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**Guy Raymond Feuya Tchouya**  
Department of Chemistry,  
Faculty of Science,  
Scientific and Technical  
University of Masuku,  
Box. 223(Potos),  
Franceville, Gabon.

**Edouard Akono Nantia**  
Department of Biological Science,  
Faculty of Science,  
University of Bamenda,  
Box. 39, Bambili, Cameroon.

**Correspondence:**  
**Guy Raymond Feuya Tchouya**  
Department of Chemistry,  
Faculty of Science,  
Scientific and Technical  
University of Masuku,  
Box. 223(Potos),  
Franceville, Gabon.  
Email: [gfeuya@yahoo.fr](mailto:gfeuya@yahoo.fr)

## Phytochemical analysis, antioxidant evaluation and total phenolic content of the leaves and stem bark of *Musanga cecropioides* R.Br. ex Tedlie (Cecropiaceae), growing in Gabon

Guy Raymond Feuya Tchouya, Edouard Akono Nantia

### Abstract

*Musanga cecropioides* R.Br. ex Tedlie (Cecropiaceae) is a plant used in the Gabonese traditional medicine in the treatment of various infectious and systemic diseases. In order to evaluate the pharmacological importance of this plant, phytochemical constituents of aqueous alcoholic extracts of the stem bark and leaves were screened using standard procedures. The *in vitro* antioxidant and total phenolic content of these extracts were determined using the free radical scavenging activity based on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical and the Folin-Ciocalteu assay, respectively. Phytochemistry results revealed the presence of phenols, coumarins, gallic tannins and triterpenes in both parts of the plant, while flavonoids and alkaloids were specifically present in leaves and in stem bark, respectively. *M. cecropioides* stem bark extract displayed the highest DPPH free radical scavenging activity ( $IC_{50} = 0.29 \pm 0.02 \mu\text{g/ml}$ ), while stem bark and leaves extracts showed comparable polyphenol content ( $40.26 \pm 3.12 \text{ mg/g}$  and  $40.69 \pm 6.43$  of extract, respectively). This study demonstrated that *M. cecropioides* stem bark and leaf extracts contain many phytochemicals, with an important amount of phenolic compounds, and possess antioxidant activities.

**Keywords:** *Musanga cecropioides*, phytochemistry, antioxidant activity, phenolic compounds.

### 1. Introduction

Living beings for their sanitary and nutritional needs, explore the forests which contain thousands of plant species capable to synthesize essential substances for their survival and some of these plants are known for their uses in traditional medicine. The use of medicinal plants is very ancient and about 80% of the world population are still relying on crude plants and extracts for the treatment of various ailments.

These recent years the interest on natural antioxidants, in relation with their therapeutic properties, has increased considerably. Scientific researches in many domains have been developed for extraction, identification and quantification of these compounds from natural sources, including medicinal plants and food products [1-3].

Antioxidant activity of a compound is laid on its capacity to resist against oxidation, neutralize or prevent oxidative processes. Most known antioxidants are  $\beta$ -carotene (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E) and phenolic compounds. These antioxidants possess hydroxyphenolic groups in their structures, which is essentially responsible for the scavenging of free radicals such as hydroxylic radicals ( $\text{OH}^{\bullet}$ ) and superoxydes ( $\text{O}_2^{\bullet-}$ ) [4-7].

*Musanga cecropioides* R.Br. ex Tedlie (Cecropiaceae) is a medicinal plant widely used in Cameroon, Gabon, South west of Nigeria and the Democratic Republic of Congo in the treatment of many diseases, among which, cough, constipation, schizophrenia, chest infection, rheumatism, leprosy, trypanosomiasis, hypertension, toothache, malaria, wounds and jaundice [8-10]. Some scientific work have shown the hypotensive, hypoglycemic, antidiabetic, antidiarrheal and antibacterial properties of *M. cecropioides* extracts and extractives [10-13]. In addition, various parts of the plant have been reported to contain some triterpenoid acids like kalaic, musangic and cecropioic acid, and a host of other biochemical compounds [14-22].

In line with the search of new and more potent antioxidants, the present study aimed to evaluate the phytochemical content and antioxidant potential of the leaves and the stem bark of *M. cecropioides*.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Plant material

Stem bark and leaves of *Musanga cecropioides* R.Br. ex Tedlie (Cecropiaceae) were collected in April 2012 near Franceville, in the Haut Ogooué Province, south-east of Gabon, and identified by Mr Yves ISSEMBE, a botanist of the National Herbarium of Gabon. The plant materials were then dried for four days at room temperature and finely powdered. Powders obtained were extracted by maceration at room temperature in an aqueous alcoholic solution (ethanol/water: 1/1) for two days and the extracts were freeze-dried.

#### 2.1.2. Chemicals

The 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, vitamin C (ascorbic acid), and the Folin–Ciocalteu reagent were obtained from Sigma Aldrich (Taufkirchen, Germany). Other reagents were of high quality grade.

### 2.2. Phytochemical screening

The plant extracts were screened for their qualitative chemical composition, using standard methods described in the literature [23, 24]. The identification of the following groups was considered: alkaloids, coumarins, flavonoids, phenols, saponosides, sterols-triterpenes, sugars and tannins.

**Alkaloids:** 0.5 g of each extract was agitated with 5 ml of hydrochloric acid in a steam bath, and then 1 ml aliquots of filtrate were treated with a few drops of Mayer's reagent or Dragendorff's reagent. The presence of a precipitate after treatment with either reagent is a preliminary indicator of the presence of alkaloids. To remove non-alkaloid compounds that could lead to false-positive reactions, part of the extract was alkalinized with 40% ammonia solution then treated twice with chloroform. The second chloroform extract was concentrated and then retested with the Mayer and Dragendorff reagents.

**Coumarins:** Examined in ultraviolet light, the TLC of drugs with coumarins present spots whose colouring, in the presence of ammonia atmosphere, varies from blue to yellow and purple.

**Flavonoids:** Were detected by using the Shibata reaction or cyanide test. Briefly, 3 ml of extract was evaporated and the residue was dissolved in 2 ml of 50% methanol, then a few magnesium shavings and a few drops of concentrated hydrochloric acid were added. The development of a red-orange or purplish color indicates the presence of flavones aglycones.

**Polyphenols and tannins:** Boiled aqueous extract (1 ml) was mixed with 1% ferric chloride. A black-blue color indicates the presence of gallic tannins and a dark green color, tannin catechists. Polyphenols are revealed by a violet-blue, pink-orange, pink-violet, or red coloration.

**Saponosides:** 1% of each sample decoction was returned gradually in 10 ml test tubes for a final volume of 10 ml. After two vigorous shakes, the tubes were left to stand for 15 min and the height of foam was measured. The tube in which the height of the foam was at least 1 cm, showed the presence of saponosides. However, the height of the foam indicated the value of the foam index.

**Sterols and triterpenes:** These families of compounds were identified by using the Liebermann–Burchard reaction. Briefly, 0.5 g of extract was dissolved in 0.5 ml of chloroform with 0.5 ml of acetic anhydride, and cooled on ice before carefully adding sulfuric acid. A change in color from purple to blue indicates the presence of sterols, while a green or purple-red color indicates the presence of triterpenes.

**Sugars:** A little quantity of extract was dissolved in an ethanol/ $\alpha$ -naphthol (99%/1%) solution contained in a test tube, then allowed to run on the tube wall few drops of concentrated sulphuric acid. Sugars presence was detected by the emergence of a red ring at the interface.

### 2.3. Determination of free radical scavenging activity

The antiradical activities of *M. cecropioides* extracts were determined according to the method described by Nantia *et al.* [25]. Briefly 980  $\mu$ l of freshly prepared DPPH solution (40  $\mu$ g/ml) was introduced in tubes and the extract or standard vitamin C (0.02, 0.2, 2, 20, and 200  $\mu$ g/ml) was added. After 30 min, the change from the radical to the non-radical form leads to the disappearance of the purple coloration of DPPH, which was recorded by spectrophotometry at 517 nm. The inhibitory potential of extracts was expressed through their inhibitory concentration fifty (IC<sub>50</sub>).

### 2.4. Determination of total phenolic content

The amounts of total phenolics in *M. cecropioides* extracts were determined with Folin–Ciocalteu reagent according to the method of Singleton and Rossi [26] with slight modification using gallic acid as a standard. Briefly, in 200  $\mu$ l of extract (2  $\mu$ g/ml) was added 500  $\mu$ l of 1/10 diluted Folin reagent and 20 % Na<sub>2</sub>CO<sub>3</sub>. The mixture was allowed to stand for 30 min with intermittent shaking, and the absorbance was measured at 730 nm using a UV-Vis spectrophotometer (Jenway 6100, Dunmow, Essex, U.K). The total phenolic content was determined as mg of gallic acid equivalent per gram of plant extracts using an equation obtained from the standard gallic acid calibration graph.

### 2.5. Data analyses

Data were expressed in mean  $\pm$  standard deviation, and for the anti-radical activity, the inhibitory concentration fifty (IC<sub>50</sub> value is the amount of the antioxidant required to decrease the initial DPPH radical concentration to 50%) of extract was determined using Graph Pad Prism software. Differences between extracts were assessed by one factor ANOVA followed by the Student-Newman-Keuls test.

For all the families of phytochemicals tested, the presence of a given phytochemical was graded according to the precipitation or color intensity as +, ++ or +++.

## 3. Results

### 3.1. Phytochemicals

Phytochemical screening using qualitative analysis showed the presence of many constituents, including phenols, coumarins and gallic tannins, in both parts of the plant. Flavonoids and alkaloids however, showed plant part richness specificity, with flavonoids being found in leaves and alkaloids in stem bark (Table 1). Both leaves and stem bark extracts did not contain saponosides and sugars.

**Table 1:** Phytochemical constituents of the leaves and stem bark extracts of *M. cecropioides*.

Phytochemical constituents	Plant extracts	
	Stem bark	leaves
Alkaloids	++	-
Coumarins	+++	+++
Flavonoids	-	+++
Phenols	++	+++
Saponosides	-	-
Sterols / Triterpenes	+++ (sterols)	+++ (triterpenes)
Sugars	-	-
Tannins	+++ (gallic)	+++ (gallic)

+++ Very intense, ++ intense, + weak, - absent.

### 3.2. Total phenolic content and radical scavenging activity

The DPPH radical scavenging activity of the aqueous alcoholic extracts of *M. cecropioides* stem bark ( $IC_{50} = 0.29 \pm 0.02 \mu\text{g/ml}$ ) was significantly high ( $P < 0.001$ ) than that of the leaves ( $IC_{50} = 6.36 \pm 0.89 \mu\text{g/ml}$ ), and similar to that of the reference compound, vitamin C ( $IC_{50} = 0.267 \pm 0.009 \mu\text{g/ml}$ ). Both extracts displayed comparable total phenolic content ( $40.26 \pm 3.12 \text{ mg/g}$  and  $40.69 \pm 6.43 \text{ mg/g}$  of extract for bark and leaves extracts, respectively).

### 4. Discussion

The phytochemical screening of *M. cecropioides* plant parts, revealed a consistency in composition for most phytochemicals, with other studies done on the same species collected in other countries [14-22]. These phytochemicals included tannins, flavonoids and triterpenes that have been shown to be active against diarrhea, dermal ulcers, skin rashes and abdominal pains [27-30]. Similarly, alkaloids are stimulants, antibiotics, antifungals and pest-destroying [31]. The saponins have antitussive, expectorant, analgesic, immunomodulatory and cytoprotective properties [31]. The presence of these phytochemicals in *M. cecropioides* extracts supports the common usage of this plant in the Gabonese traditional medicine for the treatment of several diseases. However, this study did not reveal the presence of triterpenes in the stem bark extract of *M. cecropioides*, though largely demonstrated in the literature [14-15]. This discrepancy could be attributed to variability in plant chemical contents according to the regions and countries as demonstrated in some studies [32]. Moreover the less sensitivity of the Liebermann Burchard test could also be considered for the interpretation of this result.

Phenolics are documented in the literature as antibacterials, antifungals and antiseptic [31]. The present study showed significant phenolic contents of *M. cecropioides* extracts. Phenolics compounds are known to act as antioxidants, which can neutralize unstable and reactive molecules, thereby protecting membrane lipids from oxidation [33]. DPPH is a synthetic free radical and has been used to mimic the cell intrinsic oxidative process and to test the antioxidant capacity of a given product [34]. In this study, *M. cecropioides* extracts showed significant antioxidant activity through the inhibition of DPPH free radical. Such antioxidant activity could be attributed to the phenolic content of the extracts [33]. Antioxidants have been shown to be effective in the treatment of various health problems, including neurodegenerative, systemic and infectious diseases [35-36]. Therefore, the phenolic content and antioxidant activity of *M. cecropioides* extracts could therefore support the use of this species in folk medicine for the treatment of ailments such as cough, schizophrenia, chest infection, rheumatism, trypanosomiasis, hypertension, wounds and jaundice [8-10].

### 5. Conclusion

These findings demonstrated the presence of various phytochemicals and phenolic compounds in *M. cecropioides* extracts, in addition to the antiradical activity. The data supports the use of *M. cecropioides* in the Gabonese traditional medicine for the treatment of various diseases.

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