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Evaluation of *in vitro* Antioxidant Activities of Ethanol Extracts of *Datura innoxia* Mill. Leaves and Seeds Harvested in Mali

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Authors' contributions

This work was carried out in collaboration among all authors. Author IT designed the study, participated in phytochemical analyzes, wrote the protocol and wrote the first draft of the manuscript. Author MK did the botanical identification at laboratory of tropical ecology. Authors AAD and ND collected samples, performed extractions and chemical screening and performed the determination of antioxidant activities. Author MAK performed the statistical analysis and participated in determination of antioxidant activities. Author FT supervised the determination of antioxidant activities. Authors SZM and DD managed the literature searches, verified the statistical analysis.

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ABSTRACT

Aims: The purpose of this study was to evaluate the *in vitro* antioxidant activities of *Datura innoxia* Mill. leaves and seeds harvested in Mali.
Place and Duration of Study: Collection of plant materials were done at Kolondieba (Mali) in june 2016. Evaluation of polyphenols and flavonoids contents, determination of antioxidant activities

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were done at Laboratory of Plant and Food Biochemistry and Biotechnology of University of Sciences, Techniques and Technologies of Bamako (Mali) between august 2016 and March 2017.
Methodology: The leaves and seeds were collected in Kolondieba, Mali. The phytochemical screening based on the standard methods of tube reactions has been performed with ethanol extracts. The quantitative estimation of total polyphenols was made by the Folin-Ciocalteu method and that of flavonoids by the use of aluminum trichloride. The *in vitro* antioxidant activities of the ethanol extracts was determined by three methods: the 1,1- diphenyl - 2- picrylhydrazyl (DPPH) test, the ferric reducing ability power (FRAP) test and the Phosphomolybdate test (PPM).
Results: The phytochemical screening revealed that leaves and seeds of this plant contains alkaloids, polyphenols, flavonoids, coumarins, tannins, triterpenes and saponin. Phenolic contents of ethanol extracts are 30.97 ± 0.33 mg equivalent gallic acid / g in leaves and 14.02 ± 0.15 mg equivalent gallic acid / g in seeds; those of flavonoids are 15.13 ± 0.2 mg equivalent of quercetin / g in the leaves and 4.93 ± 0.41 mg equivalent of quercetin / g in the seeds. The three tests showed that the leaves have a higher level of antiradical activity *in vitro* than seeds.
Conclusion: The results of this work showed that *Datura innoxia* Mill. has a good antioxidant activity which would justify its use as a potential source of natural antioxidants.

Keywords: Antioxidant activities; Datura innoxia Mill; flavonoids; polyphenols.

1. INTRODUCTION

Datura innoxia Mill. is a herb belonging to the Solanaceae family. In Mali, it is called" almoukaykay" and there are dramatic stories incriminating the genre Datura. This would be related to the hallucinogenic effect provided by the plant, therefore, it is generally avoided or even mystified. Indeed, the plant contains tropanic alkaloids (atropine, scopolamine and hyoscyamine) endowed with hallucinogenic power [1,2]. However, an ethnobotanical survey that we conducted in Kolondièba in southern Mali revealed that many traditional healers use the Datura (alone or in combination with other plant species) for the treatment of snake bites and poisonous insects, dermatoses, bewitching, neuropathies. Datura species are used for the treatment of asthma, inflammation, seizures, pain, rheumatism and gout. The specie *Datura innoxia* Mill. would be used as suppositories during difficult deliveries and for the treatment of hemorrhoids [3,4,5]. The anticancer, antibacterial and hypoglycemic potential of the Datura genus have been suggested by Arulvasu et al. [6] and Tandon et al. [7]. Several recent studies have shown the involvement of reactive oxygen species in the etiopathogenesis of diseases such as Parkinson's, diabetes, Alzheimer's and cancer [8]. Thus, for the treatment of these diseases, synthetic molecules with antioxidant activity are used as sensors of these free radicals [9].

On the other hand, the high cost of health services and drugs, as well as socio-economic factors, are causing a large part of the population to use medicinal plants for treatment [10,11,12, 13,14,15,16,17]. Despite these many virtues and the growing interest of the rural population in this species for health care, there are no (to our knowledge) data on the assessment of the antioxidant potential of Datura species encountered in Mali. For all these reasons, we decided to conduct a study to evaluate the *in vitro* antioxidant activities of ethanol extracts of the leaf and seeds of *Datura innoxia* Mill harvested in Mali.

2. MATERIALS AND METHODS

2.1 Plant Materials

Plant material consisting of leaves and seeds of *Datura innoxia* Mill was collected at Kolondièba (11°05'00"N, 6°54'00"W), in southern Mali in June 2016. The plant was identified by Moussa Karembé in the tropical ecology laboratory of the University of Sciences, Techniques and Technologies of Bamako. The leaves and seeds were thoroughly washed, dried at room temperature before being reduced to powder (0,2 mm) and stored away from light and moisture.

2.2 Methods

2.2.1 Preparation of ethanol extracts

The extracts were obtained by maceration of 50 g of powder in 500 mL of ethanol. The mixture was stirred for 48 hours at 30°C and then filtered under vacuum. The filtrates obtained were evaporated to dryness under reduced pressure using a rotary evaporator at 45°C (heidolph).

2.2.2 Phytochemical screening

The detection of the main chemical groups of the different Datura extracts was carried out using conventional methods based on tubes reactions by specific chemical reagents [18,19,20]. Thus, the alkaloids were highlighted by the Dragendorff reagent. The characterization of the tannins was carried out by ferric chloride. For the determination of triterpenes, we used acetic anhydride and concentrated sulfuric acid. Diluted hydrochloric alcohol, magnesium chips and isoamyl alcohol were used to search for flavonoids. The search for coumarins was made by the UV fluorescence method at 365 nm. The foam test revealed the saponins. We used the reagents of Baljet, Kedde and Raymond-Marthoud which respectively give an orange, purplish red, violet coloring in the presence of cardiotonic heterosides.

2.2.3 Determination of total phenolic compounds

The content of phenolic compounds of the various extracts of *Datura innoxia* Mill. was estimated by the method of Folin-Ciocalteu described by Balkan et al. [21]. Thus, 500 µL of the Folin-Ciocalteu reagent (diluted to 10% in distilled water) are added to 100 µL of extract and 400 µL of disodium carbonate (Na_2CO_3) at 75 mg / mL are added to the reaction mixture. After incubation for 2 hours at room temperature and protected from light, the absorbance was read at 765 nm. A calibration curve was carried out under the same operating conditions using a dilution series of gallic acid. The results are expressed in milligram equivalent of gallic acid by gram of extract (mg EAG / g).

2.2.4 Determination of total flavonoids

The estimation of total flavonoids was carried out according to the method described by Fofié et al. [22]. 500 µL of each extract to be analyzed are added to 1500 µL of 95% methanol, 100 µL of 10% (w / v) $AlCl_3$, 100 µL of 1 M sodium acetate and 2.8 mL of distilled water. The mixture was stirred and then incubated in the dark at room temperature for 30 minutes. The blank was made by replacing the extract with 95% methanol and the absorbance was measured at 415 nm using a UV spectrophotometer. The results are expressed in mg equivalent quercetin / g by dry matter.

2.2.5 *In vitro* Antioxidant activity

2.2.5.1 Test Phosphomolybdate (PPM) of ethanol extracts

The phosphomolybdate (PPM) test was carried out according to the method described by Sivaraj et al. [23]. Thus, at 1mL of extract at concentrations (20-120 µg/mL) was added the reagent composed of H_2SO_4 (600 mM), NaH_2PO_4 (28mM) and aluminum molybdate (4mM). The mixture was then incubated at 90°C for 90 minutes. Absorbance was measured at 695 nm. Ascorbic acid was used as a reference standard. The antioxidant capacity is expressed as mg ascorbic acid equivalent per gram of dry matter (mgAA / gMS).

2.2.5.2 DPPH radical scavenging activity of ethanol extracts

We used the method that uses DPPH (diphenylpicryl-hydrayl) [22]. Briefly, 50 µL of each ethanol solution, at different concentrations (12.5, 26, 40, 60, 80 µg / mL) are added to 1.95 mL of the ethanolic solution of DPPH (0.024 g / L). In parallel, a negative control is prepared by mixing 50 µL of ethanol with 1.95 mL of the ethanolic solution of DPPH. The absorbance reading was made spectrophotometrically against a blank at 515 nm after 30 min incubation in the dark at room temperature. The positive control is represented by a solution of a standard antioxidant, ascorbic acid whose absorbance was measured under the same conditions as the samples.

The antioxidant activity related to the DPPH radical scavenging effect is expressed as percentage inhibition (PI%) calculated from the absorbances obtained according to the following formula:

$$[PI\%] = \frac{A0-A1}{A0} \times 100$$

A0 = DPPH absorbance;
A1= sample absorbance.

The IC_{50} (concentrations that inhibit 50% of the DPPH radical) were deduced from the linear regression line obtained from the graph representing the percent inhibition of DPPH [22].

2.2.5.3 Ferric Reducing Ability Power (FRAP) of ethanol extracts

The reducing power of ethanolic extracts of leaf and seeds of *Datura innoxia* Mill was determined

[24]. Thus, 1 mL of sample at different concentrations (20-120 µg / mL) is mixed with 1 mL of phosphate buffer (0.2 M, pH = 6.6) and 1 mL of 1% potassium hexacyanoferrate. The whole was incubated at 50°C. in a water bath for 20 minutes. A volume of 1 mL of 1% trichloroacetic acid was then added and the mixture was centrifuged at 3000 rpm for 10 minutes. 1mL of the supernatant was then mixed with 0.2 mL of a 0.1% $FeCl_3$ solution. The mixture is allowed to stand in the dark for 30 minutes. A blank without sample was prepared under the same conditions. Absorbance measurements were made at 700 nm and ascorbic acid was used as a positive control. The antioxidant activity related to the reducing power of the extracts is expressed as reducing power (PR %) using the following formula:

$$PR\ (\%) = \frac{Aa-Ab}{Aa} \times 100$$

Aa = Absorbance of the extract;
Ab = Absorbance of blank.

2.2.6 Data analysis

The data obtained was processed with Excel® version 2013 and Minitab 18.1 software. The single-factor ANOVA test using the Fisher test was chosen to compare the means at significance level α = 0.05.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Screening

Phytochemical screening results of ethanol extracts of leaves and seeds from *Datura innoxia* Mill. are summarized in Table 1.

Table 1. Phytochemical screening of both parts of the plant

Chemical groups	Leaf	Seeds
Alkaloids	+	+
Polyphenols	+	+
Triterpens	+	+
Flavonoids	+	+
Coumarins	+	+
Tannins	+	+
Saponins	+	-
Cardiotonic heterosides	-	-

** (+) = Present; (-) = Absent*

Thus, it is clear from this characterization that the leaves and seeds of *Datura innoxia* Mill. contain alkaloids, polyphenols, triterpenes, flavonoids, coumarins and tannins. However, cardiotonic heterosides were absent in both parts of the plant. Saponins are present in the leaves; on the other hand they are absent in the seeds. The presence of these metabolites has also been reported in previous work [2,25,26]. Saponins were not revealed in the seeds as well as cardiotonic heterosides in both organs. However, a study conducted in Nigeria on the same species revealed the presence of both metabolites in leaves and seeds [27]. These same authors reported the absence of coumarins and tannins in their samples.

3.2 Determination of Total Phenolic Compounds and Flavonoids

The contents of total phenolic compounds and flavonoids are shown in Table 2.

Table 2. Total phenolic compounds and flavonoid contents of leaf and seeds

	Total phenolic compounds (mg EAG/g)	Total flavonoids (mg EQ/g)
Leaves	30.97 ± 0.33	15.13 ± 0.52
Seeds	14.02 ± 0.15	4.93 ± 0.41

These results revealed that the ethanol extracts leaf of *Datura innoxia* Mill. are richer in total phenolic compounds (p-value = 3.0E-7) and flavonoids (p-value = 2.1E-5) than the ethanol extracts of seeds. Thus, the contents of total phenolic compounds and flavonoids are respectively 30.97 ± 0.33 mg EAG / g and 15.13 ± 0.52 mg EQ / g in the leaf and 14.02 ± 0.15 mg EAG / g and 4.93 ± 0.41 mg EQ / g in the seed. These results corroborate with those obtained by Bagewadi et al. [21] with 38 ± 0.27 mg gallic acid / g of total phenolic compounds and 19 ± 0.17 mg rutin equivalent / g of flavonoids for leaf extracts and Fatima et al. [28] with 29.91 ± 0.12 mg / g of polyphenols and 15.68 ± 0.18 mg / g of flavonoids for leaf extracts. On the other hand, the values observed are lower than those of Bhardwaj et al. [29] who recorded 70.26 ± 1.12 mg gallic equivalent / g of polyphenols and 34.24 ±1.28 mg quercetin equivalent / g of flavonoid for leaf extracts versus 51.01±0.58 mg equivalent of gallic / g polyphenols and 6.99 ± 1.11 mg quercetin equivalent / g of flavonoids with *Datura innoxia* Mill seed extract.

However, a slight difference is noted between our results and the authors cited above. According to Bajalan et al. [30], variation in levels of total phenolic compounds could possibly be

explained by both genetic variation and geographical origins of plants. Studies have shown that the presence of a high content of phenols and flavonoids in the extracts of leaf and seeds of *Datura innoxia* would contribute directly to their antioxidant activity [24,29]. According to Badiaga [31], flavonoid-rich plants would play a positive role in the treatment of cardiovascular and neurodegenerative diseases and also have antitumor activity.

3.3 *In vitro* Antioxidant Activities

3.3.1 Test Phosphomolybdate (PPM) of ethanol extracts

Fig. 1 illustrates the values of the total antioxidant capacity obtained with the extracts at a concentration of 100 µg / mL.

As shown in Fig. 1, at 100 µg / mL of extract, we recorded with the leaves a TAC of 281.28 ± 6.81 mg EAA / g extract versus 141.63 ± 4.12 mg EAA / g with the extract seeds. Therefore *ethanol* extracts of *Datura innoxia* leaves show better antiradical activity than seeds (p-value = 4.0E-7). The results of this work are in agreement with those found by Bhardwaj et al. [25] who obtained 221.25 ± 1.06 mg equivalent of ascorbic acid / g with the methanolic extracts of the leaf and 130.50 ± 2.12 mg equivalents of ascorbic acid / g with the seeds. On the other hand Benabderrahim et al. [28], obtained 435.52 ± 30.99 mg trolox equivalent of *methanol leaves* extracts, which is higher than ours.

3.3.2 DPPH test of ethanol extracts

The values of IC_{50} obtained are translated and shown in Fig. 2.

Fig. 2 shows that ethanolic leaf extracts with an IC_{50} of 104.57 ± 4.03 µg / mL have a higher anti-radical activity than seeds with an IC_{50} of 133.60 ± 5.64 µg / mL (p-value = 1.4E-4), the antiradical activity being inversely proportional to the value of the IC_{50}. However, ascorbic acid with a lower IC_{50} (39.02 ± 1.83 µg / mL) has a greater anti-radical activity than the extracts. This trend is confirmed by the work of Bhardwaj *et* al. (2016) [25] obtained with methanolic extracts of leaf 146.69 ± 8.46 µg / mL and seeds 152.4 ± 1.85 µg / mL. Bagewadi et al. [21] had 80% inhibition at 1 mg / mL with the methanol extracts of the leaf. On the other hand, with the aqueous extract of the leaf, Akhtar et al. [22] have obtained an IC_{50} higher than ours, 401 ± 35 µg / mL.

3.3.3 FRAP test of ethanol extracts

The reducing powers of our extracts and the standard calculated at different concentrations are shown in Table 3.

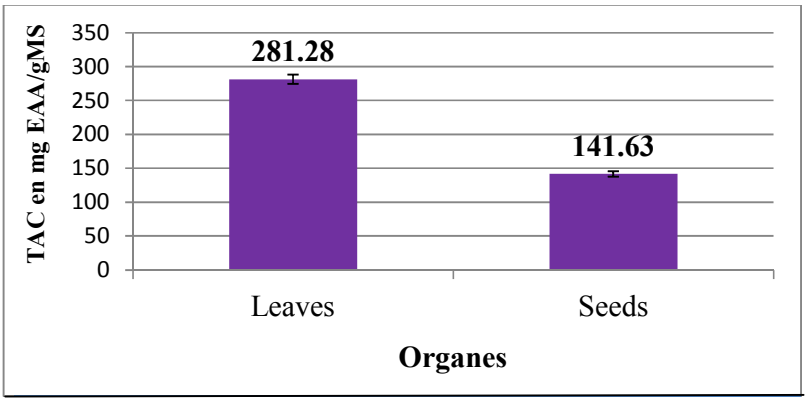

Fig. 1. Total Antioxidant Capacity (TAC) of leaves and seeds

Table 3. Reducing power (%) of our extracts and the standard at different concentrations

Concentrations (µg/ml)	Reducing powers (%)		
	Leaves	Seeds	Standard
20	11.03 ± 1.24	5.35 ± 0.49	24.20 ± 0.31
40	18.45 ± 1.82	9.07 ± 1.13	29.30 ± 0.42
60	24.70 ± 3.15	12.15 ± 0.59	35.22 ± 1.24
80	38.10 ± 2.72	21.50 ± 1.99	58.03 ± 0.65
100	53.44 ± 0.79	29.20 ± 2.16	72.01 ± 1.40
120	59.13 ± 2.50	33.09 ± 1.29	73.05 ± 1.98

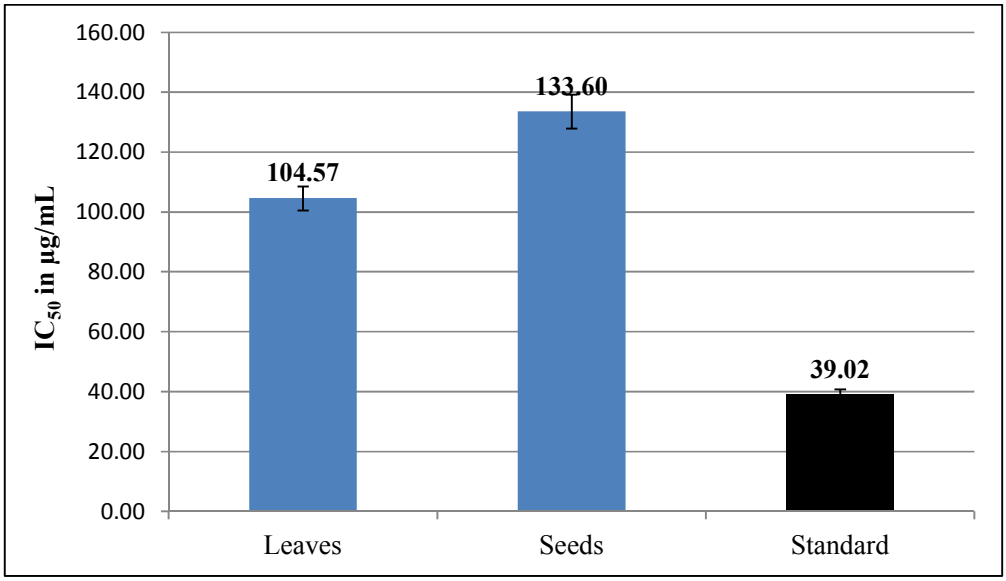

Fig. 2. IC_{50} variations of samples and standard

The data in Table 3 show that the reducing power of extracts increases with concentrations. Thus, at the concentration of 100 µg / mL of extract, we obtained a reducing power of 29.2 ± 2.16% for the seeds against 53.44 ± 0.79% for the leaves (p-value = 5.7E-4) and 72.01 ± 1.40% for the standard (p-value = 1.27E-4); which confirms the trend seen through the first two methods. The results in this study are similar to those reported by the work of Benabderrahim et al. [28] on the antioxidant activity of *Datura innoxia* Mill leaves pushing in Turkey. These authors recorded 446.38 mg equivalent Trolox / g extract. Iqbal et al. [20] obtained the maximum absorbance (0.883) at a concentration of 120 µg / mL with the methanol extract of the seeds of *Datura stramonium* L.

Thus, through these three tests, we find that the antiradical activity of the leaf is higher than that of seeds. This could be explained by the high levels of total phenolic compounds and flavonoids in the leaf, which are twice as high as those of seeds. A direct correlation between the antioxidant activity and the reducing power of plant extracts has been reported by many authors [20,21,24].

4. CONCLUSION

The present study has highlighted the richness of the leaves and seeds of *Datura innoxia* Mill. in several metabolites such as alkaloids, polyphenols, triterpenes, flavonoids, coumarins and tannins in the leaf and seeds of *Datura innoxia* of Kolondieba, Mali. The three tests show that the leaves and seeds possess good antioxidant activities. However, this activity is more pronounced in the ethanol extracts of leaves than in the seeds. This richness in various metabolites and antioxidants could justify the use of this species in traditional medicine. It would then be necessary to test these extracts of *Datura innoxia* on microbial and parasitic strains in order to confirm or deny the therapeutic virtues attributed to it. It will also be necessary to carry out *in vivo* tests to confirm the antioxidant activity of the extracts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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