In Vitro Antioxidant Potentials and total Phenolic Content of Tunisian Datura Metel Extracts from Leaves and Seeds

Leyla Tahrani[a,c], Fathia Harzallah-Skhiri[d], Mahjoub Aouni[a] and Karima Bel Hadj Salah [a,b*]
[a] *Laboratoire des maladies transmissibles et substances biologiquement actives (LR99ES27), Faculté de pharmacie, Université Sde Monastir, Tunisie.*
[b] *Department of biology, Faculty of Sciences and Arts Rabigh. King Abdul Aziz University (KAU). KSA.*
[c] *Laboratoire de Biotechnologie et Valorisation de Bio Géo Ressources Institut Supérieur de Biotechnologie (LR11-ES31), Université de la Manouba BioTech Pole Sidi Thabet, 2020, Sidi Thabet, Ariana, Tunisie.*
[d] *Laboratoire de Génétique Biodiversité et Valorisation des Bio-ressources, Institut Supérieur de Biotechnologie de Monastir (ISBM), Université de Monastir, Avenue Tahar Haddad, 5000, BP74, Monastir, Tunisie.*

Abstract

The objective of this study is to determine the antioxidant activity and total phenols of Datura metel, a medicinal plant collected in Tunisia. Leaves and seeds of the plant were subject to cold maceration with solvent extracts: petroleum ether (PE), dichloromethane (CH_2Cl_2), ethyl acetate (ACOEt) and methanol (MeOH). Total phenolic contents were investigated by the method of Folin-Ciocalteu. Antioxidant activities of different extracts were determined by DPPH radical scavenging, ABTS assay and reducing power. All tested D. metel extracts showed interesting antioxidant activities and a difference was observed between leaves and seeds antioxidant potential extracts. Our results showed that the methanolic seeds extracts exhibited the highest total phenolic content. At 1 mg/ml concentration, the DPPH radical scavenging activities of leaves and seeds were 66.4% and 63.3% respectively, and the inhibition percentages of ABTS radical were between 96.54% and 97.01%. Our results suggest that D. metel extracts from both leaves and seeds could be used as potential sources of new antioxidant agents, useful in pharmacological and food industries.

Key words: *Datura metel, antioxidant activity, total phenolic content, seeds, leaves.*

I. INTRODUCTION

Medicinal plants play a crucial role as sources of antioxidant, antimicrobial, and anti-inflammatory agents [1-2]. In recent times, several human diseases are caused by reactive oxygen species (ROS) such as Parkinson, diabetes, Alzheimer and cancer. Additionally, antioxidants from natural sources are of great interest to food technologists because of extended shelf life and reduced nutritional values losses [3-4]. The inherent antioxidant potential of plant extracts is due to presence of phenolic compounds including flavonoids, chalcones, lignoids, and tannins which can reduce the oxidative stress [5-7]. Antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative reactions that conduct to degenerative diseases or in neutralizing the free radicals interactively and synergi stically [8]. Also, antioxidants are indispensable in food industries because of the lengthening of the shelf life of products and the ability to keep their nutritional values for longer time [9].

D. metel is known as "Hchich Elfadda" in Tunisia [10]. In folkloric medicine, *D. metel* has contributed various pharmacological actions and it is used to treat fever, cough, cerebral complications, convulsion, diarrhea, skin diseases, antiseptic, animal bites, anti helmenthic, antibacterial, antifungal, in antiviral diseases and burn wounds [11-15]. Although a very large number of researches have been investigated on this medicinal plant [16-18], the antioxidant properties of the Tunisian *D. metel* have surprisingly not been investigated to our knowledge. The aim of the present work is to study the antioxidant activity of different organic extracts from seeds and leaves of *D. metel* native to Tunisia using four different tests.

II. MATERIAL AND METHODS

A. Collection of Plant Materials:

The plant leaves and seeds of *Datura metel* were harvested from Monastir region in Tunisia. Leaves and seeds were washed and dried at room temperature, then powdered and stored separately in sterile and airtight container for future use.

B. Preparation of Plant Extracts:

100 g of powder were extracted with increasing polarity organic solvents; petroleum ether (PE), dichloromethane (CH_2Cl_2), ethyl acetate (ACOEt) and methanol (MeOH) for one week of maceration in each solvent. The filtrates of plant extracts were evaporated under vacuum in a rotary evaporator (BUCHI, Germany) and the dried extracts were stored at 4 °C in amber bottles until further process.

C. Determination of the Total Phenolic Content

Total phenolic content of *D. metel* solvent extracts was determined according to the method of Singleton et al. [19]. In brief, a solution of 100 µl of each extract (1 mg/ml), 100 µl of diluted Folin-Ciocalteu reagent (50%) and 2 ml of sodium carbonate (Na_2CO_3, 7.5%) was prepared in triplicate. The reaction mixture was incubated at room temperature for 30 minutes and absorbance recorded at OD 720 nm. Gallic acid was used for constructing the standard curve (0 to 350 µg/ml; Y= 0.0029X-0.0121; R^2= 0.995) (Fig. 1) and the total phenolic content in extracts in gallic acid equivalents (GAE) were calculated by the following formula: C= c. V/m, where C is the total content of phenolic compounds in mg/g of plant extract in GAE; c is the concentration of gallic acid established from the calibration curve (mg/ml), V is the volume of extract (ml); and m denotes the weight of pure plant organic extract (g).

D. Antioxidant activity of Tested Extracts

1) DPPH radical scavenging

The DPPH scavenging effects of plant extracts were determined according to the method of Brand-Williams et al. [20]. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is a free radical stable at room temperature which produces a violet solution in ethanol. In the presence of antioxidant compounds, the DPPH gets reduced producing a colorless ethanol solution. In order to estimate the DPPH radical scavenging activity, one ml of each extract at a final concentration of 1, 0.5, 0.25, 0.125 mg/ml was taken in independent test tubes and 1 ml of 0.2 mM DPPH - ethanol solution was added. Ethanol and DPPH alone served as control. The mixture was vortexed vigorously and kept in dark for 30 minutes. Following incubation, absorbance of each sample was measured at 517 nm. All tests were performed in triplicate and the percent inhibition was calculated using the following formula:

% Inhibition = A control – A extract / A control × 100

A: absorbance at 517 nm.

The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at four different concentrations of the extract.

2) ABTS radical scavenging assay

The ABTS assay was conducted as described earlier [21]. ABTS and potassium persulfate were dissolved in distilled water to obtain a final concentration of 7 mM and 4.9 mM respectively. Equal amount of these two solutions were mixed and incubated in darkness for 12 hours at room temperature. Different concentrations (1, 0.5, 0.25 and 0.125 mg/ml) of *D. metel* extracts were tested and absorbance was measured at 734 nm. The absorbance was recorded after 30 minutes incubation in dark at room temperature and the percentage of radical scavenging capacity was calculated using the following formula:

% Inhibition =A control – A extract / A control × 100.

The control contained only ethanol and ABTS solution. The concentration of the plant extracts required to scavenge 50% of the radicals (IC50) was calculated by using the percentage scavenging activities at four different concentrations of the extract.

3) Reducing Power assay

The reducing power of extracts was determined using the method reported earlier [22]. Briefly, a solution of 500 µl of different concentration (2, 1, 0.5, 0.25, 0.125 mg/ml) of *D. metel* extracts, 500 µl of phosphate buffer (0.2 M, pH 6.6) and 500 µl of potassium ferricyanide (1 %) was incubated at 50°C for 20 min. Reaction was terminated by addition of 500 µl of trichloroacetic acid (10 %), followed by centrifugation at 3,000 rpm for 10 min. The supernatant (500 µl) obtained was mixed with equal amount of distilled water (500 µl) and 100 µl of ferric chloride (0.1 %) and allowed to stand for 10 min. Trolox was used as standard and the absorbance was recorded at 700 nm. The higher absorbance indicates stronger reducing power.

III. RESULTS

A. Total phenolic content

The results of the total phenolic contents of eight organic *D. metel* extracts are summarized in Table 1. Methanol extracts of leaves and seeds exhibited the highest total phenolic content with 118 and 108.5 mg gallic acid equivalent/g dry weight respectively. The maximum phenolic content was obtained from the seed extracts. Whereas, the lowest amount total phenolic compounds was found in ACOEt extracts.

(Please insert table 1 here)

B. Antioxidant activity of tested extracts

1) DPPH Radical Scavenging

All the *D. metel* extracts displayed promising DPPH radical scavenging activity. Both leaves and seeds extracts anti-radical activities were found to be significantly high when compared to the trolox. We noted that the radical scavenging activity of extracts increases with the increase in concentration of tested sample (Table 2).

(Please insert table 2 here.)

2) ABTS Assay

Table 2 also summarizes the data obtained for ABTS assay on *D. metel* extracts. Results showed that all the extracts were active against ABTS radicals and these activities depend on sample concentration and time of measurement. The methanolic extracts of leaves and seeds displayed highest activity, with percent inhibition close to 100%, significantly higher

than that of standard, trolox. Additionally, the IC50 values confirmed this ascertainment, since the methanolic extracts revealed the lowest IC50 values even to the control.

3) Measurement of reducing power

The results of reducing power assay of tested *D. metel* extracts are presented in Figure 1. All the extracts tested have shown a significant antioxidant potential. The methanolic extracts of leaves and seeds displayed the best reducing power when compared to other extracts. However, reducing power was weaker in comparison to the standard (Trolox). Reducing power was linearly proportional to the concentration and time of measurement.
(Please insert Fig. 1here.)

IV. DISCUSSION

This study represents to our knowledge one of the few reports investigating the antioxidant capacities of *D. metel.* Total phenolic compounds and antioxidant potentials of Tunisian *D.metel* organic extracts are investigated for the first time.

Our results showed a high phenolic content in *D. metel* organic extracts. Interestingly, the leaves extracts showed maximum phenolic content when compared to seeds, in accordance with other reports [23-24]. An earlier phytochemical screening of 70 plant extracts reported the presence of phenolic contents in different plants [25]. As secondary metabolites, phenolics are very well identified in the vegetal kingdom and even in high concentrations [26-27].

Additionally, the relationship between the phenolic content and their antioxidant potential in plants is well established [28-29]. This fact incites to determine the antioxidant activity of Tunisian *D. metel* extracts. Our results revealed that the antioxidant activity, obtained with the DPPH radical scavenging assay, exhibited more potent when compared to the finding of [7,30]. In fact, the tested extracts from the leaves and seeds of *D. metel* possess hydrogen donating capabilities which act as an antioxidant. The results of free radical scavenging potentials of both organic extracts were found to be in the order of MeOH extract > ACOEt extract > CH_2Cl_2 extract > PE extract. According to several reports, the *D. metel* extracts contain flavonoid, saponins, tannins, phenolics and aromatic compounds. All these bioactive compounds can de-colorize DPPH solution by their hydrogen donating ability [7,30- 32]. Regarding the ABTS radical scavenging assay, the seed methanolic extracts of *D. metel* showed the highest activity with the lowest IC50 values. Moreover, a promising antioxidant potential of *D. metel* extracts was confirmed by the reducing power assay, which is in agreement to previous findings [24]. The antioxidant activity of phenolics is mainly attributed to their phenol group functions, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides, and thus help protect cells from oxidative toxicity [32].

In conclusion, *D. metel* extracts from leaves and seeds could be used as potential sources of new antioxidant agents useful in pharmacological and food industries.

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Table 1: Total phenolic contents of *D. metel* organic extracts

Organic extracts	GAE (mg GAE/g dry weight)
PE leaves	88.2
PE seeds	66.6
CH2Cl2 leaves	25.7
CH2Cl2 seeds	29.1
ACOEt leaves	19.4
ACOEt seeds	17.5
MeOH leaves	118
MeOH seeds	108.5

GAE: Gallic Acid Equivalent; PE: Petroleum Ether; CH_2Cl_2: Dichloromethane; ACOEt: Ethyl Acetate; MeOH: Methanol

Table 2: Antioxidant activity of *D. metel* organic extracts

Sample	Concentration (mg/mL)	DPPH assay		ABTS assay			
		Inhibition (%)	IC50 (mg/ml)	Inhibition (%) at 5 min	Inhibition (%) at 20 min	IC50 (mg/ml) at 5 min	IC50 (mg/ml) at 20 min
PE leaves	0.125	20	1.1	7.12	13.68	4.16	1.37
	0.25	31		6.81	14.78		
	0.5	38		9.13	24.68		
	1	39.8		13.6	28.33		
PE seeds	0.125	18	1.19	5.48	16.86	2.84	1.41
	0.25	23.5		7.79	20.25		
	0.5	34.6		9.66	23.95		
	1	38.1		18.72	26.10		
CH_2Cl_2 leaves	0.125	23.3	1.08	15.37	17.67	2.18	1.31
	0.25	28		25.99	37.59		
	0.5	36.4		27.22	40.76		
	1	41.5		39.63	46.21		
CH_2Cl_2	0.125	24	1.11	4.10	12.31	1.21	0.82

seeds	0.25	30		6.80	15.48		
	0.5	36		12.23	15.48		
	1	40.6		23.03	31.88		
ACOEt	0.125	10.6	0.94	5.62	15.13	3.77	1.5
leaves	0.25	36		11.91	20.71		
	0.5	42		13.78	20.71		
	1	44		13.92	26.70		
ACOEt	0.125	7.5	0.89	8.69	19.94	1.76	1.46
seeds	0.25	35.6		20.08	23.20		
	0.5	44.8		23.89	26.04		
	1	47.2		27.23	28.23		
MeOH	0.125	37.3	0.59	82.67	87.07	0.044	0.008
leaves	0.25	41.6		84.89	87.71		
	0.5	47.8		84.89	87.71		
	1	66.4		87.88	96.54		
MeOH	0.125	39	0.56	85.94	87.93	0.009	0.001
seeds	0.25	42.2		95.19	96.46		
	0.5	56.6		95.09	96.82		
	1	63.3		95.59	97.01		
Trolox	0.125	30.6	0.16	80.07	70.5	0.14	0.009
	0.25	35.5		85.21	94.5		
	0.5	39.7		86.84	95.1		
	1	60		87.2	97.3		

PE: Petroleum Ether; CH_2Cl_2: Dichloromethane; ACOEt: Ethyl Acetate; MeOH: Methanol.

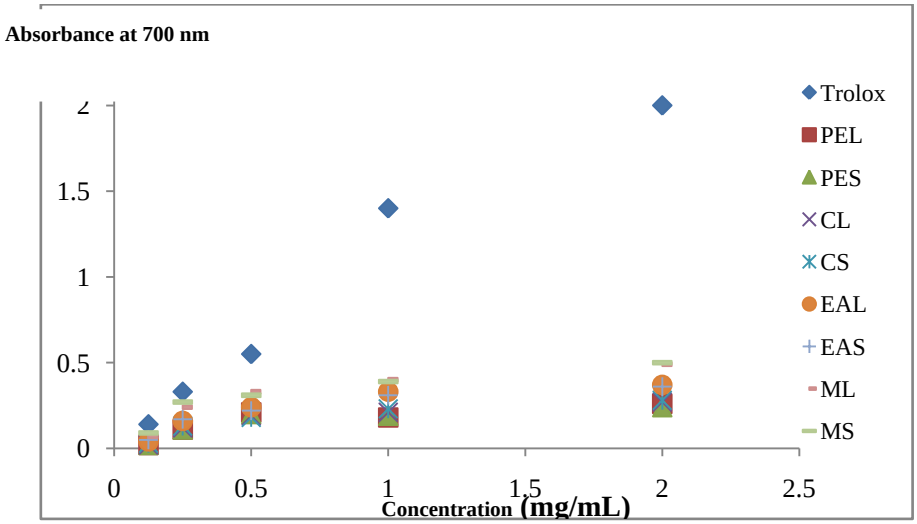

Figure 1: Reducing power of *D. metel* organic extracts compared with Trolox as standard.

PEL: Petroleum ether of leaves; PES: Petroleum ether of seeds; CL: CH_2Cl_2 of leaves; CS: CH_2Cl_2 of seeds; EAL: ACOEt of leaves; EAS: ACOEt seeds; ML: MeOH leaves; MS: MeOH seeds.