Original Research Article

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 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. 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 decoctates and macerates showed that the plant contains antioxidant phytoactives.
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, 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 phytosubstances, commonly known as secondary metabolites, which they synthesise [1]. Despite this, few plant species have been studied for medicinal applications [2]. To date, 400.000 known plant species have been the subject of chemical and pharmacological studies. [3]. *Macaranga* is a genus of the Euphorbiaceae. This botanical family includes around 300 species from tropical Africa, south-east Asia, Australia and the Pacific region [4]. Previous work on this genus has demonstrated various bioactivities, including antioxidant [5, 6], antimicrobial [7, 8], anti-inflammatory [9, 10], anticancer [11, 12] and other types of biological activity [12, 13]. 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 [14-18]. This work aims to investigate the phytochemical composition and antioxidant potential of the leaves of *M. heterophylla*.

2. MATERIAL 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.

2.2Methods

2.2.1 Preparation of extracts

Aqueous and ethanolic (70 %) decoctates: 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 decoctates were concentrated in vacuum using a rotary evaporator (BÜCHI R100), and oven-dried at 45°C until the dry extracts DA (aqueous decoctate) and DE (ethanolic decoctate) 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 (3×50 ml) and ethyl acetate (3×50 ml). The different organic fractions obtained constituted the petroleum ether (DA_1, DE_1, MA_1, ME_1) and ethyl acetate (DA_2, DE_2, MA_2, ME_2) extracts.

2.2.2Qualitative tests

Phytochemical screening to identify the phytocompound families present in the leaves of *M. heterophylla* was carried out on decoctates and macerates, as well as on selective extracts, using either precipitation and color reaction tests [19-23] or thin-layer chromatography (TLC) [20, 24]. These tests focused on phenolic compounds (phenolic acids, coumarins, flavonoids, tannins), quinones, saponosides, glycosides, sterols and polyterpenes, alkaloids and sugars.

2.2.3Quantitative tests

2.2.3.1 Evaluation of total phenolic (TP) contents

Total phenolic contents were determined on decoctates 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 (Na_2CO_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) [21, 25].

2.2.3.2Evaluation of total flavonoid (TF) contents

Total flavonoid contents were determined on decoctates and macerates as previously described [26]. 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 ($AlCl_{3)}$ (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).

2.2.3.3Evaluation of condenses tannins (CT) or proanthocyanidols contents

Condensed tannins or proanthocyanidols contents were determined on decoctates and macerates as previously described with slight modifications [27]. 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).

2.2.4Evaluation of antioxidant potential

2.2.4.1 DPPH test

The antioxidant potential of decoctates and macerates was assessed using the stable radical 2,2'-diphenyl-1-picrylhydrazyl (DPPH) reduction assay with slight modifications [28]. A concentration range (0.5; 0.25; 0.125; 0.0625; 0.03125; 0.015625 mg/ml) of extracts (decoctates 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).

2.2.4.2Reducing power assay

The ability of decoctates and macerates to reduce Fe^{3+} to Fe^{2+} was determined according to the methodologies described [29-31] 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 ($K_3[Fe(CN)_6]$) (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 thichloroacetic acid (CCl_3CO_2H) 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 ($FeCl_{3)}$ (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 (VarioskanTM LUX, Thermo Fisher Scientific). A higher absorbance value indicated greater reducing power.

2.3Statistical 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 decoctates on the one hand and the macerates on the other hand). Differences at $P<0.05$ were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1Extraction 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 [32], which could explain the high yields obtained from ethanolic extractions (70%).

Table 1. Extraction yields

Extracts	Yields (%)
DA	24.30
DE	28.90
MA	21.10
ME	24.70

3.2Qualitative phytochemical profile

3.2.1 Phytochemical composition using precipitation and color reaction tests

Phytochemical screening revealed the presence of most phytoconstituents found in decoctates and macerates, namely phenolic compounds (coumarins, flavones, tannins), quinones, sterols and polyterpenes, saponosides, glycosides including cardiotonics, sugars (oses and diholosides) ; 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 usingprecipitation or colorreaction tests

Phytoconstituents	Tests	DA	DE	MA	ME
Phenolic compounds	$FeCl_3$ (2%)	+	+	+	+
Flavonoids	Shinoda (Mg) / HCl	+	-	+	+
	NH_4OH (Flavones)	+	+	+	+
Coumarins	NaOH (10%)	+	+	+	+
Tannins	$FeCl_3$ (5%)	+	+	+	+
Quinones	H_2SO_4	+	+	+	+
Alcaloids	Bürchard	+	+	+	+
	Dragendorff	-	-	-	-
	Mayer	+	-	-	-
	Picric acid	-	-	-	-
Phlobatannins	HCl	-	-	-	-
Sterols et polyterpènes	$CH_3CO_3CH_3$, H_2SO_4	+	+	+	+
Saponosides	Fi	200			
Glycosides	$CHCl_3$, NH_4OH	+	+	+	+
Cardiotnic glycosides	Liebermann-Bürchard	+	+	+	+
Sugars (oses, diholosides)	Molish	+	+	+	+
	Seliwanoff (ketoses)	-	-	-	-

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

3.2.2 Phytochemical composition by 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 (decoctates and macerates). The results are presented below (Table 3). Several secondary metabolites such as sterols, terpenes, triterpenes of the oleane 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 oleane or ursane-type triterpenes as red spots under UV/365 nm [33, 34].Sulfuric vanillin revealed terpenes as purple spots and sterols as blue spots in visible [34, 35]. Aluminium chloride ($AlCl_3$, 1%) revealed flavonoids as green, yellow and blue spots under UV/365 nm [19, 34]. Flavonoids were also highlighted by ammonia under UV/365 nm as green spots [19, 36]. 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 [19, 37]. In addition, basic lead acetate ($C_4H_{10}O_6Pb$, 5%) highlighted them as blue and green spots fluorescing under UV/365 nm [38]. Tannins were revealed by $FeCl_3$ (2%) as gray spots in visible [34, 39]. 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_1	**Sterols[e,f] :** [0,16] Y^e ; [0,33] B^f ; **Terpenes[f] :** [0,83] O^f ;
	DE_1	**Terpenes[f] :** [0,66] P^f ; [0,85] P^f ; [0,96] P^f ;
	MA_1	**Sterols[f] :**[0,44] B^f ; **Terpenes[f] :** [0,15] P^f ; [0,29] P^f ;
	ME_1	**Sterols[e,f] :** [0,16] Y^e ; [0,19] B^f ; [0,30] Y^e ; [0,44] Y^e ; [0.85] Y^e ; **Triterpenes oleane or ursane[e]** : [0,41] R^e ; [0,55] R^e ; [0,69] R^e ;
Ethyl acetate extracts	DA_2	**Coumarins[a,h] :** [0,14] G^h ; [0,16] B^a ; [0,43] Br^a ; **Flavonoids[b] :** [0,14] G^b ; [0,40] G^b ; **Tannins[c] :** [0,40] Gy^c ;
	DE_2	**Coumarins[a,h] :** [0,05] G^a ; [0,26] YO^a ; [0,61] Bf^h ; [0,65] B^a-B^h ; [0,69] B^h ; [0,75] B^h ; **Flavonoids[b] :**[0,05] G^b ; [0,49] B^b ; [0,59] B^b ; [0,65] B^b ; [0,69] B^b ; **Tannins[c] :** [0,26] Gy^c ; [0,40] Gy^c ;
	MA_2	**Coumarins[a,h] :** [0,06] G^a ; [0,25] YO^a ; [0,65] Bf^a ; **Flavonoids[b] :** [0,06] G^b ; [0,19] B^b ; [0,51] B^b ; **Tannins[c] :** [0,25] Gy^c ;
	ME_2	**Coumarins[a,h] :** [0,16] B^a-B^h ; [0,40] YO^a ; [0,59] Y^a ; [0,63] YO^a ; [0,75] Bf^a-Bf^h ; **Flavonoids[b,g] :** [0,11] G^b ; [0,44] G^g ; [0,61] Bf^b ; [0,68] G^g ; [0,69] Bf^b ; [0,75] Y^b ; [0,85] Y^b ; [0,89] G^g ; **Tannins[c] :** [0,05] Gy^c ; [0,44] Gy^c ;

a : KOH ; b : $AlCl_3$: c : $FeCl_3$; 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

3.3Quantitative phytochemical profile

3.3.1TP 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 decoctates, 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 [40, 41]. 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 *Helichrysum stoechas* [40], indicating that estimated total phenolic contents are higher in ethanolic maceration (70%) than in aqueous maceration. Previous studies [42-44] 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 [40, 41], 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 [45], 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 ($y = 1.6546x + 0.0086$; $R2 = 0.998$) established with a concentration ranges of catechin. They range from 0.59 to 0.87 (mg EC/g DM). For decoctates, 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 *Cynara scolymus* L. In fact, CT contents were higher in the aqueous macerate than in the ethanolic macerate. In addition, previous investigations [44, 46] 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 [47].

Table 6. CT contents

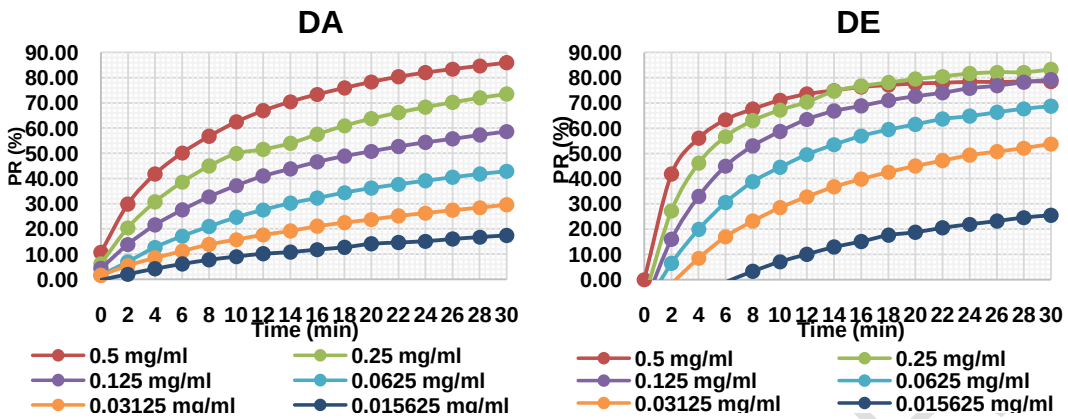
Extracts	CT contents(mg EC/g DM)
DA	0,59 ± 0,03 a
DE	0,59 ± 0,03 a
MA	0,87 ± 0,02 a
ME	0,61 ± 0,09 b

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

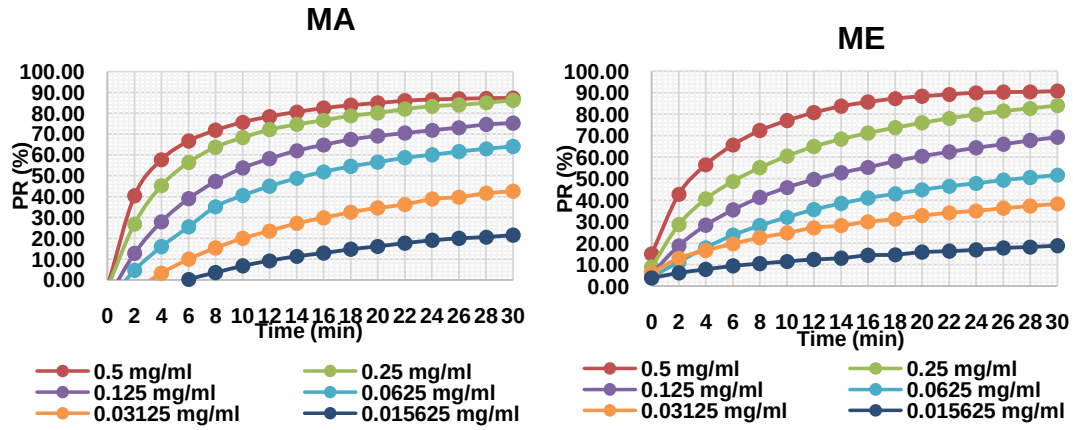
3.4Antioxidant profile

3.4.1 DPPH antioxidant activity

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

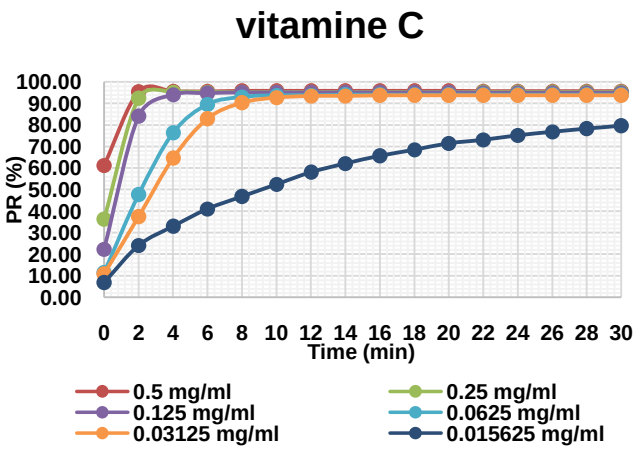


Figure 3: Percentages reduction of DPPH radical by vitamine C as a function of time

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 [48]. The CR_{50} values of decoctates and macerates were determined graphically. (Figure 4).

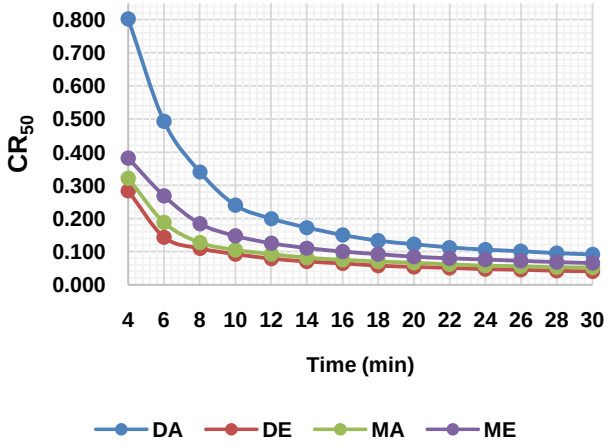


Figure 4: CR_{50}values of decoctates and macerates as a function of time

Concerning the decoctates, 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+} [49]. Figure 5 shows the reducing power of decoctates and macerates, compared with ascorbic acid (AA), used as a reference antioxidant.

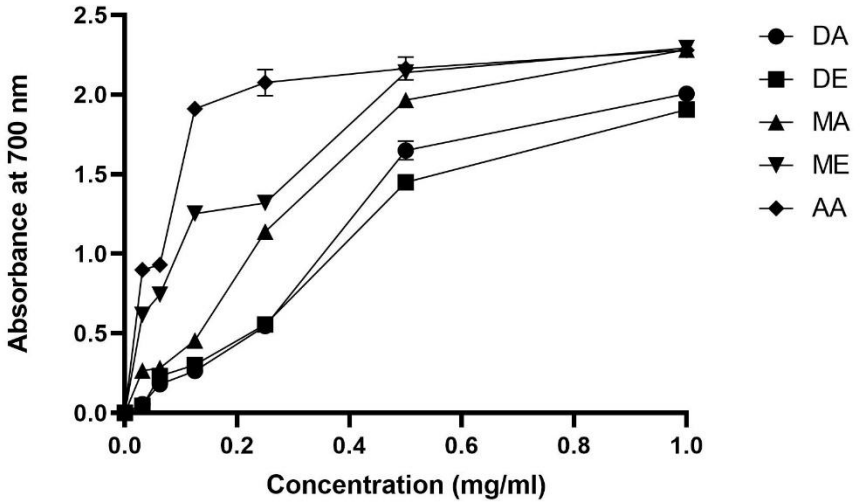


Figure 5 : Reducing power of decoctates, macerates and ascorbicacid

The reducing power of decoctates and macerates, like that of AA, is concentration-dependent. Indeed, increasing analyte concentrations induce a progressive variation in reducing power. Concerning the decoctates, 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 [50]. 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 [51, 52]. 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, cardiotonic glycosides and sugars (oses and diholosides). 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 decoctates 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.

REFERENCES

1. Fouché JG, Marquet A, Hambuckers A. The medicinal plants from plant to medicine. Temporary exhibition Liège. French. 2000; 17.
2. Strzemski M, Wójciak-Kosior M, Sowa I, Załuski D, Verpoorte R. Historical and traditionalmedical applications of Carlinaacaulis L.-A criticalethnopharmacologicalreview. Journal of ethnopharmacology. 2019; 239:111842.
3. Balick MJ, Cox PA. The science of plants, people, and culture: the science of ethnobotany. 2nd ed. New York: HPHLP. 2020; 228.
4. Davies SJ. Photosynthesis of ninepioneer Macaranga speciesfromBorneo in relation to life history. Ecology. 1998; 79(7):2292–2308.
5. Phormmart S, Sutthivaiyakit P, Chimnoi N, Ruchirawat S, Sutthivaiyakit S. Constituents of the Leaves of Macaranga tanarius. Journal of Natural Products. 2005; 68(6):927-930.
6. Matsunami K, Otsuka H, Kondo K, Shinzato T, Kawahata M, Yamaguchi K, Takeda Y. Absolute configuration of (+)-pinoresinol 4-O-[6″-O-galloyl]-β-d-glucopyranoside, 200 . Macarangiosides E, and F isolatedfrom the leaves of Macaranga tanarius. Phytochemistry. 2009; 70(10):1277–1285.
7. Schütz BA, Wright AD, Rali T, Sticher O. Prenylatedflavanonesfromleaves of Macaranga pleiostemona. Phytochemistry. 1995; 40(4):1273-1277.
8. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. Food Chemistry. 2009; 114(2):594-599.
9. Jang DS, Cuendet M, Hawthorne ME, Kardono LB, Kawanishi K, Fong HHS, Mehta RG, Pezzuto JM, Kinghorn AD. Prenylatedflavonoids of the leaves of Macaranga coniferawithinhibitoryactivityagainst cyclooxygenase-2. Phytochemistry. 2002; 61(7):867-872.
10. Ngoumfo RM, Ngounou GE, Tchamadeu CV, Qadir MI, Mbazoa CD, Begum A, Ngninzeko FN, Lontsi D, Choudhary MI. Inhibitoryeffect of macabarterin, a polyoxygenatedellagitanninfrom Macaranga barteri, on humanneutrophilrespiratoryburstactivity. Journal of naturalproducts. 2008; 71(11):1906-1910.
11. Yoder BJ, Cao S, Norris A, Miller JS, Ratovoson F, Razafitsalama J, Andriantsiferana R, Rasamison VE, Kingston DGI. AntiproliferativePrenylatedStilbenes and Flavonoidsfrom Macaranga Alnifoliafrom the Madagascar Rainforest. Journal of naturalproducts. 2007, 70(3):342–346.
12. Zakaria I, Ahmat N, Jaafar FM, Widyawaruyanti A. Flavonoidswithantiplasmodial and cytotoxicactivities of Macaranga triloba. Phytotherapy. 2012; 83(5):968-972.
13. Trinh Thi Thanh V, Doan Thi Mai H, Pham VC, Litaudon M, Dumontet V, Gueritte F, Nguyen VH, Chau VM. Acetylcholinesteraseinhibitorsfrom the leaves of Macaranga kurzii. Journal of naturalproducts. 2012; 75(11):2012-2015.
14. Bouquet A, Debray M. Medicinal plants of the Ivory Coast. French. 1974; 231.
15. Kerharo J, Bouquet A. Medicinal and toxic plants of the Ivory Coast – Upper Volta. Bulletin of the ExoticPathology Society. French. 1950; 43(1/2):56-6

16. Three J, Adam JG. Second inventory of the medicinal and poisonous plants of Casamance. French Pharmaceutical Annals. 120 St. Germain Boulevard, 75280 Paris 06, France: Masson Publisher. French. 1963; 21(11):7
17. Three J, Adam JG. The TraditionalSenegalesePharmacopoeia. Medicinal and toxic plants. Journal of Traditional Agriculture and AppliedBotany. French. 1974; 21(1-3): 76-7
18. Magassouba FB, Diallo A, Kouyaté M, Mara F, Mara O, Bangoura O, Camara A, Traore S, Keita A, Camera MK, Barry R, Keita S, Oulare K, Barry MS, Donzo M, Camera K, Totte K, Vanden Bherge D, Totte J, Pieters L, Vlietinck AJ, Baldé AM. Ethnobotanicalsurvey and antibacterialactivity of some plants used in Guineantraditionalmedicine. Journal of ethnopharmacology. 2007; 114(1):44-53.
19. Ladiguina EY, Safronitch LN, Otriachenkova VE, Balandina IA, Grinkevitch NI. Chemical analysis of medicinal plants. Moscow Edition, VischayaChkola. Moscow (Russia). French. 1983; 347.
20. Bekro YA, Mamyrbekova-Bekro JA, Boua BB, Tra Bi FH, Ehilé E. Ethnobotanicalstudy and phytochemical screening of Caesalpiniabenthamiana (baill.) herend and zarucchi (caesalpiniaceae). Science & nature. 2007; 4(2):217-2
21. N'Gaman KCC. Phytochemicalstudy and effect of extracts of GmelinaarboreaRoxb. (Verbenaceae) from Côte d'Ivoire on osmoticstability of erythrocytes. Single PhD thesis. University of Abobo-Adjamé Ivory Coast. French. 2013; 152.
22. Roghini R, Vijayalakshmi K. Phytochemical screening, quantitative analysis of flavonoids and minerals in ethanolicextract of Citrus paradisi. International Journal of Pharmaceutical Sciences and Research. 2018; 9(11):4859–4864.
23. Melenteva GA, Antonova LA. Pharmaceutical chemistry. Moscow MedicalPublishing, Moscow (Russia). 1993; 574.
24. Brou KG, Mamyrbekova-Becko JA, Dogbo DO, Gogbeu SJ, Becko YA. On the Qualitative Phytochemical Composition of CrudeHydromethanolicExtracts of the Leaves of 6 Varieties of Manihot esculentaCrantz of Ivory Coast. European Journal of Scientific Research. 2010; 45(2):200-211.
25. Singleton VL, Ortofer R, Lamuela-Raventos RM. Analysis of total phenols and otheroxidationsubstrates and antioxidants by means of Folin-Ciocalteureagent. In methods in enzymology, Orlando Academic Press. 1999; 299:152-178.
26. Arvouet GA, Vennat B, Pourrat A, Legret P. Standardisation d'un extrait de propolis et identification des principaux constituants. Journal de pharmacie de Belgique. French. 1994; 49(6):462-468.
27. Heilmer D, Vigndini P, Dini MG, Vincieri FF, Romani A. Antiradicalactivity and polyphenol composition of local Brassicaceaeediblevarieties. Food chemistry. 2006; 99(3):464-469.
28. Espin JC, Soler-Rivas C, Wichers HJ. Characterization of the total free radical scavengercapacity of vegetableoils and oil fractions using 2, 2-diphenyl-1-picrylhydrazyl radical. Journal of Agricultural and Food chemistry. 2000; 48(3):648-656.
29. Oyaizu M. Studies on products of browning reactionantioxidativeactivities of products of browning reactionpreparedfrom glucosamine. The Japanese journal of nutrition and dietetics. 1986; 44(6):307-315.

30. Boonchum W, Peerapornpisal Y, Kanjanapothi D, Pekkoh J, Pumas C, Jamjai U, Amornlerdpison D, Noiraksar T, Vacharapiyasophon P. Antioxidantactivity of someseaweedfrom the Gulf of Thailand. International Journal of Agriculture and Biology. 2011; 13(1):95-99.
31. Luqman S, Srivastava S, Kumar R, Maurya AK, Chanda D. Experimentalassessment of Moringa oleiferaleaf and fruit for its antistress, antioxidant, and scavengingpotentialusing in vitro and in vivo assays. Evidence-BasedComplementary and Alternative Medicine. 2012.
32. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, Ju YH. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidantactivity of Limnophilaaromatica. Journal of food and druganalysis. 2014; 22(3):296-302.
33. Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. 3ème Edition, Lavoisier Tec. & Doc. Paris (France). French. 1999; 1120.
34. Lagnika L. Etude phytochimique et activité biologique de substances naturelles isolées de plantes Béninoises. Thèse de doctorat. Université Louis Pasteur, Starsbourg (France) /Université d'Abomey, Calavi (Bénin). French. 2005; 280.
35. Meliani N, Dib MEA, Allali H, Tabti B. Hypoglycaemiceffect of Berberis vulgaris L. in normal and streptozotocin-induceddiabetic rats. Asian Pacific Journal of Tropical Biomedicine. 2011; 1(6):468-471.
36. Ramamurthy V, Schanze KS. SemiconductorPhotochemistry and Photophysics vol 10. Edition Marcel-Dekker Inc, CRC Press. New-York (USA). 2003; 384.
37. Georgievskii BP, Komissarenko NF, Dmitrour CE. Les composés bioactifs des plantes médicinales. Edition Naouka. 1990; 336.
38. Wagner H, Bladt S. Plant druganalysis:athin layer chromatography Atlas. 2ème Edition, Springer Science & Business Media. 1996; 384.
39. Ekoumou C. Etude phytochimique et pharmacologique de 5 recettes traditionnelles utilisées dans le traitement des infections urinaires et de la cystite. Université de Bamako, (Mali). French. 2003; 58.
40. Boubakeur H, Rebbas K, Belhattab R. Activités antioxydante et antibactérienne des extraits d'Helichrysumstoechas (L.) Moench. Phytothérapie. French. 2017; 16(3):122.
41. Lezoul NEH, Belkadi M, Habibi F, Guillén F. Extraction processeswithseveralsolvents on total bioactive compounds in differentorgans of threemedicinal plants. Molecules. 2020; 25(20):4672.
42. Katalinić V, Možina SS, Skroza D, Generalić I, Abramovič H, Miloš M, Ljubenkov I, Piskernik S, Pezo I, Terpinc P, Boban M. Polyphenolic profile, antioxidantproperties and antimicrobialactivity of grape skin extracts of 14 Vitis viniferavarietiesgrown in Dalmatia (Croatia). Food chemistry. 2010; 119(2):715-723.
43. Koffi E, Sea T, Dodehe Y, Soro S. Effect of solvent type on extraction of polyphenolsfromtwentythreeIvorian plants. Journal of Animal & Plant Sciences. 2010; 5(3):550-558.

44. Mahmoudi S, Khali M, Mahmoudi N. Etude de l'extraction des composés phénoliques de différentes parties de la fleur d'artichaut (Cynarascolymus L.). Nature &Technology. French. 2013; 5(2):35-40.
45. Mulinacci N, Prucher D, Peruzzi M, Romani A, Pinelli P, Giaccherini C, Vincieri FF. Commercial and laboratoryextractsfromartichokeleaves: estimation of caffeoyl esters and flavonoidic compounds content. Journal of Pharmaceutical and BiomedicalAnalysis. 2004; 34(2):349-357.
46. Benchaachoua A, Bessam HM, Saidi I, Bel-abbes S. Effects of different extraction methods and solvents on the phenolic composition and antioxidantactivity of Silybummarianumleavesextracts. International Journal of Medical Science and Clinical Invention. 2018; 5(3): 3641-3647.
47. Aires A, editor. Tannins: extraction from plants. Tannins-structural properties, biologicalproperties and currentKnowledgeIntechOpen; 2020.
48. Tanoh SK, N'Gaman-Kouassi C, Boa D, Mamyrbekova-Békro JA, Békro YA. Activité antioxydante des extraits bruts hydroéthanoliques et hydroacétoniques des organes de quatreplantes de Côte d'Ivoire médicinales. Nature &Technology. French. 2019; (21):28-34.
49. Bhalodia NR, Nariya PB, Acharya RN, Shukla VJ. In vitro antioxidantactivity of hydro alcoholicextractfrom the fruit pulp of Cassia fistula Linn. AYU (An international quarterly journal of research in Ayurveda). 2013; 34(2):209-214.
50. Rice-Evans C, Miller N, Paganga G. Antioxidantproperties of phenolic compounds. Trends in plant science. 1997; 2(4):152-159.
51. Mohamed H, Ons M, Yosra ET, Rayda S, Neji G, Moncef N. Chemical composition and antioxidant and radical-scavengingactivities of Periplocalaevigata root barkextracts. Journal of the Science of Food and Agriculture. (2009). 89(5):897-905.
52. Irshad M, Zafaryab MD, Singh M, Rizvi M. Comparative analysis of the antioxidantactivity of Cassia fistulaextracts. International journal of medicinalchemistry, 2012; 6.