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Research Article

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Comparative Study of the Antibacterial and Antioxidant Properties of Raw and Tannic Extract of the Bark of *Musanga cecropioides* R.Br. & Tedlie (Urticaceae)

Ngoupayo Joseph[1]*, Sonkoue M. S. Laure[2], Chelea Matchawe[3], Ndelo Joseph[4]

[1]Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon
[2]Université des Montagnes, Bagangté, Cameroon
[3]Institute of Medical Research and Medicinal Plants (IMPM), Yaoundé, Cameroon
[4]Faculty of pharmaceutical sciences, University of Kinshasa, Democratic Republic of Congo
*Corresponding author: Ngoupayo Joseph, FMSB, University of Yaoundé I, ngoupayo@gmail.com

Abstract The general objective of this study was to evaluate a comparative study of the antimicrobial and antioxidant activities of the crude and tannic bark extracts of *Musanga cecropioides*. The plant material was extracted by maceration using three solvents namely, ethanol, hydro-ethanolic and aqueous solvents. The phytochemical profile of the plant extracts was determined to identify the different secondary metabolites present in *M. cecropioides*. Antimicrobial activity was determined following the micro dilution method in a liquid medium on the reference strains of *Staphylococcus aureus* ATCC 43300; *Klebsiella pneumoniae* NR 4119; *Shigella flexineri* SF 518; *Salmonella Enteritidis* NR 4311; *Escherichia coli* ATCC 25922; *Salmonella Typhimurium* CPC and *Salmonella Typhi* CPC as described in the Clinical Laboratory Standard Institute guidelines. The antioxidant and cytotoxic activities were evaluated following the modified method of Bassene (2012) and Mosmann (1983). The extraction yield was between 9.56% and 5.4% respectively for the hydro-ethanolic and tannic extracts. The phytochemical analysis revealed the presence of alkaloids, polyphenols, flavonoids, tannins, steroids and terpenes in almost all the extracts irrespective of the extraction solvents. The antimicrobial activity showed that the tannic bark extracts of *M. cecropioides* were active on almost all the tested bacteria with MICs and MBCs ranging from 12.5 to 6.25 mg/mL. The ethanolic extracts were active against *S. aureus* ATCC 43300, *K. pneumoniae* NR41916, and S. Typhimurium while the hydro-etanolic extracts were active on *S. aureus* ATCC 43300 and *K. pneumoniae* NR 41916. Similarly, the aqueous extract was active on *S. aureus* ATCC4330, S. Typhimurium and *S. enteritidis* NR 4311. All the extracts exhibited antioxidant activity with the tannic extract being the most active. However, the latter was not cytotoxic on normal cells with a selectivity index > 10. These antibacterial and antioxidant properties partly justify the traditional use of *M. cecropioides* for the treatment of certain pathologies.

Keywords *Musanga cecropioides*, infectious diseases, antibacterial, antioxidant

1. Introduction

Throughout the ages, man has been able to rely on nature for his basic needs including food, shelter, clothing and especially for healthcare. The therapeutic use of plants for the treatment of most human diseases is of old. With the evolution of humanity, the traditional use of medicinal plants that can serve as raw materials for the production of drugs has increased. According to WHO, 80% of the population uses plants for their primary healthcare (1) due to

difficult access to pharmaceutical drugs promoted by modern medicine. Moreover, these plants have often demonstrated a real therapeutic efficiency [2, 3]. Nevertheless, people are the more and more confronted with the emerging of new oxidative stress-related diseases. Oxidative stress is an imbalance of the equilibrium between the antioxidant defense system and the production of reactive oxygen species (ROS). This leads to biochemical lesions in the body cells at the molecular level characterised by alterations in protein and lipid molecules, or breaks in the DNA molecules, or impairment of the integrity of the cell membrane [4]. This attack can affect hemoglobins. Moreover, under high stress the conditions, despite the presence of an effective defense system, oxyhemoglobin can generate a superoxide radical, which will lead to the transformation of hemoglobin into methemoglobin inducing hemolysis [5]. Oxidative stress is involved in many pathologies. It can act as triggering factor or as a factor favouring disease complications. Most oxidative stress-induced diseases age-dependent as aging decreases antioxidant defense system and increases the production of free radicals in the mitochondria [6]. Oxidative stress appears as one of the main causes of several pathologies such as diabetes, atherosclerosis, rheumatoid arthritis and many forms of cancer. It is also thought to be responsible for certain neurodegenerative pathologies such as Parkinson's and Alzheimer's [7]. Therefore, it would be essential to use natural antioxidants to restore balanced metabolic activities in the body. The use of synthetic antioxidants in food technology, cosmetics and pharmaceuticals that could have solved this problem has been widely criticised in the recent years. Indeed, the long-term use of synthetic antioxidants is believed to be teratogenic, mutagenic and carcinogenic [8]. *M. cecropioides* is a plant from the Cameroonian pharmacopoeia known for its antimicrobial virtues in traditional medicine for the treatment of toothache, especially upper respiratory infections, cough However, to our knowledge, little scientific work on the antibacterial and antioxidant properties have been performed on the bark of *M. cecropioides*. It is the motivation for this research work to highlight the antibacterial and antioxidant properties of these extracts, do laboratory analysis, allow access to new molecules to fight the pathogenic bacteria responsible for infectious diseases.

2. Material and Methods

2.1. Plant material

The plant material consisted of the bark of *M. cecropioides* that was harvested in VEGBE 2 forest (BIKOK), a village located in the MEFOU AKONO division, Centre region of Cameroon. The plant was identified at the National Herbarium as *M. cecropioides* R.Br. ex Tedlie (Urticaceae) in comparison with the sample of J. L Betti No. 246 registered at the National Herbarium under No. 66358 /HNC.

2.2. Preparation of raw extracts

After harvest, the samples were cleaned, cut and dried under the shade for two weeks. The dried bark was reduced to powder in an electric mill. The resulting powder was divided into three equal parts and extracted by macerating 500 g of the crude extracts for 24 hours in 2 L distilled water, ethanol and an ethanol-water mixture (70/30 v/v) followed by mechanical stirring. Subsequently, the mixture was filtered using Whatman # 4 paper; the filtrates were concentrated and then evaporated using a rotary evaporator at 70 °C for the hydroethanolic and ethanol extracts and oven dried. The aqueous extracts were oven dried at 42 °C. The process was repeated three times until for the plant material was exhausted. The obtained extracts were weighed and the yield calculated according to the following formula:

$$\text{Yield (\%)} = \frac{\text{masse de l'extrait brut (g)}}{\text{masse de la poudrel (g)}} \text{ X } 100$$

2. 3. Extraction of tannins

The extraction of tannins was carried out according to the modified method of Gedir *et al.* [9]. The powder (500g) was made by introducing into 2L of water-acetone mixture (70/30). After mechanical stirring, the mixture was allowed to settle for 24 hours, followed by filtration with Whatman No. 4 filter papers. The process was repeated three times; the solvent was then recovered followed by the introduction of the NaCl and the desiccant. After

filtration, the solution was introduced into the 1 liter separatory funnel with 500 ml of ethyl acetate. The process was repeated seven times followed by a vigorous stirring leading to the development of two phases: an upper organic phase and a lower aqueous phase. After separation, the aqueous phase was reintroduced into the separatory funnel to undergo the same process until complete exhaustion. Subsequently, the organic phase was collected and concentrated by rotary evaporation to obtain condensed tannins. The extract resulting from the organic phase was set aside; while the aqueous extract was oven dried at 42 °C. For conservation, the tannic extracts were stored at a temperature of 4 °C for further analysis.

2.4. Phytochemical Screening

The phytochemical screening was carried out to detect the presence of phenols, flavonoids, tannins, alkaloids, saponosides, steroids, terpenoids and coumarins.

2.5. Antibacterial assay

The inhibition parameters of the different crude extracts were evaluated by the determination of Minimal Inhibitory Concentrations (MIC) and Minimal Bactericidal Concentrations (MBC) by the micro dilution technique in liquid medium as described by CLSI (2012) (protocol M07-A9) [10].

2.5.1. Principle of microdilution

It is based on the ability of the microorganism to survive in a medium supplemented with antibiotic, materialized by visual observation of the pellet formed by the deposition of the microorganism at the bottom of the wells, or photometric reading of the turbidity at 630 nm in length at the end of incubation (CLSI, 2012) [10].

2.5.2. Determination of the minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of the different extracts

2.5.2.1. Determination of minimal inhibitory concentrations

The tests were carried out in triplicates in 96-well microplates with 12 columns (from number 1 to 12) and 8 lines (from letter A to H) as follows:

- 150 µl of Mueller-Hinton Broth (MHB) were introduced into the first twelve wells of line A and 100 µl into the rest of the wells of the plate.
- 50 µl of a sterile solution of each extract concentrated to 100 mg/ml were taken and introduced into the corresponding wells followed by a series of six geometric dilutions of the order 2 from line A to line G.
- 100 µl of a bacterial load ($1x10^6$ cells/ml) were distributed in all the wells except those of the control. The concentrations of the extracts and that of ciprofloxacin ranged from 25 mg/mL to 0.780 mg/mL and from 5 µg/ml to 0.3125 µg/mL respectively and the final inoculum concentration in each well was 5×10^5 cells /ml.
- The first six wells of line H containing the culture medium and the bacterial suspension served as a negative control and the other six wells containing only the culture medium served as the sterility control.
- Line F containing the culture medium, the inoculum and ciprofloxacin served as a positive control.
- The microplates were covered and incubated at 37 °C for 24 hours.

At the end of incubation, the MIC was considered as the lowest concentration showing no visible bacterial growth, marked by the absence of deposit of a whitish pellet at the bottom of the cup.

In order to determine the bactericidal or bacteriostatic nature of the extracts, the minimum bactericidal concentration (MBC) parameter was evaluated.

2.5.2.2. Determination of the minimum bactericidal concentrations (MBC)

The determination of MBC was carried out by subculture in a liquid medium of the preparations taken from the plates used for the determination of MIC.

In fact, at the end of incubation of the MIC determination plates, 50 µl of aliquots of the inhibitory cupules were removed aseptically and transferred to the corresponding wells of another sterile plate containing 150 µL of MHB. The test was performed simultaneously for positive, negative and sterility control. The plates were covered and incubated at 37 °C for 48 hours. The tests were performed in triplicates. At the end of the incubation, the lowest

concentration of an extract showing no bacterial growth characterised by the absence of broth turbidity was considered as the minimal bactericidal concentration.

2.6. *In vitro* antioxidant assay

The antioxidant ability of saline extract was investigated through the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) following the modified protocol described by Bassene (2012) [11].

2.6.1. Principle

The method is based on the ability of the extract to deliver protons to DPPH. The DPPH radical is an unstable molecule that absorbs light at 517nm. In the presence of an anti-radical substance capable of providing protons, this molecule is stabilised. This stability is indicated by a change in color from violet to yellow and a decrease in absorbance at 517 nm (Bassene, 2012) [11]. The development of a yellow coloration will be characteristic of the reducing power of the extract, which is proportional to the extract concentration.

2.6.2. Procedure

We introduced 25µL of solvent (80% ethanol) into all the wells of line B to line H. Then 50µL of each extract was introduced into the wells of line A in triplicates. A series of two-fold dilutions were performed from line A to line G. The final concentrations of the extracts were 250; 125; 62.5; 31.25; 15.625; 7.8125 and 3.90625 µg/ml. Finally, 75µL of DPPH (0.01%) was added to all the wells except the controls. The negative control consisted of DPPH alone. Vitamin C was used as the positive control and was treated in the same way as the extracts. The final concentrations were 25; 12.5; 6.25; 3.125; 1.5625; 0.78125; 0.390625 µg/ml. Optical densities were read at 517 nm after 30 min of incubation at room temperature in the dark and.

2.6.3 Expression of results

The sequestration of DPPH• radicals was measured by using the following equation:

% inhibition = $\frac{Ao-As}{Ao}\times 100$

Where Ao= Absorbance of the negative control, **As**= Absorbance of the extract or positive control after

Three other parameters were introduced to better characterise the sequestration DPPH radical capacity, namely the inhibitory concentration 50 (IC_{50}), effective concentration 50 (EC50) and anti-radical power (AP).

IC_{50}: Anti-free radical concentration to capture 50% of free radicals is obtained by linear regression from the graphs representing the percentage of the sequestration of free radicals as a function of the logarithm of the concentrations.

EC50: Takes into consideration the molar concentration of DPPH • present in the reaction medium. It is calculated according to the equation.

EC50 = $\frac{SC\ 50}{[DPPH]}$.

The AP is inversely proportional to the EC50. It measures the effectiveness of the anti-radical. The higher it is, the more effective the anti-radical. It was calculated according to the following equation:

$$PA = \frac{1}{CE_{50}}$$

2.7. Cytotoxicity assay of active extracts

The cytotoxicity of the tannic extract was carried out on the Vero cell line using the colorimetric method with MTT (3- (4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide) as described by Mosmann (1983) [12].

2.7.1. Principle

This method is based on the ability of cells to survive in a medium containing the antibacterial substance. Cell viability was determined by measuring the absorbance of formazan formed at 570 nm following MTT reduction by dehydrogenases of the viable mitochondrial cells (Mosmann, 1983) [12]

2.7.2. Determination of median cytotoxic concentrations (CC_{50})

2.7.2.1. Operating mode

In a 96-well microplate, 100 µl of a cell suspension at a concentration of 5 x10^3 cells/well were introduced into all wells and incubated for 4h at 37 °C to allow cell adhesion. At the end of the incubation period, the culture medium containing the non-adherent cells was removed and then 4 µL of each dilution of the extract and 96 µL of a new medium were added to the test wells and the plates were then incubated for 48 hours at 37 °C and 5% CO_2. The cell-free wells and 0.2% DMSO without inhibitors were the negative controls, cell-containing wells and 10% DMSO were the positive control, those containing only the culture medium constituted the sterility control. After 48 hours of incubation, 20µl of a solution of MTT (Sigma) were introduced into each well and the whole was homogenized and incubated for 4 hours at 37 °C and 5% CO_2. At the end of this incubation period, the formazan formed was solubilised with 100% DMSO. The latter was quantified by reading the optical density at 570 nm using the TECAN plate reader (Infinite M200).

Expression of results

From the optical densities obtained, the percentages of viable cell were calculated using the Microsoft Excel software using the following equation:

$$\textbf{pourcentage de viabilité}\ (\%)\ \frac{\textbf{DO test}}{\textbf{DO contrôle négatif}} \times \mathbf{100}$$

Viability Percentages Using GraphPad Prism 7.0 Software helped to determine Median Cytotoxic Concentrations (CC_{50}) Using Nonlinear Regression Curves.

3. Results

3.1. Extraction yields and physical aspects of the extracts

Table 1: Extraction yields and physical characteristics extracts of *M. cecropioides*

Raw extract	Weight of the powder (g)	Weight of extract (g)	Yield in(%)	Physical characteristics
AE	500	31.6	6.32	Dark brown powder
EE	500	42.9	8.58	Light brown powder
HEE	500	47.8	9.56	Dark brown powder
TE	500	27	5.4	Brown paste

Legend: AE= Aqueous extract; EE= Ethanol extract ; HEE=Hydro-ethanolic extract ; TE= Tannic extract

The extraction yields are described in Table 1 above. This Table shows that general extraction yields varied from 5.4% to 9.56%. In addition, the extraction yield of the crude extracts varied from 6.32% to 9.56%

The aqueous extract contained less secondary metabolites (6.32%) compared to the ethanol extract (8.58%) and the hydroethanolic extract (9.56%). As a result, the water-soluble tannins represent 5.4% of the secondary metabolites present in hydroethanolic extracts. This Table also shows that the extracts had different consistency. The aqueous and hydro ethanolic extracts were in powder form while the tannic extract had a pasty consistency. Based on colour, while the tannic extract was dark in color, the aqueous extract and the hydroethanolic extract were dark brown and dark maroon, respectively.

3.2. Result of Phytochemical Screening

Table 2 reveals the presence of several families of secondary metabolites in the bark extracts of *M. cecropioides*. Ethanol and hydroethanolic extracts all the tested groups of secondary metabolites except coumarins. On the other hand, saponins were absent only in the aqueous extract. However, coumarins were absent in all the extracts.

Table 2: Results of Phytochemical Analysis

Secondary Metabolites	AE	EE	HEE
Alkaloids	+	+	+
Saponins	_	+	+
Polyphenols	+	+	+
Flavonoids	+	+	+
Tannins	+	+	+
Coumarins	_	_	_
Steroids & terpenes	+	+	+

Legend: AE = Aqueous extract; EE = Ethanol extract; HEE = Hydro-ethanolic extract; TE = Tannic extract; + = presence; - = absence.

3.3. Characterisation of Tannins in Tannic Extract

Table 3 below shows that tannins were present in the tannic extract irrespective of the test used.

Table 3: Characterisation of tannins in tannic extract

Characterisation of tannins	TE
Ferric chloride test	+
Stiasny test	+

Legend: TE; tannic extract

3.4. In vitro evaluation of the antimicrobial activity

3.4.1. MBC/MIC ratios of the bark extracts

The minimum inhibitory and bactericidal concentrations against bark extracts of *M. cecropioides* against the tested bacterial strains were shown in Table 4. The MICs of bark extracts lied between 6.25 and 12.5 mg/ml on the tested bacterial strains. Results of the antimicrobial activities of the different extracts revealed that the tannic extract exhibited an antimicrobial activity against all the tested bacteria except *P. aeruginosa* HM 601and *S. Enteritidis* NR 4311 with an MIC fluctuating between 6.25 and 12.5 mg/ml. The ethanol extract demonstrated no antimicrobial activity on *S. typhimurium E. coli* ATCC 25922 and *S, flexineri* NR 518 whereas the hydroethanolic extract failed to be active on *S. typhimurium*, *S. flexineri* NR 518, *P. aeruginosa* HM 601and *S. enteritidis* NR 4311. Moreover, the aqueous extract did not show antibacterial activity against *Escherichia coli* ATCC 25922, *S. flexineri* NR 518, *K. pneumoniae* NR 41916 and *P. aeruginosa* HM 601. Results indicate that all the bark extracts irrespective of the solvent used exhibited antibacterial against S. aureus. The mode of action of all the active extracts was bactericidal. However, MBC of the aqueous extract was not determined. The strongest bactericidal activity was demonstrated by the tannic extract and ethanol extract respectively on *E. coli* ATCC 25922 and *S. enteritidis* NR 4311 with MBC/MIC ratio equal to 1.

3.5. Cytotoxic activity

Table 5 below shows the CC_{50} values of tannic extract on Vero cells. Results indicate that the CC_{50} value was greater than 1000 µg/ml.

3.6. In vitro evaluation of antioxidant activity

3.6.1. The DPPH free radical scavenging Activity

The parameters of the antioxidant activities of the extracts are described in Table 6. It results from this Table that tannic extract had the least IC_{50} value (19.673 ± 0.555 µg/ml) with the aqueous extract having the highest (237.89 ± 0.435 µg/ml). Similarly, tannic extract registered the smallest value for EC_{50} (1.967 ± 0.055 µg/ml) with aqueous extract having the highest EC_{50} value (23.789 ± 0.044 µg/ml). In contrast, the tannic extract had the strongest antioxidant power (AP = 50.859 ± 1.432 ml/µg) with the aqueous extract having the weakest antioxidant power (4.449 ± 1.316 ml/µg).

Figure 1 below illustrates the different concentrations of extracts used to trap 50% of radicals. It appears like in Table 5 that tannic extract and ethanol extract had the lowest IC_{50} value (19.673 and 88.7 µg/ml, respectively) in opposition to the hydro-ethanolic and extracts extracts, which registered the higher IC_{50} values (146.43 and 237.89 µg/ml, respectively). However, none of the extracts had an activity comparable to that of vitamin C ($p < 0.05$).

Table 4: MBC/MIC ratios of tannic extracts

Antibacterial activities (mg/ml)						
		TE	**EE**	**HEE**	**EA**	**Cipro (µg/ml)**
Staphylococcus aureus ATCC 43300	MIC	6 .25	12.5	12.5	12 .5	O .5
	MBC	12.5	ND	ND	ND	
	R	2	_	_	_	
Klebsiella pneumoniae NR 41916	MIC	6 .25	12.5	12 .5	> 25	O .5
	MBC	12 .5	ND	ND	ND	
	R	2	_	_	_	
Salmonella Typhimurium	MIC	6 .25	> 25	ND	12.5	O.5
	MBC	12.5	ND	ND	ND	
	R	2	_	_	_	
Pseudemonas aeruginosa HM 601	MIC	ND	12.5	> 25	> 25	O.5
	MBC	ND	ND	ND	ND	
	R	_	_	_	_	
Escherichia coli ATCC 25922	MIC	12.5	ND	12.5	ND	O.5
	MBC	12.5	ND	ND	ND	
	R	1	_	_	_	
Shigella flexineri NR 518	MIC	12.5	ND	ND	> 25	O.5
	MBC	6.5	ND	ND	ND	
	R	2	_	_	_	
Salmonella Enteritidis NR 4311	MIC	ND	6.5	ND	12.5	O.5
	MBC	ND	6.5	ND	> 25	
	R	_	1	_	_	

Legend: EA: Aqueous extract**; EE**: Ethanolic extract**; HEE:** hydroethanolic extract; **TE:** Tannic extract; **MIC**: Minimal Inhibitory Concentration; **Cipro**: ciprofloxacin; **ND**: not determined

Table 5: Cytotoxic Median Concentration

Extract	CC_{50} (µg/ml)
Tannic extract	>1000

Table 6: IC_{50}, EC_{50} and PA of the different bark extracts

Sample	IC_{50} (µg/mL)	$EC_{50} \times 10^3$ (µg/mL)	AP $\times 10^{-5}$ (mL/µg)
EE	88.700 ± 0.600^{c}	8.870 ± 0.061^{c}	11.304 ± 0.715^{c}
EA	237.89 ± 0.435^{e}	23.789 ± 0.044^{e}	4.449 ± 1.316^{a}
EHA	146.43 ± 0.090^{d}	14.643 ± 0.009^{d}	7.141 ± 1.868^{b}
TE	19.673 ± 0.555^{b}	1.967 ± 0.055^{b}	50.859 ± 1.432^{d}
VitC	2.7100 ± 0.080^{a}	0.271 ± 0.008^{a}	368.886 ± 11.089^{e}

Values with the same superscript letters are not significantly different ($p > 0.05$), Waller Duncan.

Caption: Legend: EA: Aqueous Extract HEE: Hydroethanolic extract; EE Ethanol extract; TE: Tannic extract VIT C: Vitamin C

3.7. DPPH Curve

The DPPH curve below (Figure 2) shows the inhibition percentages of the extract as a function of its concentration. This curve shows us that the ability to capture the free DPPH radical was proportional to the concentration of each extract. Results described in Figure 2 reveal that at the concentration of 500 µg/ml, the scavenging activity of all extracts ranged between 61.65 and 67.9% with the ethanol bark extract having highest inhibition percentage (67.9%) and the aqueous and tannic extract registering the least (61.65%).

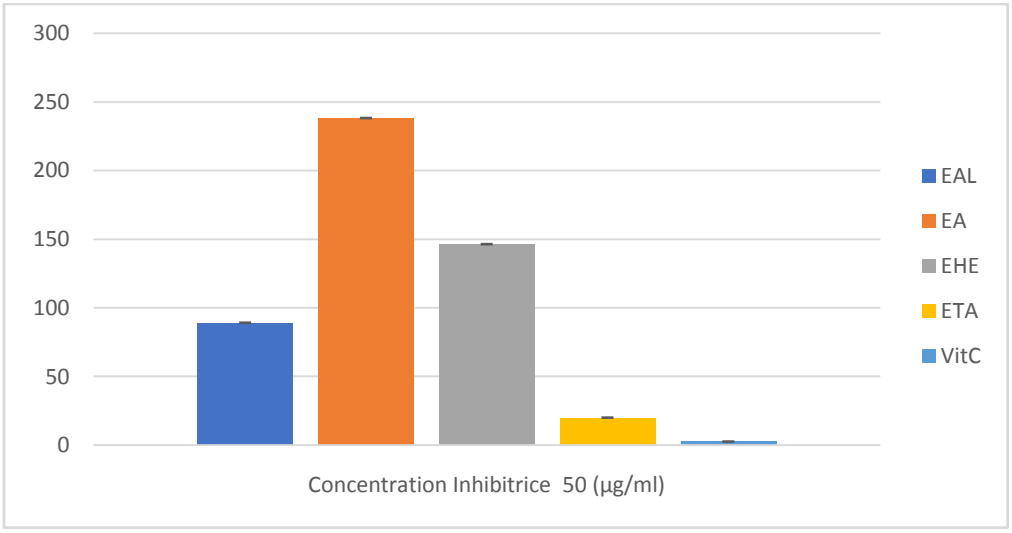

Figure 1: The IC_{50} values (Inhibitory concentration 50) of the extracts and vitamin C

Legend: EA: Aqueous extract; HEE: Hydro-ethanolic extract; EE: Ethanol extract; TE: Tannic extract; VIT C: Vitamin C.

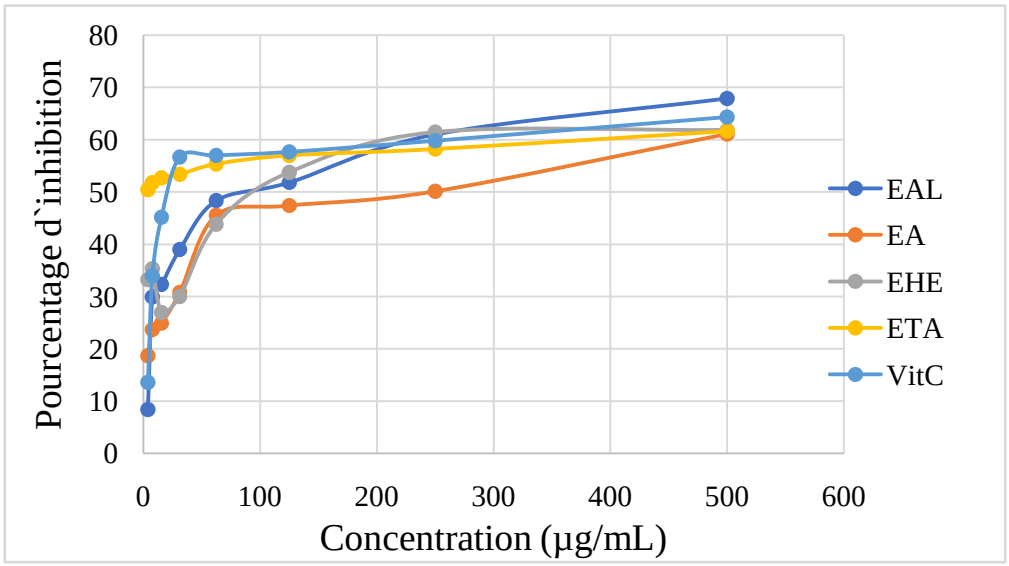

Figure 2: Inhibition Percentage of the extract as a function of the DPPH concentration

4. Discussion

M. cecropioides is a plant from the Cameroonian pharmacopoeia known for its antimicrobial virtues in traditional medicine for the treatment of toothache, especially upper respiratory infections, cough etc. It is also involved in the treatment of soiled wounds, stomach upset and gonorrhea.

The extraction yield of the raw materials varied from 6.32% to 9.56%. The tannic extract had the least extraction yield with 5.4% while the hydroethanolic extract had the greatest yield followed by the ethanol extract. This may suggest that *M. cecropioides* bark contained more secondary metabolites that could be extracted by the water/ethanol solvent system. The choice of the solvent is therefore important during extraction by maceration. These results agree with the work of Afolayan and colleagues [13] who concluded that the water/ethanol solvent system is more suitable for the extraction of secondary metabolites. Moreover, Atefeibu (2002) [14] obtained an extraction yield of 2.52% with tannin, half of what was realised in the present study (5.4%) with the bark extract of *M. cecropioides*. This difference could be attributed to the differences in the total mass of the plant material (100 g

for Atefeibu against 500 g for our sample), type of the plant material, the geographical origin of the plant, the extraction method used and the conditions in which the extraction was carried out [15].

Phytochemical screening reveals the presence of polyphenols, tannins, flavonoids, coumarines, steroids and alkaloids. The variation of the different secondary metabolites from one solvent to another can therefore be attributed to the difference in polarity of the different solvents. Beyond the bark, different parts of *M. cecropioides* seem to be rich in these secondary metabolites. For instance Uwah and his teammates detected the presence of tannins and alkaloids in the roots of *M. cecropioides* (16) while Tchouya *and* Nantia (2015) [17] recorded the presence of alkaloids, polyphenols, tannins, coumarins, steroids, and glycosides in the leaves and barks of *M. cecropioides.*

These different secondary metabolites are primarily synthesized to resist attacks from the outside by exhibiting antiphytopathogenic activities [18]. In the light of this, the antimicrobial activity of the crude extracts may therefore related to the presence of the secondary metabolites. For example, phenolic compounds such as tannins, flavonoids, phenols have shown inhibitory effect on the alteration of membrane structures [19]. The antimicrobial mechanisms of several flavonoids could be attributed to the inhibition of nucleic acid synthesis [20, 21, 22, 23]. Tannins particularly have the capacity to induce complexation with proteins [24]. Thus, their antimicrobial mode of action may be related to their ability to inactivate microbial adhesins, membrane enzymes and membrane transport proteins [25]. Steroids, which were present in all the extracts, are known to promote membrane destruction by lipophilic action [26].

The ethanol and hydroethanol extracts have the same composition in terms of secondary metabolites. Despite this similarity in secondary metabolites, there is a difference in the levels of antibacterial activity. This could be explained by the presence of certain synergistic metabolites or certain antagonistic metabolites. The tannic extract of *M. cecropioides* exhibited the best antimicrobial activity on all the tested bacteria except *P. aeruginosa* HM 601and *S. Enteritidis* NR 4311. Though Atefeibu [14] also demonstrated the lack of inhibitory effect the tannins-rich *Acacia nilotica* extract against *P. aeruginosa*, the antimicrobial spectrum of the present tannic extract is broader. On the contrary, Doss and co-workers [27] reported that tannins exert an inhibitory and lethal effect on the various strains including *E. coli*, *S. aureus* and *P. aeruginosa*. However according to Dorman [28], the phenolic structure components are strongly active against microorganisms and act as protein denaturing agents. These phenolic compounds are able to bind to certain proteins and enzymes thus modifying their enzyme equilibrium [29]. Thus, tannins have the ability to prevent the growth of bacterial colonies by destroying their cell walls [30]. The biological activity of the extracts is related to their different chemical constituents. The difference in efficacy of an extract on the strains of the same species can be explained by a genetic difference between these strains. Curiously, all the bark extracts irrespective of the solvent used exhibited antibacterial activity against *S. aureus*. This may underline the fact that Gram-positive organisms such as S aureus are generally more susceptible to antimicrobial substances. The mode of action of all the active extracts of the bark of *M. cecropioides* was interestingly bactericidal. In addition, the antimicrobial activities of some bark extracts on *S. enterica*, *E.coli* and *Shigella* appear promising in the management of foodborne pathogens.

The search for new drugs for the treatment of pathologies is increasingly criticised because of their associated undesirable side effects such as the cytotoxicity. For this reason, the evaluation of cytotoxicity is considered as a preliminary test of the safety of the plant extracts. The tannic extract which presented the best antimicrobial properties was evaluated for cytotoxicity on the *Vero* cell lines. According to the criterion for classifying the cytotoxicity of extracts defined by the National Cancer Institute in 2001, an extract is considered cytotoxic when its CC_{50} value is less than 20 µg/mL. Therefore, the tannic extract of *M.* cecropioides bark was not cytotoxic on the *Vero* cell line since its CC_{50} value was greater than 1000 µg/ml. To the best of our knowledge, no cytotoxic activity was detected with the bark extracts of *M. cecropioides*. Similarlyr, Ghasemi *et a*l, 2016 showed that *Urtica dioica* extracts from the Urticaceae family do not have a toxic effect on the normal cells of the stomach and colon [30].

With the exception of vitamin C, tannic extract had the lowest IC_{50} value (19.673 ± 0.555 µg/ml) with the aqueous extract having the highest IC_{50} value (237.89 ± 0.435 µg/ml). The IC_{50} values are used to deduce the values of the effective concentrations 50 (EC_{50}) and subsequently the antiradical power (AP) of the extracts. The higher the

effective concentration of 50, the lesser the antiradical power of the extract. Therefore, the tannic extract appears to be the most recommended in terms of antioxidant activity followed by the ethanol extract. This difference in antioxidant activity between bark extracts could be due to the variation in metabolite content extracts as well as differences in their concentration of certain specific compounds. In contrast to the study carried out by Tchouya and Nantia [17] who reported an IC_{50} (0.290 µg/ml) of ethanol bark extracts of *M. cecropioides* comparable to that of vitamin C (IC_{50} = 0.267 µg/ml), in the present research work, the lowest IC_{50} (19.67 µg/ml) registered by the tannic extract is more than seven greater that of the control (2.71 µg/ml) (vitamin C). This deviation might be due to differences in the extraction solvent and methods, place, time and method of harvest as well as the sample drying methods,

5. Conclusion and Perspectives

The hydro-ethanolic extract recorded the highest extraction yield with the tannic extract having the least yield extraction.

Phytochemical screening revealed the presence of several families of secondary metabolites in the bark extracts of *M. cecropioides* irrespective of the solvent used with the exception of the absence of coumarin in all the extracts and that of saponins in the aqueous extract.

Moreover, the tannic extract exhibited the best antibacterial and anti-oxidant activity with the highest anti-radical power (AP=50,859).

The presence of phenolic compounds such as flavonoids, polyphenols and tannins in the extracts of *M. cecropioides* provides this plant with pharmacological properties thereby supporting its traditional use for the treatment of bacterial infections.

Finally, given the results obtained, as future perspective, we intend to evaluate some biological activities such as fractionation, toxicological study as well as the formulation of phyto drugs.

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