 60 minutes).

Optimum ratio (OR) was determined by setting OT and OD. Different mass (0,5, 1, 2, 3, 4 and 5 g) were mixed with 50 mL of distillated water.

c. Extraction process

After the optimization, the optimal settings were fixed to make a proper extraction in water. Four organic solvents (Methanol, Ethanol, Acetone and Ethyl Acetate) were also chosen to conduct an extraction; at 1g of non-defatted seed were added 50 mL of the chosen solvent. After 24 h, the mixture is filtered and completed at 50 mL. The different extracts were stored at −20°C until further notice.

d. Total polyphenol content

The total polyphenols content (TPC) was determined using the method developed by Vuong et *al*. [21] with modifications. To 200 µL of diluted sample were added 150 µL of Folin-Ciocalteu reagent, 60 µL of Na_2CO_3 20% and 2.32 mL of distillated water. The mixes were incubated in the darkness at room temperature. After 30 minutes, the absorbance were read at 760 nm using a Perkin-Elmer UV-Vis spectrophotometer Lambda 365. Gallic acid (GA) was used as standard. Results were expressed as mg GAE/g ± Standard deviations.

e. Flavonoids content

The total flavonoids content (TFC) was determined using the method described by Gaye et *al*. [22]. To 2.5 mL of sample were added 2.5 mL of an ethanolic solution of $AlCl_3$ at 2%. The mixes were incubated at room temperature for 1 h. the absorbance were read at 425 nm using a Perkin-Elmer UV-Vis spectrophotometer Lambda 365. Quercetin was used as standard, and the results were expressed as mg QE/g ± Standard deviations.

f. Antioxidant activity

i. Antioxidant capacity and IC50 determination by DPPH˙ method

The antioxidant capacity of the different extracts was determined using the method described by Sy et *al*. [5]. A working solution was prepared by adding 40 mg of DPPH• radical to 1L of Methanol. The methanolic solution of DPPH˙ is stocked at −20°C until use. Firstly, the antioxidant capacity is determined by mixing 200 µL of extracts and 3.8 mL of the methanolic solution of DPPH˙. Secondly, the IC50 were settled by making several dilutions of the extracts. The mixes are incubated for 30 minutes at room temperature in the darkness. The absorbance was

read at 517 nm using a Perkin-Elmer UV-Vis spectrophotometer Lambda 365. TROLOX was used as standard, and the results were expressed as mg TE/g ± Standard deviations.

$$\%\ Inhibition = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}}$$

ii. Antioxidant capacity by CUPRAC method

The CUPRAC method was used to determine the total antioxidant capacity (TAC) of our extracts. The method used was described by Apak et *al*. [23]. At 1.1 mL of extract were added 1 mL of $CuCl_2$ solution, 1 mL of ammonium acetate buffer (pH 7) and 1 mL of a neocuproine solution. The mixture was incubated at room temperature in the darkness for 1 h. The absorbance was read at 450 nm using a Perkin-Elmer UV-Vis spectrophotometer Lambda 365. TROLOX was used as standard, and the results were expressed as mg TE/g ± Standard deviations.

III. Results and discussion

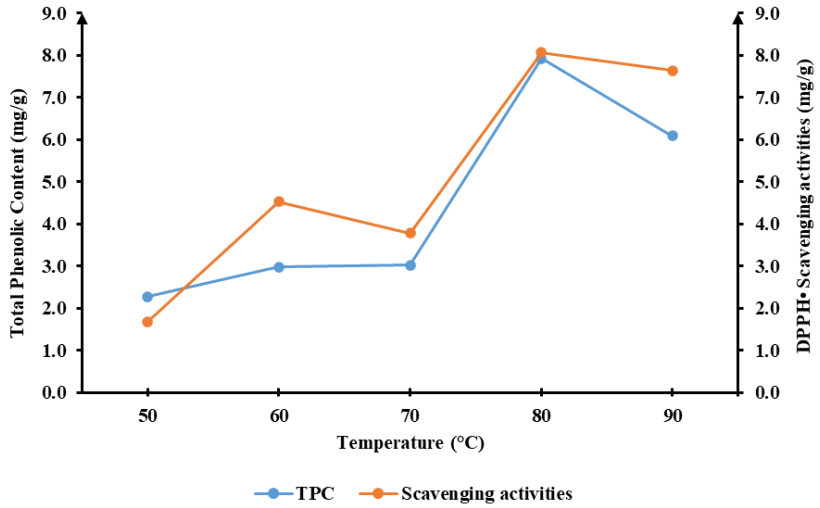
a. Optimization of the extraction

According to the described experimental conditions, Figure 1 summarizes the variations of TPC and the scavenging activities of the free radical DPPH• following the temperature (a), the duration (b) and the ratio (c). Figure 1 shows that these three parameters have an influence on the yield of the polyphenol extraction and the free radical scavenging activities DPPH• on the aqueous extracts of non-defatted seeds of *Datura stramonium*. Between 50 and 80°C, the polyphenol extraction yield increases to reach a maximum at 80°C with a TPC of 7.93±0.28 mg GAE/g. The DPPH• scavenging activity increases as the TPC and reaches a maximum at 8.07±0.15 mg TE/g. After 80°C, the TPC extraction yield as well as the scavenging activity decrease to attain, respectively, 6.10±0.28 mg GAE/g and 7.64±025 mg TE/g at 95°C (Figure 1a). In the following steps the temperature is maintained constant at 80°C. When the time of extraction is varied, the TPC extraction yield and the DPPH• scavenging activity increase until reaching a maximum at 40 minutes with 5.00±0.07 mg GAE/g and 6.05±0.07 mg TE/g, respectively. After 40 minutes, the TPC extraction yield decreases to attain a value of 4.14±0.16 mg GAE/g, and the scavenging activity increases to reach a values of 6.23±0.13 mg TE/g. The compounds extracted after 40 minutes at 80°C have no influence on the scavenging activity (Figure 1b).

The temperature is maintained at 80°C and the time of contact is fixed at 40 minutes. The ratio vegetal material/volume of water is varied from 1 to 10. Figure 1c shows that the TPC extraction yield is more efficient with a ratio of 1% with a TPC of 3.73±0.08 mg GAE/g and a DPPH• scavenging activities of 6.20±0.27 mg TE/g. In fact, when the vegetal material/volume of water increases the TPC extraction yield and the scavenging activity decrease drastically to attain the values of 1.90±0.02 mg GAE/g and 2.25±0.04 mg TE/g, respectively.

The results seem to go according with the observations of Gertenbach et *al*. [24] and Vuong *et al*. [25]. According to these authors, although mass transfer rates increase with increasing temperature, the TPC extraction yield decreases with increasing temperature due to thermal decomposition and epimerization of polyphenols that occur at high temperature. Therefore, the scavenging activity decreases as observed in Figure 1. When the duration of heating is long, decomposition of polyphenol can occur. The TPC extraction yield extraction and the scavenging activity are time dependent. Regarding the ratio, it has been shown that the higher the dilution, the better the yield. Indeed, according to Vuong *et al*. [25] and Gertenbach et *al*. [26], the kinetic of the extraction is accelerated because of the greater concentration gradient between the phenolic compounds trapped inside the leaf particles and those located on the surface.

a



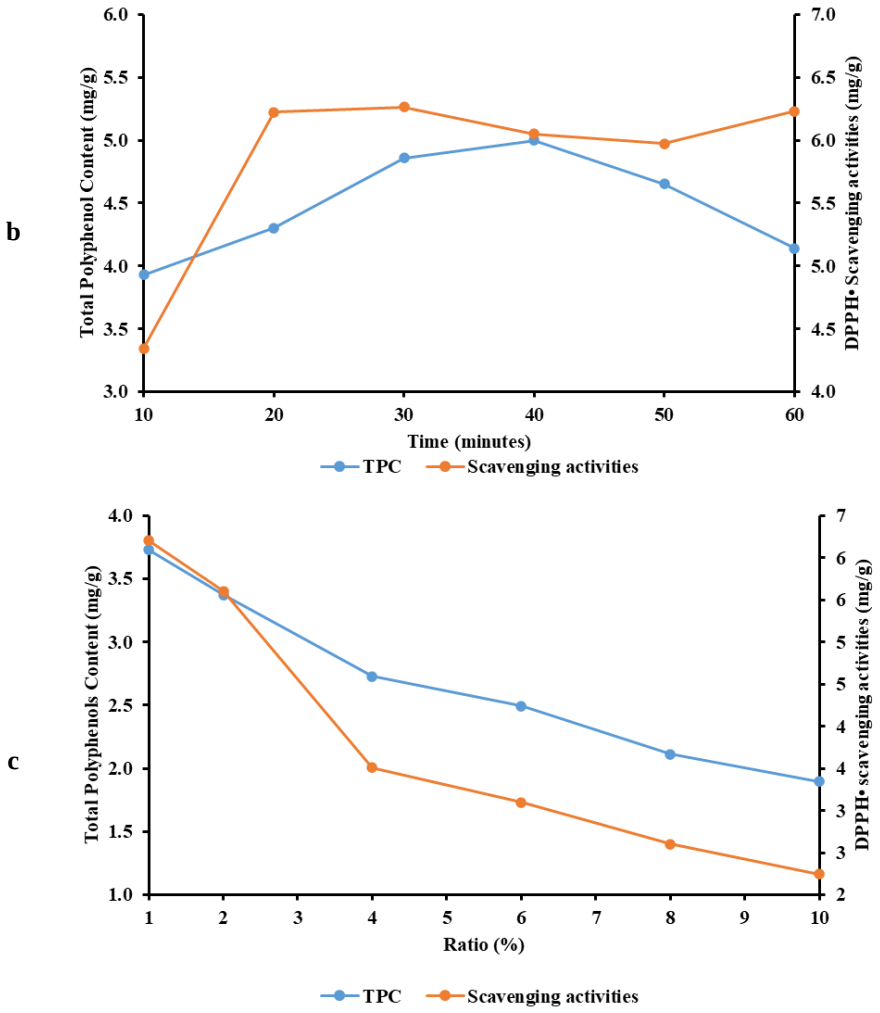


Figure 1. Optimization parameters of the aqueous extraction. Temperature (a), Duration (b) and Ratio (c).

For this present study, the following optimal parameters were used: temperature of 80°C, duration of 40 minutes and ratio of 1%. These parameters are used for the aqueous extraction of polyphenols, flavonoids, and antioxidant activities by CUPRAC and DPPH•.

b. Comparison of the hot water extraction with room temperature solvent extraction

Table 1. Total polyphenol and flavonoid content of water, methanol, ethanol, acetone and ethyl acetate

	Water	Methanol	Ethanol	Acetone	Ethyl Acetate
Total polyphenol content (mg GAE/g)	5.01±0.14	5.56±0.06	2.91±0.02	5.92±0.03	2.16±0.04
Flavonoid content (mg QE/g)	0.37±0.01	2.03±0.11	1.39±0.11	2.32±0.03	0.83±0.05

The results for the quantitative determination of the total polyphenol content are reported in Table 1 and illustrated in Figure 2. The acetone extract is richer than the other extracts with 5.92±0.03 mg GAE/g followed by the methanol extract (5.56±0.06 mg GAE/g) and water extract (5.01±0.14 mg GAE/g). The ethanol and ethyl acetate extract are less rich than the other extract with 2.91±0.02 mg GAE/g and 2.16±0.04 mg GAE/g respectively. Regarding to the polarity and dielectric constant of solvent, water is more polar (μ = 1.85 D, ε = 80) than methanol (μ = 1.70 D, ε = 33) and acetone (μ = 2.88 D, ε = 21). Polyphenols which are polar must be better extracted by polar solvent, therefore TPC extraction yield must be greater in water. The TPC extraction yield by water at 80° C is slightly lower than the yields of extraction of TPC carried out at room temperature by methanol or acetone, despite the stronger polarity of water. This is explained by the partial degradation of polyphenols at

80°C in water. The TPC extraction yield in methanol and acetone are comparable. The less polar solvent such as ethyl acetate (μ = 1.78 D, ε = 6) and ethanol (μ = 1.695 D, ε = 24) gave the lower TPC extraction yield. These results shows that *Datura stramonium* are richer than *Datura metel* seeds [27].

Flavonoids contents are listed in Table 2 and illustrated in Figure 2. Flavonoids are non-polar compounds and must be better extracted by less-polar solvent. The TFC extraction yield is lower in water which is the more polar solvent (0.37±0.01 mg QE/g). The acetone and methanol extract are the richest with 2.32±0.03 mg QE/g and 2.03 mg QE/g respectively. These results are higher than those reported for *Datura metel* [28], [29].

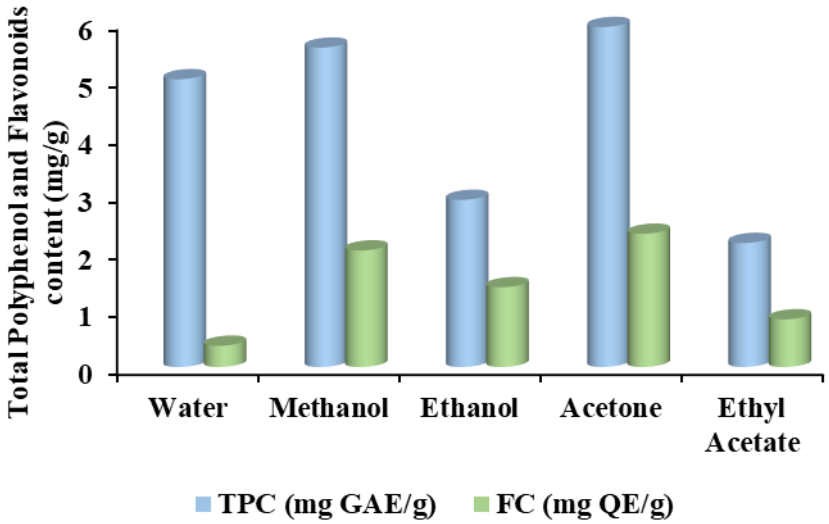


Figure 2. Total Polyphenol and flavonoid content of hot water and solvent extraction

The radical DDPH• is suitable for studying the antioxidant capacity of phenolics compounds which are known for their ability to scavenge free radicals. We conducted a study to measure the effectiveness of extracts for antiradical activity. The methanolic and acetonic extracts are richer in polyphenols and flavonoids and give the best activities of 3.55±0.13 mg TE/g and 3.58±0.02 mg TE/g, respectively (Table 2). The water extract gives a low value of 1.95±0.19 mg TE/g. Comparatively to methanol and extract, the decreases of the scavenging activity in water is due to the partial degradation of polyphenol at 80°C. The ethyl acetate extract have the worst antioxidant capacity by DPPH• (0.17±0.01 mg TE/g) followed by the ethanol extract (1.03±0.11 mg/g). The IC50s were scanned for the free radical DPPH•. For whole extracts no IC50 is obtained. The maximum percentage inhibition as determined for the methanol extract has a value of 28.04% at a concentration of 71.12 µg/mL.

The CUPRAC method is based on the capacity of a sample to reduce Cu^{2+} neocuproine complex in Cu^{+} neocuproine complex. Therefore in the CUPRAC method polyphenols as well as thiol, lipophilic and hydrophilic antioxidants species are incorporated to the reaction [30]. As expected, the methanol and the acetone extracts show high values of 11.34±0.22 mg TE/g and 10.89±0.10 mg TE/g, while water extract show low value of 3.38±0.08 mg TE/g (Table 2).

Table 2. Antioxidant capacity determined by free radical DPPH• and CUPRAC method

	Water	Methanol	Ethanol	Acetone	Ethyl Acetate
Antioxidant capacity by DPPH• (mg TE/g)	1.95±0.19	3.55±0.13	1.03±0.11	3.58±0.02	0.17±0.01
Total antioxidant capacity by CUPRAC (mg TE/g)	3.38±0.08	11.34±0.22	1.91±0.08	10.89±0.10	2.11±0.11

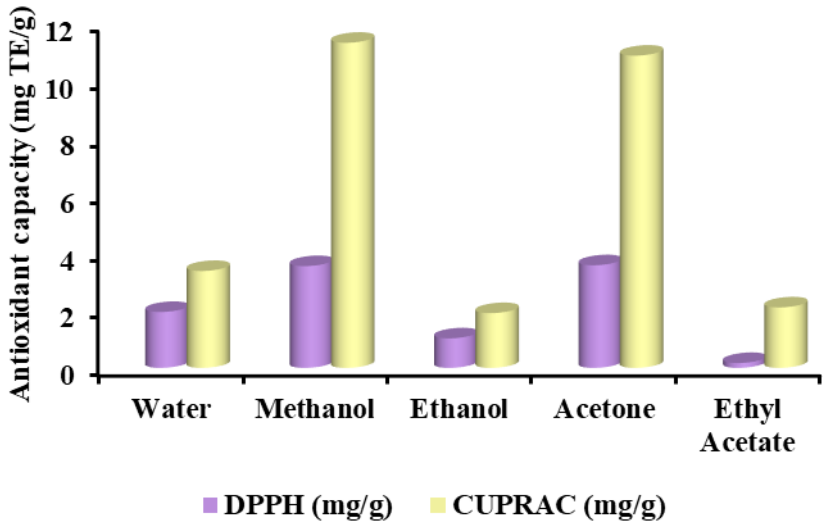


Figure 3. Antioxidant capacity determined by free radical DPPH˙ and CUPRAC method for hot water and different solvents extraction.

IV. Conclusion

In this review, we optimized the extraction in aqueous medium of the polyphenols contained in the non-delipidated seeds of *Datura stramonium*. The optimum parameters are 80° for the temperature, 40 minutes for the contact time and 1% for the plant matter/water volume ratio. We compared extraction in aqueous medium at 80° and extraction with organic solvents. This study showed better results for the use of methanol and acetone for the extraction of polyphenols and flavonoids. These solvents show a greater antiradical capacity with both DPPH and CUPRAC. Moreover, the combination of scavenging activity measurements with a more specific analysis of individual antioxidants would provide a better knowledge of the antioxidant status of non-delipidated *Datura stramonium* seeds.

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