

# Phytochemical and antioxidant investigations of extracts from the leaves of *Macaranga heterophylla* (Müll. Arg.) (Euphorbiaceae), a medicinal species used in Côte d'Ivoire

## ABSTRACT

**Aims:** This work aims to investigate the phytochemical composition and antioxidant potential of the leaves of *Macaranga heterophylla*.

**Methodology:** For this purpose, phytochemical screening by detection tests and thin layer chromatography (TLC), determination of total phenols content, total flavonoids content and condensed tannins content, and assessment of antioxidant potential by DPPH and reducing power tests were carried out on aqueous crude extracts, ethanolic crude extracts and selective extracts of the leaves of *M. heterophylla*

**Results:** The percentage yields obtained with ethanol (70%) (28.90 and 24.70% for the ethanolic decoction and ethanolic macerate respectively) are higher than those obtained with water (24.30 and 21.10% for the aqueous decoction and aqueous macerate respectively). The phytochemical screening highlighted the presence of several phytochemical families such as phenolic compounds (coumarins, flavones, tannins), quinones, sterols and polyterpenes, saponosides, glycosides, cardiotoxic glycosides and oligosaccharides. Quantitative analysis of total phenolics, total flavonoids and proanthocyanidols showed that their respective levels in the leaves of *M. heterophylla* varied depending on the solvent and the extraction technique. Concerning total phenolics, the aqueous decoction and ethanolic macerate gave the best total phenol contents ( $129.04 \pm 9.53$  and  $119.82 \pm 2.63$  mg EAG/g DM respectively); for total flavonoids, the aqueous decoction gave the best content ( $33.03 \pm 1.61$  mg EQ/g DM), while for condensed tannins, the aqueous macerate gave the best content ( $0.87 \pm 0.02$  mg ECT/g DM). With regard to DPPH antioxidant activity, the results showed that the ethanolic decoction has more pronounced antioxidant activity than the aqueous decoction, while the aqueous macerate showed better antioxidant activity than the ethanolic macerate. Concerning the reducing power test, the opposite trend was observed.

**Conclusion:** The present study demonstrated that *M. heterophylla* is a concentrate of secondary metabolites with antioxidant properties, which would explain its use in traditional medicinal practice.

**Keywords:** *Macaranga heterophylla*, leaves, phytoconstituents, antioxidant activity.

## 1. INTRODUCTION

“Essential natural resources, including medicinal plants, have always been the main source of medicines because of their wealth of biologically active phytochemicals, commonly known as secondary metabolites, which they synthesize” [1]. “Secondary metabolites are produced as an outcome of primary metabolism” [2]. “They are not essential for the growth and reproduction of the plant but they play many roles including defense against pathogens, pests and herbivores, response to environmental stresses and mediating organismal interactions” [3-5]. “As mentioned above, these metabolites also have a wide range of biological activities (antioxidants, antimicrobials, anticancer agents, etc.), making them highly valuable for human health and well-being, hence the use of plants in traditional medicine in

many ancient communities" [6-8] "Despite this, few plant species have been studied for medicinal applications" [9]. "To date, 400.000 known plant species have been the subject of chemical and pharmacological studies" [10].

"*Macaranga* is the largest genera in the Euphorbiaceae family. It is known to consist of about 300 species from tropical Africa, south-east Asia, Australia and the Pacific region" [11].

Previous work on this genus has demonstrated various bioactivities, including antioxidant [12, 13], antimicrobial [14, 15], anti-inflammatory [16, 17], anticancer [18, 19] and other types of biological activity [20-22]. Notwithstanding the great interest in this genus, there is a lack of information about the phytochemical and antioxidant properties of the *M. heterophylla* species we are investigating. It is an ornamental species used for its captivating foliage and flowers and traditionally used to treat snake bites, coughs, certain infectious diseases, as a purgative and to facilitate childbirth [23-27]. This work aims to investigate the phytochemical composition and antioxidant potential of the leaves of *M. heterophylla*.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

The plant material used consists of the leaves of *M. heterophylla*. They were harvested in April 2021 at Petit Yapo (5° 47' 51" N, 4° 8' 21" W), a locality near Azaguié that is a town in the Agnéby-Tiassa region of southern Côte d'Ivoire. After identification and authentication at the Centre National de Floristique (CNF) of the Félix HOUPHOUËT-BOIGNY University (Cocody/Abidjan) in accordance with the existing herbarium (N° UCJ006139), the leaves were cleaned with water and then dried under air conditioning (18° C) for 2 weeks. After drying, they were ground to a powder using an electric grinder (Vorwerk, Thermomix 3300) to improve the contact area with the solvents.

### 2.2 Methods

#### 2.2.1 Preparation of extracts

**Aqueous and ethanolic (70 %) decoctions:** 30 g of powder were boiled in 600 ml of distilled water and 600 ml of ethanol (70%) for 10 min, respectively. After cooling to room temperature and filtration through a büchner, the decoctions were concentrated in vacuum using a rotary evaporator (BÜCHI R100), and oven-dried at 45°C until the dry extracts DA (aqueous decoction) and DE (ethanolic decoction) were obtained.

**Aqueous and ethanolic (70 %) macerates:** 30 g of powder were macerated in 600 ml of distilled water and 600 ml of 70% ethanol for 24 hours, respectively. The solutions obtained were filtered through a büchner. The macerates were concentrated under vacuum using a rotary evaporator (BÜCHI R100), then kept in an oven (45°C) until the dry extracts MA (aqueous macerate) and ME (ethanolic macerate) were obtained.

**Selective extracts:** an aliquot of each dry DA, DE, MA, ME extract taken up in 100 ml of distilled water was successively exhausted with petroleum ether (3x50 ml) and ethyl acetate (3x50 ml). The different organic fractions obtained constituted the petroleum ether (DA<sub>1</sub>, DE<sub>1</sub>, MA<sub>1</sub>, ME<sub>1</sub>) and ethyl acetate (DA<sub>2</sub>, DE<sub>2</sub>, MA<sub>2</sub>, ME<sub>2</sub>) extracts.

#### 2.2.2 Qualitative tests

Phytochemical screening to identify the phytochemical families present in the leaves of *M. heterophylla* was carried out on decoctions and macerates, as well as on selective extracts, using either precipitation and color reaction tests [28-32] or thin-layer chromatography (TLC) [29, 33]. These tests focused on phenolic compounds (phenolic acids, coumarins,

flavonoids, tannins), quinones, saponosides, glycosides, sterols and polyterpenes, alkaloids and oligosaccharides.

### **2.2.3 Quantitative tests**

#### ***2.2.3.1 Evaluation of total phenolic (TP) contents***

Total phenolic contents were determined on decoctions and macerates using the Folin-Ciocalteu colorimetric method, with slight modifications. To 1 ml of each extract, diluted 1/10th with distilled water, were added 1.5 ml of sodium carbonate ( $\text{Na}_2\text{CO}_3$  (17%, m/v) and 0.5 ml Folin-Ciocalteu reagent (0.5N). The mixture was dark-incubated at room temperature for 30 min. Absorbance was read at 760 nm with a UV-visible spectrophotometer (Spectro AL 800) against a blank without extract. Total phenolics were quantified according to a regression equation ( $y = ax + b$ ) using gallic acid as standard at different concentrations (0.005 to 0.0375 mg/ml) under the same conditions as the sample. Results were expressed in milligrams of gallic acid equivalent per gram of dry matter (mg EAG/g DM) [30, 34].

#### ***2.2.3.2 Evaluation of total flavonoid (TF) contents***

Total flavonoid contents were determined on decoctions and macerates as previously described [35]. 0.01 g of each crude extract were solubilized in 10 ml of distilled water to give the stock solution, which was diluted 1 :10. To 2 ml of the diluted solution, 2 ml of aluminium chloride ( $\text{AlCl}_3$ ) (2%, m/v) in methanol (MeOH) were added. The mixture was dark-incubated for 15 min. Absorbance was read at 415 nm with a UV-visible spectrophotometer (Spectro AL 800), with distilled water used as the blank. A calibration line was drawn with quercetin at different concentrations (0.0005 to 0.0375 mg/ml). Total flavonoid contents were expressed in milligrams of quercetin equivalent per gram of dry matter (mg EQ/g DM).

#### ***2.2.3.3 Evaluation of condenses tannins (CT) or proanthocyanidols contents***

Condensed tannins or proanthocyanidols contents were determined on decoctions and macerates as previously described with slight modifications [36]. To 0.2 ml of each sample, 1.5 ml of a methanolic solution of vanillin (4%, m/v) and 0.75 ml of concentrated hydrochloric acid (HCl) were added. The mixture was dark-incubated for 15 min and the absorbance was read at 500 nm with a UV-visible spectrophotometer (Spectro AL 800). Condensed tannins contents were determined from calibration ranges established with catechin at various concentrations (0.009 to 0.15 mg/ml), and expressed in milligrams of catechin equivalent per gram of dry matter (mg EC/g DM).

### **2.2.4 Evaluation of antioxidant potential**

#### ***2.2.4.1 DPPH test***

The antioxidant potential of decoctions and macerates was assessed using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) reduction assay with slight modifications [37]. A concentration range (0.5; 0.25; 0.125; 0.0625; 0.03125; 0.015625 mg/ml) of extracts (decoctions and macerates) and ascorbic acid (vitamine C, used as antioxidant reference) was prepared in ethanol (EtOH). The ethanolic solution of DPPH at concentration of 0.03 mg/ml was also prepared. The reaction mixture used for the test consisted of the extract (1 ml) and the DPPH solution (2.5 ml), introduced into the cuvette of the UV-visible spectrophotometer (Spectro AL 800). The absorbance of the reaction mixture was read at

517 nm every 2 min (from 0 to 30 min) against a blank (1 ml EtOH and 2.5 ml DPPH solution).

#### **2.2.4.2 Reducing power assay**

The ability of **decoctions** and macerates to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  was determined according to the methodologies described [38-40] with slight modifications. Extracts and standard agent (0.5 ml) of different concentrations (1 ; 0.5 ; 0.25 ; 0.125 ; 0.0625 and 0.03125 mg/ml) were mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml potassium ferricyanide ( $\text{K}_3[\text{Fe}(\text{CN})_6]$ ) (1%, m/v), then incubated at 50°C in a water bath for 20 min. The reaction was stopped by adding 0.5 ml of trichloroacetic acid ( $\text{CCl}_3\text{CO}_2\text{H}$ ) solution (10%) and the mixture was centrifuged at 3000 rpm for 10 min. To 0.15 ml of the mixture were added 0.15 ml of distilled water and 0.04 ml of ferric chloride ( $\text{FeCl}_3$ ) (0.1%, m/v). Then, the mixture was incubated for 10 min and the absorbance of the reaction mixture was measured at 700 nm with a multimode microplate reader (Varioskan™ LUX, Thermo Fisher Scientific). A higher absorbance value indicated greater reducing power.

### **2.3 Statistical analysis**

Statistical analysis of the results was carried out using Graph Pad prism version 8.4.2 software to compare total phenols, total flavonoids, total condensed tannins and antioxidant activity. The Student's t-test was used to check whether the means of the variables differed between the samples (the **decoctions** on the one hand and the macerates on the other hand). Differences at  $P < 0.05$  were considered statistically significant.

## **3. RESULTS AND DISCUSSION**

### **3.1 Extraction yields**

Extraction yields obtained with 30 g of plant powder are respectively 24.30% for DA, 28.90% for DE, 21.10% for MA and 24.70% for ME (Table 1). We note that the yields obtained with ethanol (70%) are higher than those obtained with water. Indeed, under the same conditions of extraction time and temperature, solvent and sample composition are the most important parameters affecting yield. The use of a hydroalcoholic solvent may therefore facilitate the extraction of phytoconstituents that are soluble in water and/or in the organic solvent [41], which could explain the high yields obtained from ethanolic extractions (70%).

**Table 1. Extraction yields**

<b>Extracts</b>	<b>Yields (%)</b>
DA	24.30
DE	28.90
MA	21.10
ME	24.70

### **3.2 Qualitative phytochemical profile**

#### **3.2.1 Phytochemical composition using precipitation and color reaction tests**

Phytochemical screening revealed the presence of most phytoconstituents found in **decoctions** and macerates, namely phenolic compounds (coumarins, flavones, tannins),

quinones, sterols and polyterpenes, saponosides, glycosides including cardiotonics, **oligosaccharides**; with the exception of flavones for DE, phlobatanins and ketoses. The presence or absence of alkaloids in extracts depends on the type of reagent used (Table 2).

**Table 2. Results of phytochemical screening using precipitation or color reaction tests**

Phytoconstituents	Tests	DA	DE	MA	ME
Phenolic compounds	FeCl <sub>3</sub> (2%)	+	+	+	+
Flavonoids	Shinoda (Mg) / HCl	+	-	+	+
	NH <sub>4</sub> OH (Flavones)	+	+	+	+
Coumarins	NaOH (10%)	+	+	+	+
Tannins	FeCl <sub>3</sub> (5%)	+	+	+	+
Alkaloids	H <sub>2</sub> SO <sub>4</sub>	+	+	+	+
	Bürchard	+	+	+	+
	Dragendorff	-	-	-	-
	Mayer	+	-	-	-
	Picric acid	-	-	-	-
Phlobatannins	HCl	-	-	-	-
Sterols and polyterpenes	CH <sub>3</sub> CO <sub>3</sub> CH <sub>3</sub> , H <sub>2</sub> SO <sub>4</sub>	+	+	+	+
Saponosides	Fi		200		
Glycosides	CHCl <sub>3</sub> , NH <sub>4</sub> OH	+	+	+	+
Cardiotonic glycosides	Liebermann-Bürchard	+	+	+	+
Oligosaccharides	Molish	+	+	+	+
	Seliwanoff (ketoses)	-	-	-	-

(+): positive ; (-): negative ; Fi: Foam index

### 3.2.2 Phytochemical composition by thin layer chromatography (TLC)

Phytochemical screening by precipitation or color reaction tests does not specify the exact type of phytomolecules sought. That is why we also decided to determine the phytochemical composition of extracts by TLC. This analytical planar chromatographic technique enables phytocompounds to be separated according to their migration properties. It was carried out on selective extracts, which are less complex in terms of phytochemical content than matrix extracts (decoctions and macerates). The results are presented below (Table 3). Several secondary metabolites such as sterols, terpenes, triterpenes of the oleanane or ursane type, coumarins, flavonoids and tannins were identified using TLC. The Liebermann-Bürchard reagent revealed sterols as yellow spots under UV/365 nm. This reagent also revealed oleanane or ursane-type triterpenes as red spots under UV/365 nm [42, 43]. Sulfuric vanillin

revealed terpenes as purple spots and sterols as blue spots in visible [43, 44]. Aluminium chloride (AlCl<sub>3</sub>, 1%) revealed flavonoids as green, yellow and blue spots under UV/365 nm [28, 43]. Flavonoids were also highlighted by ammonia under UV/365 nm as green spots [28, 45]. Methanolic solution of potassium hydroxide (KOH, 5%) was used to detect coumarins as yellow-orange spots in visible and as yellow, green, blue and blue fluorescent spots under UV/365 nm [28, 46]. In addition, basic lead acetate (C<sub>4</sub>H<sub>10</sub>O<sub>6</sub>Pb, 5%) highlighted them as blue and green spots fluorescing under UV/365 nm [47]. Tannins were revealed by FeCl<sub>3</sub> (2%) as gray spots in visible [43, 48]. Examination of the TLC phytochemical profile confirms the results obtained after screening by precipitation and color reaction tests.

**Table 3. Results of phytochemical screening using TLC**

Selective extracts	Secondary metabolites identified : [Rf], color
Petroleum ether extracts	DA <sub>1</sub> Sterols <sup>e,f</sup> : [0,16] Y <sup>e</sup> ; [0,33] B <sup>f</sup> ; Terpenes <sup>f</sup> : [0,83] O <sup>f</sup> ;
	DE <sub>1</sub> Terpenes <sup>f</sup> : [0,66] P <sup>f</sup> ; [0,85] P <sup>f</sup> ; [0,96] P <sup>f</sup> ; Sterols <sup>f</sup> : [0,44] B <sup>f</sup> ;
	MA <sub>1</sub> Terpenes <sup>f</sup> : [0,15] P <sup>f</sup> ; [0,29] P <sup>f</sup> ;
	ME <sub>1</sub> Sterols <sup>e,f</sup> : [0,16] Y <sup>e</sup> ; [0,19] B <sup>f</sup> ; [0,30] Y <sup>e</sup> ; [0,44] Y <sup>e</sup> ; [0,85] Y <sup>e</sup> ; Triterpenes oleanone or ursane <sup>e</sup> : [0,41] R <sup>e</sup> ; [0,55] R <sup>e</sup> ; [0,69] R <sup>e</sup> ;
Ethyl acetate extracts	DA <sub>2</sub> Coumarins <sup>a,h</sup> : [0,14] G <sup>h</sup> ; [0,16] B <sup>a</sup> ; [0,43] Br <sup>a</sup> ; Flavonoids <sup>b</sup> : [0,14] G <sup>b</sup> ; [0,40] G <sup>b</sup> ; Tannins <sup>c</sup> : [0,40] Gy <sup>c</sup> ;
	DE <sub>2</sub> Coumarins <sup>a,h</sup> : [0,05] G <sup>a</sup> ; [0,26] YO <sup>a</sup> ; [0,61] Bf <sup>h</sup> ; [0,65] B <sup>a</sup> -B <sup>h</sup> ; [0,69] B <sup>h</sup> ; [0,75] B <sup>h</sup> ; Flavonoids <sup>b</sup> : [0,05] G <sup>b</sup> ; [0,49] B <sup>b</sup> ; [0,59] B <sup>b</sup> ; [0,65] B <sup>b</sup> ; [0,69] B <sup>b</sup> ; Tannins <sup>c</sup> : [0,26] Gy <sup>c</sup> ; [0,40] Gy <sup>c</sup> ;
	MA <sub>2</sub> Coumarins <sup>a,h</sup> : [0,06] G <sup>a</sup> ; [0,25] YO <sup>a</sup> ; [0,65] Bf <sup>a</sup> ; Flavonoids <sup>b</sup> : [0,06] G <sup>b</sup> ; [0,19] B <sup>b</sup> ; [0,51] B <sup>b</sup> ; Tannins <sup>c</sup> : [0,25] Gy <sup>c</sup> ;
	ME <sub>2</sub> Coumarins <sup>a,h</sup> : [0,16] B <sup>a</sup> -B <sup>h</sup> ; [0,40] YO <sup>a</sup> ; [0,59] Y <sup>a</sup> ; [0,63] YO <sup>a</sup> ; [0,75] Bf <sup>a</sup> -Bf <sup>h</sup> ; Flavonoids <sup>b,g</sup> : [0,11] G <sup>b</sup> ; [0,44] G <sup>g</sup> ; [0,61] Bf <sup>b</sup> ; [0,68] G <sup>g</sup> ; [0,69] Bf <sup>b</sup> ; [0,75] Y <sup>b</sup> ; [0,85] Y <sup>b</sup> ; [0,89] G <sup>g</sup> ; Tannins <sup>c</sup> : [0,05] Gy <sup>c</sup> ; [0,44] Gy <sup>c</sup> ;

a : KOH ; b : AlCl<sub>3</sub> ; c : FeCl<sub>3</sub> ; e : Liebermann-Bürchard ; f : sulfuric vanillin ; g : ammoniac ; h : basic lead acetate ; O : orange ; YO : yellow orange , B : blue ; Y : yellow ; G : green ; Bf : blue fluorescent ; P : purple ; R : red ; Gy : gray

### 3.3 Quantitative phytochemical profile

#### 3.3.1 TP contents

The TP contents (Table 4) were obtained from the linear regression equation ( $y = 6.7469x - 0.0353$ ;  $R^2 = 0.994$ ) established with a concentration ranges of gallic acid. They range from 84.99 to 129.04 mg (EAG/g DM). In the case of **decoctions**, TP contents are significantly higher ( $P < 0.05$ ) in DA (129.04 mg EAG/g DM on average) than in DE (84.99 mg EAG/g DM on average). An opposite trend was observed in previous studies [49, 50]. In the case of

macerates, TP contents are significantly higher ( $P < 0.05$ ) in ME (119.82 mg EAG/g DM on average) than in MA (107.90 mg EAG/g DM on average). A similar trend was observed in work carried out on the species *Helichrysumstoechas* [49], indicating that estimated total phenolic contents are higher in ethanolic maceration (70%) than in aqueous maceration. Previous studies [51-53] have reported that ethanol combined with water provides better extraction of phytophenols. From these results, we deduce that the total phenolic content depends on the type of extraction and the polarity of the solvent used. As a result, aqueous decoction appears to be suitable for better extraction of total phenolics.

**Table 4. TP contents**

Extracts	TP contents (mg EAG/g DM)
DA	129.04 ± 9.53 a
DE	84.99 ± 4.44 b
MA	107.90 ± 3.15 a
ME	119.82 ± 2.63 b

Results are expressed as mean ± SD (n=3). There is a significant difference between values followed by a different letter ( $P < 0.05$ ) as measured by the Student's t-test

### **3.3.2TF contents**

The TF contents (Table 5) were determined from the linear regression equation ( $y = 22.779x - 0.0159$ ;  $R^2 = 0.999$ ) established with a concentration ranges of quercetin. They range from 13.42 to 33.03 (mg EQ/g DM). They are significantly higher ( $P < 0.001$ ) in DA (33.03 mg EQ/g DM on average) than in DE (13.42 mg EQ/g DM on average). An opposite trend has been reported by other authors [49, 50], mentioning that total flavonoid contents are higher in ethanolic decoction (70%) than in aqueous decoction. For macerates, no significant difference was found between total flavonoid contents ( $P > 0.05$ ). However, according to these authors [54], an ethanol/water mixture (70, 30 ; v/v) would increase the quantity of flavonoids. From the results we have obtained, we deduce that aqueous decoction would be the most suitable method for flavonoid extraction.

**Table 5. TF contents**

Extracts	TF contents (mg EQ/g DM)
DA	33.03 ± 1.61 a
DE	13.42 ± 1.28 b
MA	23.56 ± 1.33 a
ME	23.42 ± 1.48 a

Results are expressed as mean ± SD (n=3). There is a significant difference between values followed by a different letter ( $P < 0.05$ ) as measured by the Student's t-test

### **3.3.3CT or proanthocyanidols contents**

The CT contents (Table 6) were determined from the linear regression equation ( $y = 1.6546x + 0.0086$ ;  $R^2 = 0.998$ ) established with a concentration ranges of catechin. They range from 0.59 to 0.87 (mg EC/g DM). For **decoctions**, no significant difference was found between CT

contents ( $P > 0.05$ ). Regardless of the solvents used, decoctions extracted practically the same quantities of CT. However, CT contents were significantly higher ( $P < 0.05$ ) in MA (0.87 mg EC/g DM on average) than in ME (0.61 mg EC/g DM on average). A similar trend was observed in the bracts of *Cynarascolumus* L. In fact, CT contents were higher in the aqueous macerate than in the ethanolic macerate. In addition, previous investigations [53, 55] have shown that decoction is more effective at extracting condensed tannins than maceration. However, according to our results, the high content of condensed tannins is obtained by aqueous maceration. This can be explained by the fact that decoction, which consists of immersing the plant in a solvent and boiling for a few minutes, leads to the degradation of heat-sensitive tannins [56].

**Table 6. CT contents**

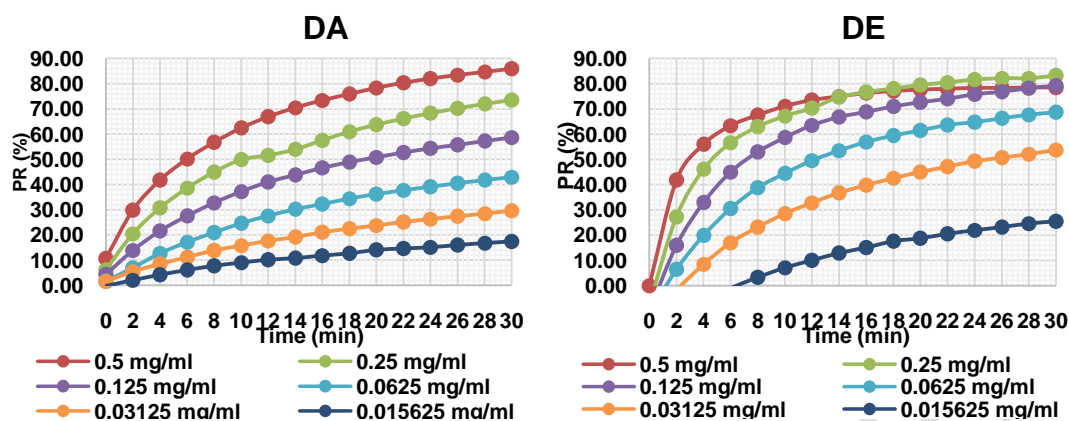
Extracts	CT contents(mg EC/g DM)
DA	0,59 ± 0,03 a
DE	0,59 ± 0,03 a
MA	0,87 ± 0,02 a
ME	0,61 ± 0,09 b

Results are expressed as mean ± SD (n=3). There is a significant difference between values followed by a different letter ( $P < 0.05$ ) as measured by the Student's t-test

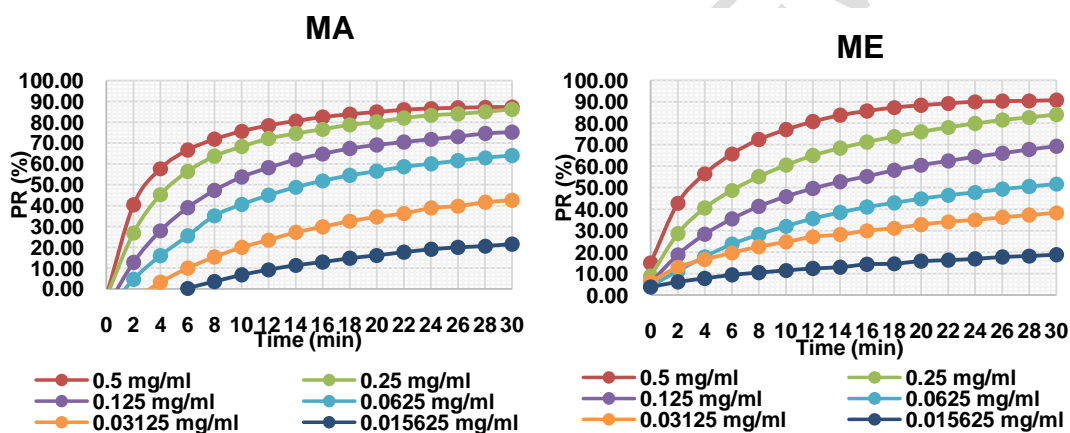
### 3.4 Antioxidant profile

#### 3.4.1 DPPH antioxidant activity

**Decoctions** and macerates showed antioxidant potential with concentration-dependent percentages reduction (PR) of DPPH radical as a function of time. In the first few minutes, a rapid drop in the absorbance of the DPPH radical is observed, followed by a slow step until equilibrium is reached. Concerning the PR of DPPH radical by the **decoctions** (Figures 1), DA showed a maximum DPPH scavenging activity of 86% at 0.5 mg/ml (30 min), whereas for DE was found to be 78.55% at the same concentration and time. Concerning the PR of DPPH radical by the macerates (Figures 2), MA showed a maximum DPPH scavenging activity of 87.27% at 0.5 mg/ml (30 min), whereas for ME was found to be 90.75% at the same concentration and time.



Figures 1: Percentages reduction of DPPH radical by the decoctions (DA and DE) as a function of time



Figures 2: Percentages reduction of DPPH radical by the macerates (MA and ME) as a function of time

A slight drop of PR was observed with decoctions, which could be explained by the loss of heat-sensitive phytoconstituents. However, the PR of DPPH radical by the decoctions and the macerates are lower than that of vitamin C (95.75%) at 0.5 mg/ml (30 min) (Figure 3).

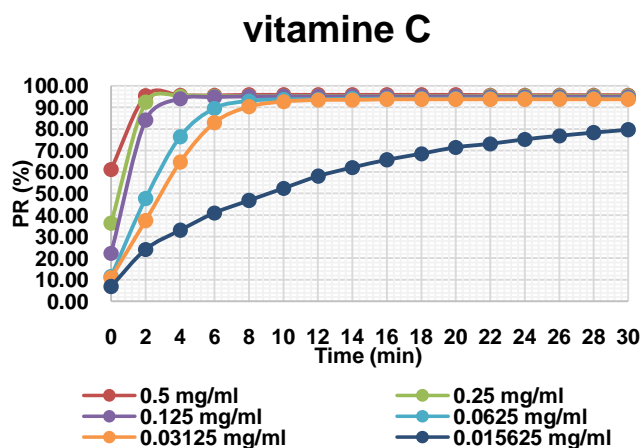


Figure 3: Percentages reduction of DPPH radical by vitamine C as a function of time

Determination of the  $CR_{50}$  (median concentration of the sample that reduces 50% of the DPPH) enabled a better assessment of the antioxidant potential of the extracts. The lower its value, the more pronounced the antioxidant activity [57]. The  $CR_{50}$  values of decoctions and macerates were determined graphically. (Figure 4).

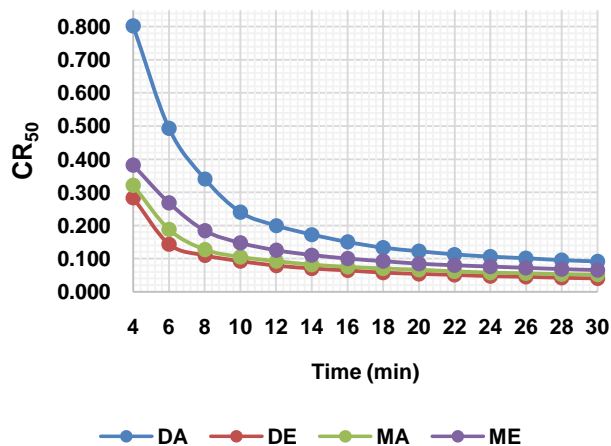


Figure 4:  $CR_{50}$  values of decoctions and macerates as a function of time

Concerning the decoctions,  $CR_{50}$  values of DE are lower than those of DA, demonstrating that DE has a more pronounced antioxidant activity than DA. For the macerates,  $CR_{50}$  values of MA are lower than those of ME. MA therefore exhibits a better antioxidant activity than ME.

### 3.4.2 Reducing power

The reducing power assay is often used to assess the ability of compounds to reduce  $Fe^{3+}$  to  $Fe^{2+}$  [58]. Figure 5 shows the reducing power of decoctions and macerates, compared with ascorbic acid (AA), used as a reference antioxidant.

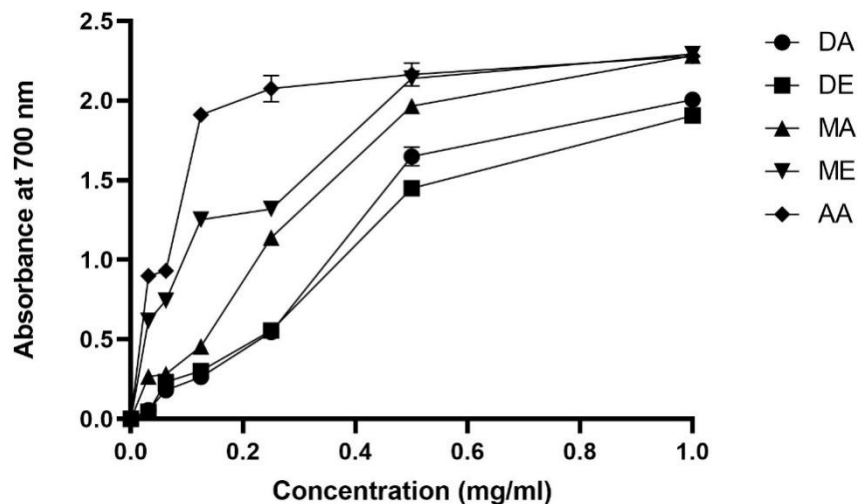


Figure 5 : Reducing power of decoctions, macerates and ascorbic acid

The reducing power of decoctions and macerates, like that of AA, is concentration-dependent. Indeed, increasing analyte concentrations induce a progressive variation in reducing power. Concerning the decoctions, DA showed a significantly higher reducing power ( $P < 0.001$ ) than DE at 0.5 mg/ml. For the macerates, ME showed a significantly higher reducing power ( $P < 0.001$ ) than MA, suggesting that the high levels of total phenolics in DA and ME are the reason. Phytophenols have antioxidant properties as hydrogen donors. They are excellent free radical scavengers [59]. These results are in contrast to those of the DPPH test, which shows the antioxidant profile of DE to be better than that of DA, and that of MA to be better than that of ME. The judicious choice of these two methods seems to explain this difference with regard to the type of reduction mechanism [60, 61]. In any case, the presence of antioxidant phytoconstituents in decocted and macerated of the leaves of *M. heterophylla* justifies its traditional use in the treatment of various diseases.

#### 4. CONCLUSION

This work has enabled us to determine the phytochemical and antioxidant profiles of the leaves of *M. heterophylla*. The study of the chemical composition and effects of a plant matrix is based on a rational approach: firstly, the choice of solvent for better extraction of the phytochemicals, and secondly, the choice of extraction method. The percentages of phytoconstituents extracted from the leaves of *M. heterophylla* varies according to solvent and extraction method. The phytochemical screening highlighted the presence of several phytochemical families, including phenolic compounds (coumarins, flavones, tannins), quinones, sterols and polyterpenes, saponosides, glycosides, cardiogenic glycosides and oligosaccharides. Quantitative analysis of total phenolics, total flavonoids and proanthocyanidols showed that their respective levels in the leaves of *M. heterophylla* varied depending on the solvent and the extraction technique. Assessment of the antioxidant power of the decoctions and macerates showed that the plant contains antioxidant phytoactives. These results suggest that the local species could be developed into a phytopreparation for therapeutic use in the form of an herbal tea as water was able to extract phytoconstituents better.

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