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Optimization of extraction conditions by the dosage of polyphenols and determination of antioxidant activity: Case of *Melothria maderaspatana* organs, a plant used in traditional African medicine for the treatment of diabetes

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Abstract

Melothria maderaspatana is a medicinal plant of Asian origin belonging to the Cucurbitaceae family. It has a wide range of phytochemicals such as alkaloids, flavonoids, tannins, saponins, steroids, terpenoids, phenolic compounds. The leaves, roots and fruits are considered to have stomachic activities. It is a plant very rich in phenolic compounds, generally responsible for the biological activity of plants. In this study, we optimized the extraction parameters, including temperature, duration and solvent mass/volume ratio, by determining the total polyphenol contents and the antiradical activity at DPPH●. The Folin-Ciocalteu reagent was used to evaluate the content of phenolic compounds, with gallic acid as a reference. The determination of flavonoids with $AlCl_3$ was carried out with quercetin as a reference. The DPPH● radical made it possible to measure the antioxidant power of the extract, with reference to Trolox. This study showed that the optimal conditions for extraction of bioactive molecules, responsible for antioxidant activity, are obtained for a temperature of 80 °C, for a period of 30 min in a ratio of 1 g for 50 mL of extraction solvent which is distilled water. Under these conditions, the contents of total polyphenols and flavonoids are evaluated for the other organs of the plant. The values obtained show that the three parts of the plant contain approximately the same polyphenol contents and exhibit the same DPPH● antioxidant activity. As for the flavonoid content, the leaves are revealed to be the richest part, compared to the stems and fruits. These physicochemical indicators in secondary metabolites and biological activity justify the primary choice reserved for this plant by traditional practitioners for the treatment of numerous pathologies.

Keywords: *Melothria maderaspatana*; Optimization; Extraction; Polyphenols; Flavonoids; Antioxidant activity

1 Introduction

The plant is man's friend for his survival, providing him with food and medicine since the earliest times of civilization [1]. Plants continue to be a major source of medicine, as they have been throughout human history [2]. For a long time, they have been a valuable source of natural products to maintain human health, especially in recent decades with more intensive studies on natural therapies. Nowadays, the use of phytochemicals for pharmaceutical purposes has gradually increased in many countries. According to the World Health Organization (WHO), plants are the best source for obtaining a variety of medicines. About 80 % of people in developing countries rely on traditional medicine, which contains compounds derived from medicinal plants [3]. *Melothria maderaspatana* is one of them. It belongs to the Cucurbitaceae family. It is a monoecious annual plant found in mountainous regions of India. It is traditionally used to

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treat dysuria, tonsillitis, dizziness and indigestion [4]. The plant has been scientifically evaluated for its anti-inflammatory [5], hypolipetamic [6], antioxidant [7], larvicidal [8], antiplatelet [9], antihypertensive [10], and hepato-protective [11] properties, as well as its effectiveness against skin diseases [12]. Current research has shown that *Melothria maderaspatana* is a good source of natural and effective antioxidants such as polyphenols (phenolic acids, flavonoids, etc.).

The aim of this work is to optimize the extraction of total polyphenols (temperature, duration and mass/volume ratio) from the leaves of *Melothria maderaspatana* by the dosage of total polyphenols and flavonoids and the evaluation of the antioxidant activity of the aqueous extract of the different organs of this plant. To this end, a qualitative analysis through phytochemical screening is carried out in order to know the nature of the secondary metabolites present in the different organs of the plant. The optimization of the extraction conditions is based on the determination of the values of the polyphenol content and the anti-radical activity as a function of the temperature, time and ratio parameters. These optimal extraction conditions are applied to the different organs of the plant in order to compare them in terms of therapeutic values.

2 Material and methods

2.1 Plant material

The plant was harvested in December 2021 in the commune of Taïba Ndiaye, a town located in the region of Thiès, in western Senegal, with geographical coordinates 15°3'0" N and 16°52'60" W. The plant material consisted of the leaf, stem and fruit of *Melothria maderaspatana*. After harvest, the different parts of the plant were dried away from light at room temperature in the GRSB laboratory (Bioactive Substances Research Group) of the Cheikh Anta Diop University of Dakar after identification at IFAN (Fundamental Institute of Black Africa). They were ground using an electric grinder to obtain a fine powder then kept well in jars to avoid any contamination.

Figure 1 Fine powder of leaves, stems and fruits

2.2 Extraction Methods

2.2.1 Solid-liquid extraction

This is the general case of the extraction of a biological organ (plant or animal) using the solvent. The sample to be extracted is most often in powder form. Depending on the extraction temperature, we distinguish: maceration, infusion, decoction and soxhlet extraction [13].

2.2.1.1 Maceration

The sample is placed in prolonged contact with the solvent, at room temperature (20 °C-25 °C). This method can be done continuously, the solvent being renewed automatically, maceration is preferably applied to thermolabile compounds such as proteins.

In maceration, frequent renewal of the solvent is fundamental. In fact, the solvent is quickly saturated with solute and becomes inefficient; it must therefore be renewed to achieve a new balance. Maceration carried out in a percolator is called percolation or leaching. Maceration is done cold, it is a slow process and consumes a lot of solvent [*13*].

2.2.1.2 Operating mode

Indeed, 20 g of fine powder from the leaves of the stems and fruits of *Melothria maderaspatana* were introduced into an Erlenmeyer flask with 100 mL of hexane. The mixture is covered with aluminum foil. After 24 hours in the dark at room temperature, we carried out filtration, a physical method which allows the solvent and the residue to be separated from

the material. With the residue obtained, a new maceration is carried out using a solvent of greater polarity. This same operation is repeated with all the solvents used, ethyl acetate, methanol and water.

2.2.2 Phytochemical characterization techniques

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: polyphenols, flavonoids, alkaloids, sterols and polyterpenes, leucoanthocyanins and catechols, coumarins, saponosides, mucilages, catechic and gallic tannins. We tested the presence of these different chemical groups by referring to the techniques described in the work of Ronchetti and Russo [38].

2.3 Optimization of *Melothria maderaspatana* extraction parameters

Temperature, time and the solvent mass/volume ratio constitute determining factors for the extraction of secondary metabolites in plant material samples, as well as for the determination of their biological activity. In this study, the aim is to determine the conditions for which the contents of polyphenols, flavonoids and the antioxidant activity at DPPH are maximum. To do this, we vary one of the analytical parameters, and by fixing the other two parameters we note the value for which the polyphenol content and the anti-radical activity are maximum.

2.3.1 Temperature

In order to determine the optimal temperature, the extraction is done with 0.5 g of plant material in 50 mL of distilled water at different temperatures (50 °C, 60 °C, 70 °C, 80 °C, 90 °C and 100 °C) for 20 minutes in a water bath. At the end of this operation, the extracts are filtered and the filtrate recovered then used for the various experiments.

2.3.2 Time

Once the optimal temperature is determined, the optimal extraction time is the next parameter. We set the temperature and the extraction mass and vary the duration from 10 to 60 minutes. As before, the filtered extracts are used for the different experiments.

2.3.3 Solvent mass/volume ratio

The temperature and duration having been determined, the variation of the quantity of plant material to be extracted in 50 mL of distilled water is carried out to determine the optimal extraction ratio. The masses to study are 0.5; 1; 2; 3;4 and 5 g. As for the two previous parameters, the filtrates recovered are used for the experiments.

2.3.4 Determination of total phenolic content

The content of total phenolic compounds was determined with the Folin-Ciocalteu reagent [14]. Indeed, 40 µL of each extract are taken and made up to 200 µL with distilled water. A volume of 150 µL of Folin-Ciocalteu reagent, 600 µL of a 20% Na_2CO_3 solution and 2.32 mL of distilled water are added in addition. After 30 minutes of incubation in the dark, the absorbance is read at 760 nm from a UV/Visible spectrometer of the Perkin-Elmer Lamda 365 type. The measurement was compared to a standard curve of gallic acid prepared from a 0.1 mg/mL stock solution of gallic acid.

2.3.5 Determination of flavonoid content

The flavonoid content was calculated by the method described by Dirar and al. [14]. This method consists of adding to 500 µL of each extract, 2.5 mL of distilled water, 2.5 mL of an ethanolic solution $AlCl_3$ at 2%. The mixtures are incubated for 1 hour at room temperature and the absorbance is read at 425 nm. The flavonoid content is expressed in terms of Quercetin equivalent (EqQ) with reference to the calibration curve plotted with a concentration range obtained from a 0.1 mg/mL quercetin stock solution.

2.3.6 Determination of antiradical activity: DPPH• method [14].

In order to determine the anti-radical activity, each of the extracts is subjected to a methanolic solution of DPPH● at 0.1 mM. Antioxidants produced by the plant kingdom are capable of giving up one of their hydrogen atoms in order to render free radicals inert, such as DPPH●.

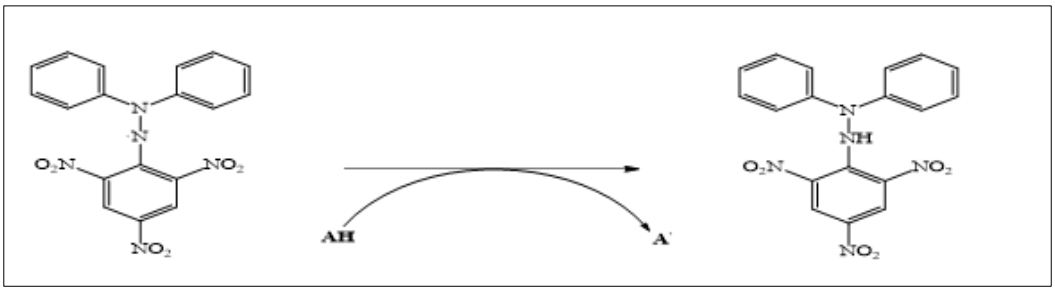

Figure 2 DPPH● radical reduction mechanism

To do this, the solutions are diluted according to the content of the stock solution and 200 µL of each extract are taken, 3.8 mL of DPPH● solution are added. The mixture is incubated for 30 min in the dark and the absorbance read at 517 nm.

3 Results and discussion

3.1 Phytochemical screening

Table 1 Results of phytochemical screening of solvent extracts from *Melothria maderaspatana* organs

		Polyphenols	Flavonoïds	Alcaloïds	Sterols and Polyterpenes	Leucoanthocyanins and catechols	Coumarins	Saponosins	Mucilages	Gallic Tannnins	Catechics Tannins
Leaves	Hexane	+	-	+	+	+++	-	-	-	-	-
	Ethyl acetate	+++	-	++	-	-	+++	-	-	-	-
	Methanol	+++	-	++	+++	+	-	-	-	-	-
	Water	+++	++	+	-	++	-	-	-	-	+++
Stems	Hexane	-	-	-	+++	+	-	-	-	-	-
	Ethyl acetate	+++	-	++	+++	-	+	-	-	-	-
	Methanol	+++	+	+	+++	+++	-	-	-	-	-
	Water	+++	++	+	-	+++	+	-	-	-	+++
Fruits	Hexane	-	-	+	+++	-	+	-	-	-	-
	Ethyl acetate	+++	-	-	+++	++	+	-	-	-	-
	Methanol	+++	-	-	+	++	-	-	-	-	-
	Water	+++	+	+		+++	-	-	-	-	+++

- = Absent + = Present ++ = Very present +++ = Very very present

3.1.1 For leaves

Phytochemical tests carried out on extracts from the leaves of *Melothria maderaspatana* revealed:

- The presence of polyphenols and alkaloids in all extracts.
- The absence of flavonoids, mucilage saponosides and gallic tannins in all extracts, except aqueous extract.
- The absence of leucoanthocyanins and catechols in the ethyl acetate extract and their presence in the other extracts.
- The absence of coumarins in all extracts except that of ethyl acetate.
- The presence of sterols and polyterpenes in hexanic and methanolic extracts and their absence in ethyl acetate and aqueous extracts.
- The flavonoid and Catechic tannins are only present in the aqueous extract

3.1.2 For the stems

Phytochemical tests carried out on extracts from the stems of *Melothria maderaspatana* revealed:

- The presence of polyphenols and alkaloids in all extracts except the hexanic one
- The presence of flavonoids in the methanol and aqueous extract and its absence in the others
- The presence of sterols and polyterpenes in all extracts except the water one
- The absence of leucoanthocyanins and catechols in the ethyl acetate extract and their presence in all the others
- The presence of coumarins in hexane and methanol extracts and its absence in the others
- The absence of mucilage saponosides and gallic tannins. Catechy tannins are only present in the aqueous extract

3.1.3 For fruits

Phytochemical tests carried out on stem extracts of *Melothria maderaspatana* revealed:

- The presence of polyphenols of leucoanthocyanins and catechols in all extracts except the hexanic one
- The presence of flavonoids in the aqueous extract and its absence in the others
- The absence of alkaloids in ethyl acetate and methanol extracts and its presence in other
- The absence of coumarins in the aqueous extracts and methanol but its absence in the others.
- The presence of sterols and poly terpenes in all extracts except the aqueous one.
- The absence of mucilage saponosides and gallic tannins. Catechy tannins are only present in the aqueous extract

3.2 Optimizing extraction parameters

3.2.1 Parameter 1: Temperature

Table 2 Evolution of absorbance as a function of temperature for total polyphenol contents

Temperature (°C)	**50**			**60**			**70**		
Mass (mg)	500			500			500		
Absorbance	0,156	0,158	0,160	0,156	0,159	0,162	0,170	0,171	0,173
Temperature (°C)	80			90			100		
Mass (mg)	500			500			500		
Absorbance	0,169	0,164	0,167	0,182	0,183	0,185	0,166	0,168	0,170

Table 3 Evolution of absorbance as a function of temperature for anti-radical activity at DPPH

Temperature (°C)	**50**			**60**			**70**		
Mass (mg)	500			500			500		
Absorbance	1,038	1,035	1,033	1,01	1,006	1,008	1,170	1,018	1,020
Temperature (°C)	80			90			100		
Mass (mg)	500			500			500		
Absorbance	1,024	1,025	1,028	1,018	1,020	1,019	1,001	1,008	1,004

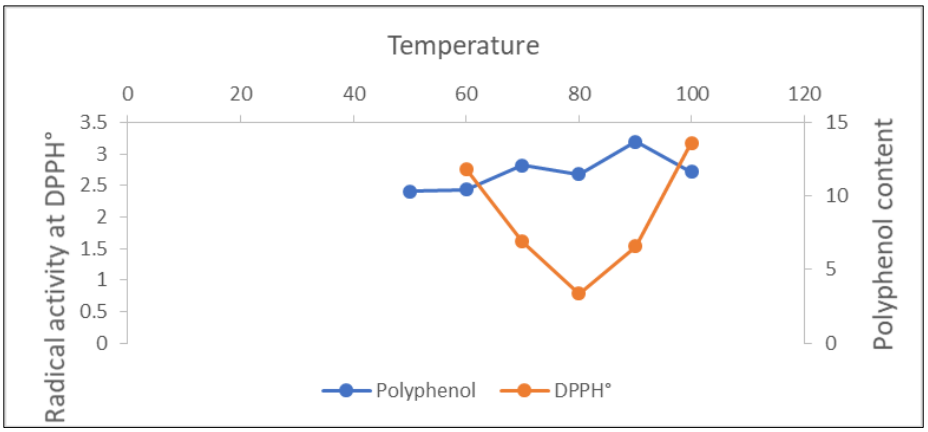

Figure 3 Evolution curve of total polyphenol contents and DPPH● anti-radical activity of the aqueous extract of *Melothria maderaspatana* as a function of temperature

The curves of evolution of the total polyphenol content and the anti-radical activity at DPPH● as a function of temperature show two particular points: that at 70°C and 90 °C

At 70 °C, we see a first maximum for the polyphenol content but the activity continues to decrease. At 90 °C, we reach a second maximum for the polyphenol content but with the activity increasing. However, we can see that from 80°C, the polyphenol content and the activity tend towards their maximum values, which allows us to take this value as the optimal extraction temperature. However, we can see that from 80°C, the polyphenol content and the activity tend towards their maximum values, which allows us to take this value as the optimal extraction temperature.

3.2.2 Parameter 2: Time

Table 4 Absorbance as a function of time for total polyphenol contents

Time (min)	**10**			**20**			**30**		
Mass (mg)	500			500			500		
Absorbance	0,185	0,185	0,183	0,147	0,147	0,145	0,179	0,180	0,181
Time (min)	40			50			60		
Mass (mg)	500			500			500		
Absorbance	0,169	0,171	0,172	0,185	0,186	0,183	0,180	0,180	0,181

Table 5 Absorbance as a function of time for antiradical activity at DPPH•

Time (min)	**10**			**20**			**30**		
Mass (mg)	500			500			500		
Absorbance	1,047	1,049	1,05	1,051	1,049	1,051	1,036	1,044	1,040
Temperature °C	40			50			60		
Mass (mg)	500			500			500		
Absorbance	1,06	1,066	1,069	1,048	1,035	1,038	1,036	1,038	1,036

The optimal temperature is set at 80 °C. By varying the extraction duration, we see an increase in the polyphenol contents and the anti-radical activity at DPPH● as a function of time (figure 4). These two indicators reach their stable values after 30 min. Beyond this date, they begin to decrease steadily. Therefore, these results allow us to conclude that the optimal extraction time for the bioactive molecules responsible for the antioxidant activity, contained in the aqueous extract of *Melothria maderaspatana*, is 30 min.

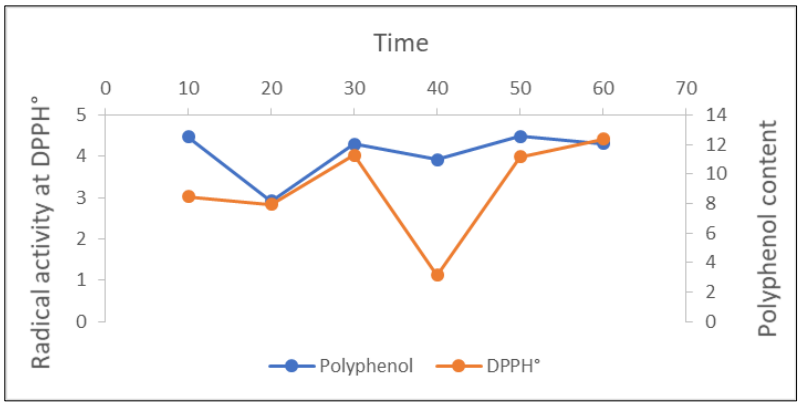

Figure 4 Evolution curve of total polyphenol contents and DPPH● anti-radical activity of the aqueous extract of *Melothria maderaspatana* as a function of time

The optimal temperature is set at 80 °C. By varying the extraction duration, we see an increase in the polyphenol contents and the anti-radical activity at DPPH● as a function of time (figure 4). These two indicators reach their stable values after 30 min. Beyond this value, they begin to decrease steadily. Therefore, these results allow us to conclude that the optimal extraction time for the polyphenols responsible for the antioxidant activity, contained in the aqueous extract of *Melothria maderaspatana*, is 30 min.

3.2.3 Parameter 3: Solvent mass/volume ratio

Table 6 Absorbance as a function of mass for total polyphenol contents

Mass (g)	**0,5**			**1**			**2**		
Absorbance	0,169	0,165	0,162	0,259	0,263	0,265	0,386	0,385	0,383

Table 7 Absorbance as a function of mass for antiradical activity at DPPH

Mass (g)	**0,5**			**1**			**2**		
Absorbance	1,153	1,154	1,58	1,110	1,117	1,115	1,061	1,067	1,064
Mass (g)	3			4			5		
Absorbance	1,001	0,999	0,996	0,936	0,929	0,933	0,880	0,880	0,883

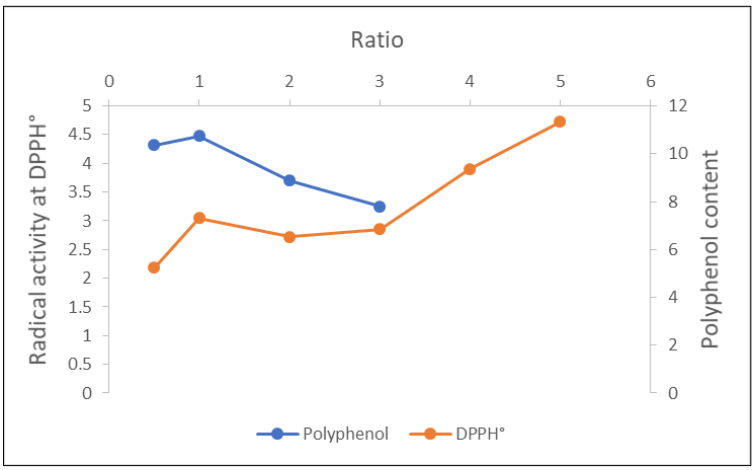

Figure 5 Evolution curve of total polyphenol contents and DPPH● anti-radical activity of the aqueous extract of *Melothria maderaspatana* as a function of the solvent mass/volume ratio

By varying the mass/volume ratio of solvent used, we see a reduction in the polyphenol content and the anti-radical activity beyond one gram (1g). These results then show a better ratio of 1g in 50 mL of solvent for optimal polyphenol content and anti-radical activity.

The results obtained, illustrated in the three previous figures, reveal that the total polyphenol content and the antioxidant activity of *Melothria maderaspatana* are optimal by dissolving 1g in 50 mL of plant material for a period of 30 min and at a temperature of 80 °C.

3.3 Application of optimal conditions in the aqueous extract of other plant organs

In this part we determine the contents of polyphenols and flavonoids then the anti-radical activity with DPPH● under the optimal extraction conditions determined previously. The extraction solvent used is water.

Temperature: 80 °C
Duration: 30 min
Ratio: 1g in 50 mL of solvent

The results obtained are recorded in the table below

Table 8 Content of polyphenols, flavonoids and the amount of antioxidant in DPPH● at mg/g

	Leaves	**Stems**	**Fruits**
Total polyphenol content	5,0112108	5,6651719	4,3759342
Flavonoid content	13,04362	6,8955496	4,9707031
Quantity of antioxidant in DPPH●	3,1541375	4,8295455	3,955496

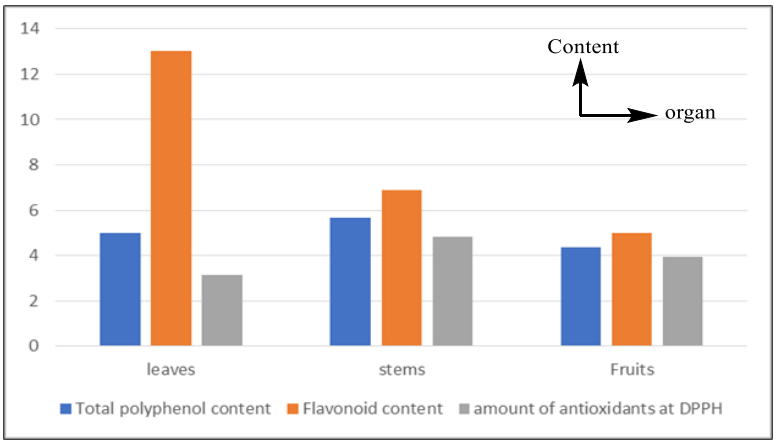

Figure 6 Diagram of evolution of the contents of polyphenols, flavonoids and the quantity of antioxidant at DPPH●

Under these extraction conditions, the total polyphenol contents are respectively equal to 5.0112108; 5.6651719; 4.3759342mg/g gallic acid equivalent, for leaves, stems and fruits. In flavonoids, they are equal to 13.04362 respectively; 6.8955496; 4.9707031mg/g Quercetin equivalent, for leaves, stems and fruits. The amount of DPPH● antioxidant evaluated revealed the values 3.1541375; 4.8295455; 3.955496 mg/g for leaves, stems and fruits respectively.

These results obtained, illustrated in Figure 6, show a significant flavonoid content in the leaves of *Melothria maderaspatana*, almost twice as high as that in the stems and fruits. Compared to polyphenols, we notice a balanced distribution in the three organs of the plant, with an average value of 5 mg/g of extract with a slight advantage for stems. Furthermore, the stems constitute the most active part for DPPH●, slightly higher than the aqueous extracts of the leaves and fruits. These results show a difference with those obtained by CHOUDHARY and al. [14]. Indeed, their work showed that the leaves are by far the most active part to the DPPH● radical and that polyphenols are the secondary metabolites most present in the aqueous extract of the leaves, in comparison to flavonoids. This result can be explained by the fact

that these authors carried out the extraction of polyphenols and flavonoids without taking into account the optimization of the conditions. In summary, we can note that the three parts of the plant have very interesting total polyphenol and flavonoid contents, with significant anti-radical activity, compared to the references.

4 Conclusion

In this work, we studied the impact of physicochemical parameters such as extraction time, temperature and solvent mass/volume ratio on the total polyphenol contents, and on the antioxidant activity of the aqueous extract. leaves of *Melothria maderaspatana*. The experimental results revealed that the optimal values are obtained at a temperature of 80°C, for 30 min and for a ratio of 1g per 50 mL of extraction solvent.

In view of the results obtained, we plan to deepen the study of the antioxidant activity, in particular the evolution of the percentage of inhibition as a function of the concentration, the evolution of the kinetics of reduction at DPPH$^{\bullet}$ and the optimization of the extraction conditions of *Melothria maderaspatana* with other organic solvents. A more complete phytochemical study is in perspective, in order to evaluate the antimicrobial and anticancer activities, up to the isolation and characterization of the bioactive molecules contained in this plant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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