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Phytochemical screening; determination of total polyphenol and flavonoid contents, and antioxidant activity of different parts of *Datura metel* L.

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Abstract

Datura metel L. is widely used by traditional healers in Senegal for the treatment of certain affections such as tuberculosis, asthma, cough, and bronchitis. This study makes the focus on the determination of the nature of the molecules presents on different organs of this plant, their content in polyphenol and flavonoids, also in the determination of their antioxidant activities by DPPH•. Different organs of Datura metel L. were collected dried and ground. Each organ was sequentially extracted using hexane, ethyl acetate, ethanol, and water. The raw material was also extract only with ethanol, hexane, methanol, or water. Phytochemical screening was realised followed by the quantification of total polyphenol content and flavonoids. At the end, the antioxidant activity was determined by calculating the IC50. The screening shows the presence of polyphenols, flavonoids, alkaloids, and sterols in certain organs such as leaves and flowers. The dosage of polyphenols by the Folin-Ciocalteu method showed that the methanolic extract of flowers exhibits the highest content at 2.974±0.0125 mg GAE/g. The aqueous extract of flowers shows the highest rate of flavonoids content at 0.253±0.0005 mg QE/g. Regarding the antioxidant activity conduct with the DPPH• radical, we can notice some extracts don't have IC50 and others have highest value like sequential water extraction for flower with IC50 = 26.000±0.919 mg/mL. The lowest IC50 were observed with the crude ethanol extract of stem at 0.200±0.007 mg/mL.

Keywords: *Datura metel L., phytochemical screening, polyphenols, flavonoids, antioxidant activities, IC50*

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I. Introduction

Traditional medicine occupies a primordial place in the Senegalese health system. Indeed, access to medications is a real problem given the precariousness in which rural populations live and the high cost of so-called modern drugs. Consequently, rural and urban populations are increasingly resorting to the use of plants to which therapeutic effects not scientifically proven are attributed. Thus, more than 80% of the world's population use so-called traditional medicine to deal with health problems [1]. According to Cox and Balick [2], plants have been a source of medicine because they possess a multitude of bioactive molecules, most of which evolve as chemical defences against predation and infection. These molecules are generally endowed with important pharmaceutical properties. For example, alkaloids have local anaesthetic properties, tannins are healing, antibacterial and antiseptic, flavonoids have anti-inflammatory and antibacterial properties. Saponosides have anti-inflammatory, anti-oedematous and analgesic properties, terpenes and steroids are analgesic and anti-inflammatory and quinone derivatives are antibacterial [3]. Among these secondary metabolites, polyphenolic compounds are highly valued molecules in research centres because of their remarkable physiological and pharmacological activities [4].

Datura metel L. is a plant widely used in Senegal by traditional healers to take care of certain pathologies such as, asthma and bronchodilator problems. *Datura metel* L. seeds are one of the parts of the medicinal plant most popular for the treatment of rheumatoid arthritis and seizures [5]. Although the seeds are poisonous, they also have potent analgesic, anthelmintic, antioxidant, antimicrobial, antiviral, and antidiabetic activities [6–10].

Various parts of plants (roots, stems, leaves, flowers, fruits, seeds) are used for different purposes in herbal medicine. *Datura metel* L. (especially leaves and seeds) has anaesthetic, antiasthma, antispasmodic, antitussive, narcotic, bronchodilator effects. The leaves are used as a local application to treat rheumatic swellings of the joints, lumbago, sciatica, neuralgia, painful tumours, scabies, eczema, allergies and glandular

inflammations, such as mumps; used externally for earaches and smoked to relieve spasmodic asthma [11]**.** In view of the beneficial effects assigned to polyphenols and flavonoids, and the extent of asthma and respiratory problems in Senegal, the determination of these compounds content as well as the antioxidant activity in *Datura metel* L. becomes a key indicator in the management of these pathologies.

II. Material and Methods

Plant material

The choice of the *Datura metel* L. is justified by an ethnobotanical survey coupled with a bibliographic study of the plants listed among certain traditional healers in the Thiès region. The plant material used consists of the powder of the *Datura metel* L. plant : leaves, flowers, seeds and stem (Figure 1). The harvest of the different organs of this plant was carried out in November 2020 in a field located in the commune of Taïba Ndiaye, located in the region of Thiès, in the West of Senegal with geographical coordinates 15°3'0" North and 16°52"60 West.

The four organs of the plant were dried away from sunlight. The dried drugs were pulverized using an electric grinder. The fine powder thus obtained after spraying is used as raw material for the rest of the work.

Fig. 1 Photographs of the leaves, flowers, seeds, and stems of *Datura metel* L.

Extraction procedures

The secondary metabolites were extracted by maceration to avoid possible degradation of the thermo-degradable molecules present in the plant.

Plant material was extracted in solvents of increasing polarity. Indeed, 20 g of powder from each organ of the plant was added to 100 mL of hexane. Maceration was done for 10 min with manual stirring at room temperature. The macerate was filtered on filter paper, the filtrate collected is called Hexane 1 and the marc obtained is subjected after drying to a new maceration with hexane. This same process was repeated twice with the marc obtained previously, giving the Hexane 2 and Hexane 3 extracts. After the three successive extractions with hexane, the marc is treated twice in the same way as the hexane extraction with 100 mL of methanol leading to the Methanol 1 and Methanol 2 extracts. The three or two filtrates obtained are combined and evaporated.

The aqueous extract was obtained by adding 5 g of drug powder to 100 mL of distilled water. Maceration was done for 10 min with manual stirring at room temperature. The macerate was filtered, the filtrate collected is called aqueous extract.

The different extracts (Hexane, Methanolic and aqueous) are used for the determination of total polyphenols and flavonoids.

For phytochemical screening and determination of antioxidant activities, a few grams of plant powder are subjected to cold maceration successively with solvents of increasing polarity (hexane, ethyl acetate, ethanol and aqueous) for 24 hours with stirring.

Phytochemical screening

Phytochemical screening is a qualitative analysis based on precipitation or coloring reactions. The latter make it possible to define the presence or absence of secondary metabolites which may be found in a plant sample. In this work, the screening concerns the search for: alkaloids, polyphenols, tannins, flavonoids, saponins, sterols and polyterpenes, coumarins, leucoanthocyanin, catechol and mucilage. The presence of these different chemical groups were examined by referring to the techniques described in the work of Ronchetti et *al.* [12] and Karim et *al.* [13]. The polyphenols and tannins were identified by the $FeCl_3$ test and Stiasny's reagent;

flavonoids, leucoanthocyanins and catechols by reaction with cyanidin; saponins by the foam test; sterols and polyterpenes by the Liebermann-Burchard test; coumarins by the test with ammonium hydroxide; mucilages by the absolute ethanol test and alkaloids by Mayer's tests [14].

Determination of the total phenolic content

The content of total phenolic compounds was determined with the Folin-Ciocalteu method [15]. At 200 µL of each extract were added 150 µL of Folin-Ciocalteu reagent, 600 µL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water. Dilutions were made when required. After 30 minutes of incubation in the dark, the absorbance is read at 760 nm with an UV/Visible spectrometer of the Perkin-Elmer Lambda 365. Concentration was determined using an acid gallic standard curve. Results were expressed as mg GAE/g.

Determination of flavonoids content

The flavonoid content was calculated by the method described by Gaye et *al*. [15]. This method consists of adding 2.5 mL of a 2% ethanol solution $AlCl_3$ to 2.5 mL of each extract. Dilutions were also made when required. The mixture are incubated for 1 hour at ambient temperature and the absorbance is read at 425 nm. The flavonoid content is expressed in terms of Quercetin equivalent (EQ) by reference to the calibration curve plotted with a concentration range obtained from a stock solution at 0.1 mg/mL of quercetin.

Antioxidant activity

The antioxidant test was carried out with the $DPPH^{\bullet}$ method [16]. A stock solution of 40 mg/mL is prepared by dissolving 80 mg of dry extract in 2 mL of methanol then a series of dilutions were made. At 0.2 mL of these solutions were added 3.8 mL of the methanolic $DPPH^{\bullet}$ solution. The tubes were stirred manually for a few seconds then incubated for 30 minutes in the dark. Absorbance were recorded using a spectrophotometer at a wavelength of 517 nm using methanol as a blank. Ascorbic acid was used as control. $DPPH^{\bullet}$ scavenging activity was determined by calculating the percentage of inhibition :

$$Scavenging\ activity = \frac{Absorbance_{control} - Absorbance_{sample}}{Absorbance_{control}} \times 100 \qquad (1)$$

III. Results and Discussion

Extraction yields

Table no 1 lists the extraction yields of the different solvents used. Ethanol used directly on the raw material gives good extraction yield which range between 19−34 %, with leaves giving the best yield. Sequential extractions, with increasing the solvent polarity (Hexane, Ethyl Acetate, Ethanol and Water), were done. For leaves, the extraction yield increase as solvent polarity. Thus, the highest yield was observed with aqueous solvent, which means the leaves are rich in high polar compounds. The same observation can be made for flowers and stems. For seeds, the ethanol solvent give the best extraction yield. The polarities of the compounds present in seed are more compatible with the ethanol polarity.

Table no 1 Extraction yields for the different parts of *Datura metel* L. by several solvents

Parts	Solvents	Volume (mL)	Sample mass (g)	Mass obtained (g)	Yield (%)
Leaves	Hexane*	300	68	1.36	2
	AcOEt*			2.04	3
	Ethanol*			3.4	5
	Aqueous*			4.76	7
	Ethanol Crude**	200	20	6.8	34
Flowers	Hexane*	250	34.28	1.03	3
	AcOEt*			1.37	4
	Ethanol*			3,428	10
	Aqueous*			3.43	10
	Ethanol Crude**	200	7.339	1,379	19
Stems	Hexane*	200	34	0.68	2
	AcOEt*			0.34	1
	Ethanol*			1.02	3
	Aqueous*			3.4	10
	Ethanol Crude**	200	20	3.74	19
Seeds	Hexane*	200	44.36	1.774	4
	AcOEt*			1.33	3
	Ethanol*			4.436	10
	Aqueous*			2.66	6
	Ethanol Crude**	200	20	5.32	27

*Sequential extract, **Crude extract

Phytochemical screening

The phytochemical screening was also conduct for different parts of *Datura metel* L. Table no 2 lists all the results concerning the screening. The phytochemical screening revealed the presence of all types of compounds that were tested. However, they are differently distributed in the parts of the plant. The screening results also revealed a higher concentration of secondary metabolites in the ethanolic and aqueous extracts. This shows the importance of the polarity of the solvent for the extraction of polar compounds. Stems are less rich in secondary metabolites. Indeed, polyphenols, flavonoids, saponosides, leucoanthocyanins, catechols, and gallic tannins are absent in the different solvent extracts. Only alkaloids, sterols and coumarins are weakly present. Polyphenols, flavonoids, alkaloids, sterols and polyterpenes, and coumarins are found in some solvent extracts of leaves, flowers, and seeds. These results are in agreement with the work of Okwu et *al*. [17]. The presence of alkaloids in the leaves and seeds is in agreement with the results reported by Dibon,g et *al*. [18]**.** Gallic tannins are only present in the leaves and flowers in small quantities. Catechic tannins are present in leaves, flowers, and seeds. Saponosides are strongly present in leaves, flowers, and seeds. The results of the phytochemical screening showed that for the sequential extraction, the ethanolic extract of the seeds and flowers of the *Datura metel* L. is the richest in secondary metabolites, compared to the other extracts of these same organs. The crude ethanol extract give the same observation as expected. The Ethyl Acetate extract of leaves is rich in polyphenols, flavonoids, sterols and saponosides.

Table no 2 Phytochemical screening

		Polyphenols	Flavonoids	Alkaloids	Sterols and Polyterpenes	Saponosides	Leucoanthocyanins	Catechols	Coumarins	Catechin tannins	Gallic tannins
Stems	Hexane*	-	-	-	-	-	-	-	+	-	-
	Ethyl Acetate*	-	-	+	-	-	-	-	-	-	-
	Ethanol*	-	-	-	+	-	-	-	-	-	-
	Aqueous*	-	-	+	+	-	-	-	-	+	-
	Ethanol crude**	-	-	+	+	-	-	-	+	-	-
Leaves	Hexane*	+	+	-	+	+++	-	-	-	+	+
	Ethyl Acetate*	+++	+++	+	+++	+++	-	-	-	-	+
	Ethanol*	-	+	+	+	+++	-	-	-	+	+
	Aqueous*	++	-	+	++	+++	-	+	-	+	+
	Ethanol crude **	+	-	+	+++	+++	-	-	-	-	+
Flowers	Hexane*	-	++	+	+	+++	-	-	-	+	-
	Ethyl Acetate*	+	++	+	+	+++	-	-	+	-	-
	Ethanol*	+++	+++	++	+	+++	-	+	-	-	+++
	Aqueous*	+	++	-	-	+++	-	+	-	++	+

	Ethanol crude **	+++	+++	+++	++	+++	-	+	+++	++	+
Seeds	Hexane*	-	-	-	+	+++	-	-	++	-	-
	Ethyl Acetate*	-	-	+	+	+++	-	-	++	-	-
	Ethanol*	+++	+	++	+++	+++	-	+++	++	-	-
	Aqueous*	+	-	++	++	+++	-	+++	++	-	-
	Ethanol crude **	+++	++	++	+++	+++	-	+	+++	++	-

*Sequential extract, **Crude extract, +++ Strong presence, ++ Moderate presence , + Weak presence, - Absence

Total polyphenols and flavonoids content

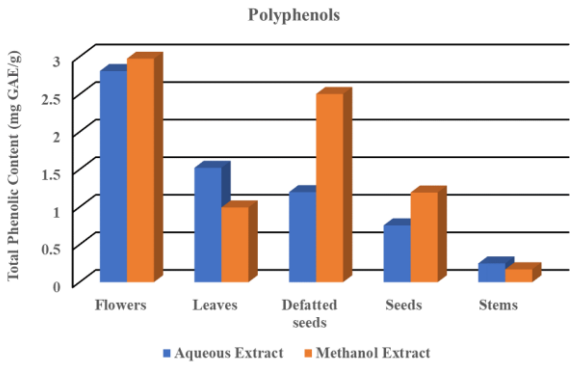
The results of the assay are presented in Table no 3 and illustrated in Figure 2A. The total polyphenol contents of the different parts of the plant vary between 0.249 and 2.810 mg GAE/g in the aqueous extracts and 0.171 and 2.974 mg GAE/g in methanolic extracts. The highest content was measured in the methanolic extract of the flowers with a rate of 2.974 mg GAE/g. The aqueous and methanolic extracts of the stems record the lowest levels of total polyphenols with respective values of 0.249 and 0.171 mg GAE/g. It is also noted that the delipidated seeds are richer in polyphenolic compounds in the methanolic and aqueous extracts with respective levels of 2.504 mg GAE/g and 1.198 mg GAE/g, compared to raw seeds with respectively 1.193 mg GAE/g and 0.756 mg GAE/g. This result can be explained by the presence of lipids in the raw seeds which can trap polyphenols, leading to a drop in their content. These results reveal the important bioactive properties of *Datura metel* L. flowers due to their high polyphenol content.

Table no 3 Total Polyphenols and Flavonoids content of different part of *Datura metel* L.

	Polyphenols (mg GAE/g)		Flavonoids (mg QE/g)	
	Aqueous Extract	Methanol Extract	Aqueous Extract	Methanol Extract
Flowers	2.810 ± 0.0069	2.974 ± 0.0125	0.253 ± 0.0005	0.171 ± 0.0003
Leaves	1.5220 ± 0.0015	0.9950 ± 0.0036	0.1610 ± 0.0002	0.0990 ± 0.0001
Defatted seeds	1.1980 ± 0.0013	2.5040 ± 0.0021	0.1110 ± 0.0002	0.1430 ± 0.0031
Seeds	0.7560 ± 0.0037	1.1930 ± 0.0035	0.0340 ± 0.0001	0.0410 ± 0.0012
Stems	0.2490 ± 0.0008	0.1710 ± 0.0023	0.0570 ± 0.0002	0.0980 ± 0.0003

The results presented in Table no 3, illustrated in Figure 2B, show that the flavonoid content vary from one part of the plant to another. The methanolic and aqueous extracts of the flowers are the richest in total flavonoids with respective rates of 0.171 and 0.253 mg QE/g. The raw seeds and the stems being the poorest in flavonoids, with respective rates of 0.041 mg QE/g and 0.098 mg QE/g in the methanolic extracts, and 0.034 and 0.057 mg QE/g in the aqueous extracts. It is also noted that the delipidated seeds are richer in flavonoids for the methanolic and aqueous extracts with respective rates of 0.143 mg QE/g and 0.111 mg QE/g, compared to raw seeds with respectively 0.041 mg QE/g and 0.034 mg QE/g. This result can be explained by the presence of lipids in the raw seeds which can trap the flavonoids, thus leading to a drop in their extraction yield.

A



B

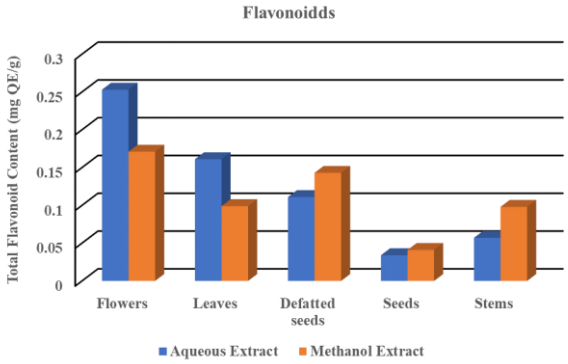


Fig. 2 Total phenolic content (mg/g plant extract of gallic acid equivalent) and Total flavonoid content (mg/g plant extract of quercetine equivalent) of the crude methanolic extract and crude aqueous extract of different organs of *Datura metel* L.

Antioxidant activity

The antiradical activity of the various extracts was evaluated using methanolic solution of DPPH$^{\bullet}$, by measuring the absorbance at a wavelength of 517 nm. DPPH$^{\bullet}$ scavenging activity was determined by calculating the percentage of inhibition (Equation (1)). The standard used is ascorbic acid. The results of the IC50 of the various extracts of *Datura metel* L. are summarized in Table no 4. Analysis of the IC50 values of the different organs of *Datura metel* L. shows that the stems in the crude ethanol extract constitute the part which has the best radical scavenging capacity with an IC50 of 0.2 mg/mL. The IC50 values recorded in Table no 4 show that the crude ethanolic extracts of the different organs of the plant, compared to the other extracts, have the best antiradical capacity with IC50 values ranging from 0.2 to 2.61 mg/mL .

Table no 4 IC50 of different parts of *Datura metel* L.

	Leaves	Flowers	Stems	Defatted Seeds
Hexane	12.600±0.141	n/a	n/a	24.400±1.131
Ethyl acetate	8.200±0.028	23.000±0.494	17.600±0.127	10.300±0.021
Ethanol	15.220±0.018	2.800±0.035	n/a	0.400±0.007
Water	n/a	26.000±0.919	n/a	39.300±0.020
Crude Ethanol	2.320±0.020	2.610±0.007	0.200±0.002	1.35±0.01

The aqueous extract of the seeds has the worst IC50 value of 39.3 mg/mL and therefore exhibits the lowest antiradical activity. As the successive extraction was done with solvents with increasing polarity, this result could be explained by previous extractions of secondary metabolites by solvents such as ethyl acetate and ethanol. Indeed, the delipidated seed sequential ethanol extract has the best antiradical capacity with a lower IC50 value of 0.4 mg/mL. This fact is in accordance with high presents of secondary metabolites such as polyphenols and flavonoids in the ethanol extract. The crude ethanolic extract of the leaves, and flowers have better IC50 than their sequential ethanolic extracts. In contrary, the sequential ethanol extract of seeds have better IC50 than the corresponding crude ethanolic extract. Using ascorbic acid as reference (IC50 = 0.085 mg/mL), we can note that the extracts of the different organs of *Datura metel* L. present a lower antioxidant activity than ascorbic acid.

In hexane leaves and seeds extracts, we note the presence of a low antiradical activity with high values of IC50 (12.6±0.141 mg/mL for leaves and 24.4±1.131 mg/mL for defatted seeds). Indeed, hexane is not the most appropriate solvent for extracting polyphenolic compounds which are considered as responsible for the biological activity of the various organs plant. However, it should be noted that this activity of the hexane extract agrees with the results obtained by Da Costa Cordeiro et al. [19] whose work focused on the hexane extract of *Spodias tuberosa*, which contains flavonoids and hydrolysable tannins from leaves. Other studies carried out on roots and seeds of *Levisticum persicum* have also revealed antioxidant activity of their hexane extracts [20].

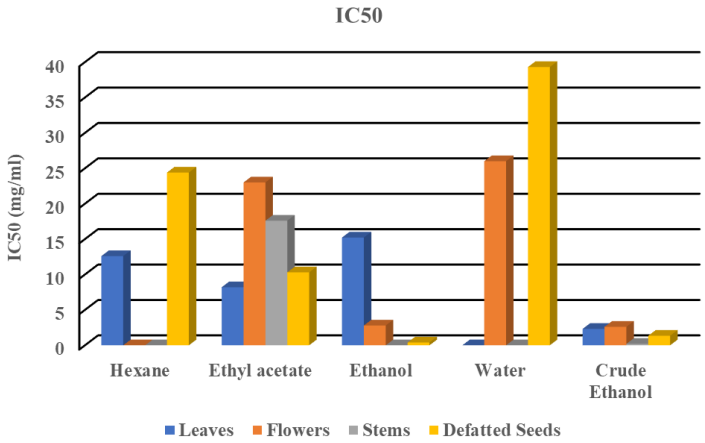


Fig.3 IC50 (mg/mL) values of crude hexane extract, ethyl acetate extract, ethanol extract water extract and crude ethanol extract of *Datura metel L.* leaves, flower, stem, and defatted seeds.

IV. Conclusion

The phytochemical screening revealed the presence of polyphenols, flavonoids, alkaloids, sterols and polyterpenes, coumarins and saponosides in the major parts of the *Datura metel* L. However, there is a moderate presence of alkaloids and strong presence of polyphenols and flavonoids in flowers and seeds. The assay results revealed that protic polar solvents such as methanol and ethanol are the most suitable for the extraction of polyphenols and flavonoids. The quantification of polyphenolic compounds and flavonoids made it possible to deduce that the recurrent use of the different parts of this plant would be linked to their relative richness in secondary metabolites based on polyphenols and flavonoids. The evaluation of the antioxidant power revealed that organs of *Datura metel* L. possess an interesting antioxidant activity which would justify the use of this plant in traditional medicine.

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