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Review, Phytochemical Assay and In Vitro Evaluation of Antioxidant and Anti-Inflammatory Activities of *Ximenia americana* L. (Olacaceae) Extracts

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MBAIHOUGADOBE Séverin[1,2*], MBAIOGAOU Abel[1], MADJITOLOUM BETOLOUM Salomon[1], NANGA YANGA Richard[1], DJEKILAMBER Azor[1], KOUDAMBAYE Mbaigogoum[1,3], TARKODJIEL Mianpereum[1], MAHMOUT Yaya[1]

[1]Laboratory of Research on Natural Substances, Faculty of Exact and Applied Sciences (F.E.A.S) of the University of N'Djamena, Chad. [2]University of Moundou, Chad. [3]University of Sarh, Chad.

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ABSTRACT

Stem and root barks of *Ximenia americana L.* from Chad were studied for their antioxidant and anti-inflammatory activities, their total polyphenol and total flavonoid contents. The contents of total polyphenols and flavonoids were estimated respectively by the Folin-Ciocalteu and iron trichloride methods. Antioxidant activities were determined by DPPH and FRAP tests and the anti-inflammatory activity was studied *in vitro*. It was found that the methanolic extract of stem bark was richer in total polyphenols (176 mg EAG/g) than that of root bark (131 mg EAG/g). Aqueous extracts of root bark and stem bark yielded total polyphenol contents of 108 mg GAE/g and 128 mg GAE/g, respectively. The total flavonoid contents of the methanolic extracts of *Ximenia americana L.* were 50.00 mg EQ/g for root barks and 38.33 mg EQ/g for stem barks. The aqueous extracts gave the following contents 49.66 mg EQ/g for roots and 37.33 mg EQ/g for stem barks. The different concentrations of the extracts gave the percentages of inhibition of protein denaturation ranging from 19.52% to 71.90% for root bark extracts and from 20% to 72.85% for stem bark extracts. Diclofenac was used as a standard to compare its anti-inflammatory activity to those of the extracts. With this standard, a percentage of inhibition of 76.66% was found. This percentage is close to those of the extracts studied.

INTRODUCTION

Since ancient times, people have used plants for their well-being. Plants are used to treat inflammatory diseases and many other pathologies. Their use in traditional medicine is even more increased in developing countries and in recent years even the so-called developed countries integrate them into their health system. In Chad, the lack of health structures, the distance to reach a hospital, the insufficient number of health care personnel and the poverty mean that most of the population uses plants to treat themselves. *Ximenia americana L.* (*Olacaceae*) locally called "Tindi" in Ngambaye and "Himete" in Arabic is a plant found in tropical areas. It is a fruit tree and thorny 4 to 5 m high. It is used to treat inflammation.

The secondary metabolites found in the different organs and extracts of *Ximenia americana L.* are phenolics, lignans, monoterpenes, sesquiterpenes, diterpenes, naphthoquinones, triterpenes and steroids, alkaloids, saponins, hydrolyzable tannins, anthraquinones and glycosides, (Lamien-Meda et al., 2008 ; Maikai et al., 2010 ; Ticiana et al., 2018 ; Maikai et al., 2009 and Hemamalini et al., 2011). Some recent works on the Ivory Coast species showed the absence of coumarins (reaction with potassium hydroxide and reaction on Lactones) and alkaloids (with Dragendorff's Reagent) but confirmed the presence of the other families (Affi et al., 2020).

All parts of the plant are used in traditional medicine in most African and Asian countries. In Chad, the leaves are used as anti-snake bite. Crush the leaves of the plant to obtain a large quantity of juice and give this juice to the victim as a drink. Spread the residue on the bitten part before tying it. The fruits are edible when ripe. In Burkina Faso, the root bark of *Ximenia americana* is used to treat pain and inflammation (Onifade et al., 2011). In Côte d'Ivoire, *Ximenia americana L.* is used to treat diseases such as jaundice, diarrhea, fever, inflammation. In tropical African countries, the leaves are used as eyewash, for toothache, constipation and angina, cough and fever. The roots can also be used to treat venereal diseases, edema and also as an antidote to poisons (James et al., 2008). In southern Uganda, the seed oil is used to prevent varicose veins, to improve skin tone and elasticity and to prevent stretch marks, in cosmetics and to relieve muscle and abdominal pain. The leaves are used either as an extract or in direct application to relieve ear pain, coughs, to treat wounds and burns, measles and skin rashes. The leaves are also used in veterinary medicine to treat cows with respiratory disorders (Valeria et

al., 2013). The plant is also used in the treatment of cancer (Ngulde et al., 2015; Sharma et al., 2011) ; tooth decay, fever, cancer (Baggnian et al., 2018).

The study conducted by Aliyu et al. (2019) on *Ximenia americana* shows that the pulp can be considered a good source of lipids, minerals such as potassium, sodium, magnesium, phosphorus and calcium and is also rich in vitamin C (ascorbic acid). Fruits also contain substances that can be harmful to health when consumed in large quantities.

All the chemical families highlighted could give this plant an antiradical activity and several other pharmacological activities.

Ximenia americana L. is a widespread plant in Chad whose fruits are widely consumed. Therefore, it is important that a phytochemical study be carried out of this plant in order to determine its composition in secondary metabolites.

The hypothesis on which this study is based stipulates that the fruits of *Ximenia americana L*. from Chad contain phenolic compounds.

The objective of this study is to valorize this plant as source of antioxidants and anti-inflammatory.

I- Bibliographic review

I.1 Compounds isolated in the different parts of *Ximenia americana L.*

The review of the literature carried out allowed to inventory several chemical compounds isolated in the different parts of Ximenia americana L. Table 1 presents the compounds isolated by previous studies in the different parts of the plant.

Table 1a: Structures of some compounds isolated in the different parts of *Ximenia americana L.*

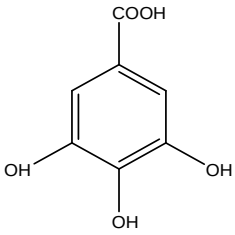
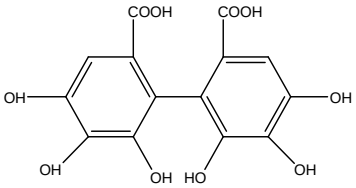
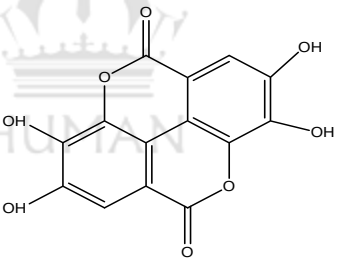
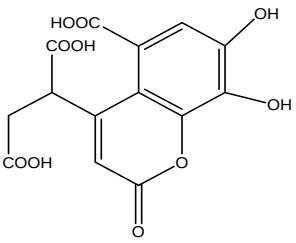
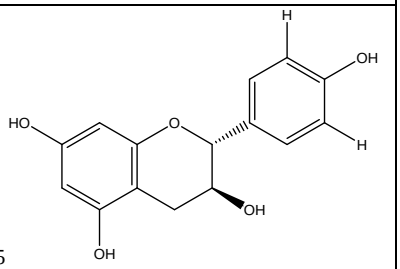
N°CAS	Compound name and molecular peak	Gross formula and structure	Reference
149-91-7	Gallic acid $m/z = 170.12$	$C_7H_6O_5$ 	Keïta, 2004
10315050 (PubChem)	(S)-hexahydroxydiphenic acid $m/z = 338.22$	$C_{14}H_{10}O_{10}$ 	Valdiléia *et al.*, 2016
476-66-4	Ellagic Acid $m/z = 302.19$	$C_{14}H_6O_8$ 	
23725-05-5	Chebulic acid $m/z = 338.22$	$C_{14}H_{10}O_{10}$ 	
2545-00-8	Afzelechol $m/z = 274.27$	 $C_{15}H_{14}O_5$	

Table 1b: Structures of some compounds isolated from the different parts of *Ximenia americana L.* (continued)

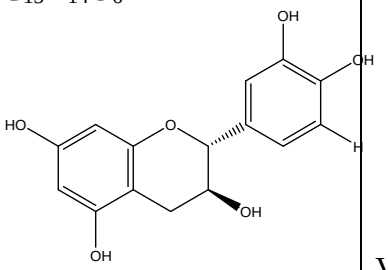
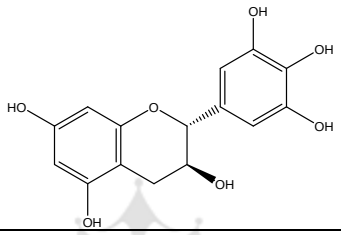
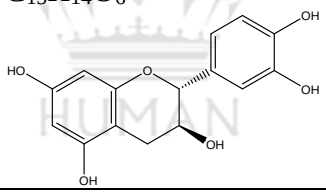
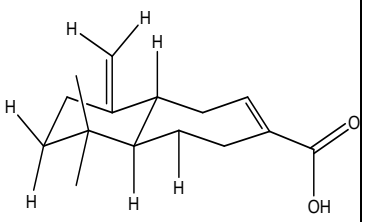
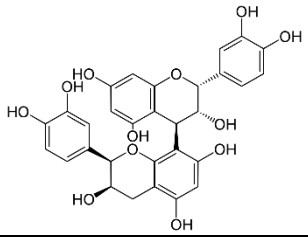
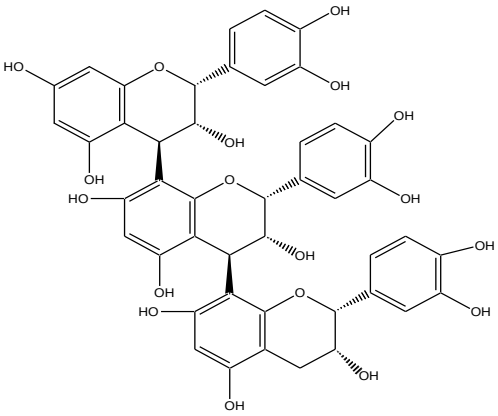
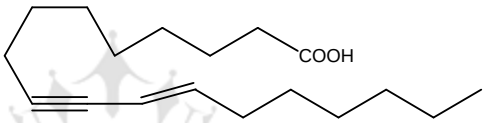
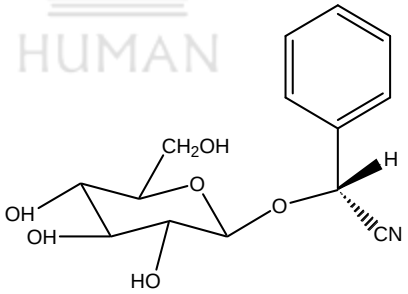
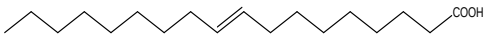
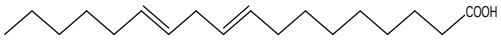
N°CAS	Compound name and molecular peak	Gross formula and structure	Reference
120-80-9	Catechol $m/z = 290.27$	$C_{15}H_{14}O_6$ 	Valdiléia *et al.*, 2016
16-17-55-6	Gallocatechol $m/z = 306.27$	$C_{15}H_{14}O_7$ 	
154-23-4	Catechin catechin/epicatechin $m/z = 290.27$	$C_{15}H_{14}O_6$ 	Valdiléia *et al.*, 2016 ; Ticiana *et al.*, 2018
	Ximonican $m/z = 235.657$	$C_{15}H_{22}O_2$ 	Ticiana *et al.*, 2018
29106-49-8 : 122738 PubChem	Procyanidin B $m/z = 578.520$	$C_{30}H_{26}O_{12}$ 	Valdiléia *et al.*, 2016 ; Ticiana *et al.*, 2018

Table 1c: Structures of some compounds isolated from the different parts of *Ximenia americana L.* (end)

N°CAS	Compound name and molecular peak	Gross formula and structure	Reference
37064-31-6	Procyanidin C2 $m/z = 866.77$	$C_{45}H_{38}O_{18}$ 	Valdiléia *et al.*, 2016
557-58-4	Ximenynic acid $m/z = 278.43$	$C_{18}H_{30}O_2$ 	
99-18-3	Sambunigrin $m/z = 295.28$	$C_{14}H_{17}NO_6$ 	
112-80-1	Acide oléique $m/z = 282.46$	$C_{18}H_{34}O_2$ 	
60-33-3	Acide linoléique $m/z = 280.45$	$C_{18}H_{32}O_2$ 	
506-32-1	Acide arachidonique $m/z = 304.24$	$C_{20}H_{32}O_2$ 	

I.2 Biological activities of the different parts of *Ximenia americana L.*

The biological activities of the extracts of the different parts of the plant are represented in the table 2

Table 2a: Biological activities of the extracts of the different parts of *Ximenia americana L.*

Parts	Solvents	Activities	References
Leaves		Analgesic	Hemamalini *et al.*, 2011
	Organic solvents	Anti - inflammatory	Siddaiah *et al.*, 2012
	Aqueous	hepatic effects, hematological effects	James *et al.*, 2008
	Ethanolic	Antiradical	Valdiléia *et al.*, 2016
	Ethanolic and aqueous	Antioxidant	Mariko, *et al.*, 2016
	Chloroform, ethyl acetate, methanol, distilled water	Anthelmintic	Arun and Ankala, 2017
	Aqueous and ethanolic	Toxicity, anti - inflammatory	Onifade *et al.*, 2011
	Aqueous	Antiulcer, gastro-duodenal activities	Aminata, 2004
		Hepatic effects, hematological effect	James *et al.*, 2008
Root bark	Aqueous and ethanolic	Toxicity, anti - inflammatory	Onifade *et al.*, 2011
	Aqueous	Antiulcer activity, gastro-duodenal	Aminata, 2004
		Hepatic effects, hematological effect	James *et al.*, 2008
Bark of the trunk	Ethanolic	Antiradical	Valdiléia *et al.*, 2016
		Antinociceptive, anti-inflammatory	Dias *et al.*, 2018
	Methanolic	Antioxidant properties	Maikai *et al.*, 2010
	Aqueous	Gastro-duodenal anti-ulcer activity	Aminata, 2004
	Hydrométhanolic 80%	Antibacterial: *Staphylococcus aureus*	Affi *et al.*, 2020
	Petroleum ether, chloroform, butanol, methanol and water	Microbial activity: *Escherichia coli, P.aeruginosa, Staphylococcus aureus, P. vulgaris, Candida albicans, B. subtilis*	Maikai *et al.*, 2009
	Aqueous	Hepatic effects, hematological effect	James *et al.*, 2008
	Fractions of the aqueous extract	Antipyretic	Soro *et al.*, 2015

Table 2b: Biological activities of extracts of the different parts of *Ximenia americana L*. (end)

Parts	**Solvents**	**Activities**	**References**
Bark of the trunk	Fractions of the ethanolic extract: Epicatechin	Antinociceptive Anti-inflammatory	Dias *et al.*, 2018
	Methanol/Water	Antioxidant activity	Lamien *et al.*, 2008 ; Maria *et al.*, 2016 ; José *et al.*, 2015.
	Aqueous	Nutritional and anti-nutritional composition	Aliyu *et al.*, 2019

In the search for phytocompounds, Indian researchers isolated champion endophytes and evaluated their biological activities. The species isolated from the fresh root and leaves of *Ximenia americana L*. was Thielaviopsis baciloca, a champion plant pathogen species. The antagonistic, antioxidant, antiurolithiasis and anticancer activities of the aqueous extract were evaluated in vitro (Rohit et al., 2020).

In 2012, Francisco José Queiroz Monte and collaborators conducted a bibliographic review of the last 10 years of work on the chemical, pharmacological and biological properties of *Ximenia americana L*. They listed in this review, 26 molecules among which terpene compounds, steroids and other chemical families are found. Several biological activities such as antimicrobial, antifungal, anticancer, antineoplastic, antitrypanosomal, antirheumatic, antioxidant, analgesic, moluscicide, pesticide, hepatic and hematological effects were also shown. In food and cosmetics, glyceride mixtures containing ximenynic acid are used.

II. Phytochemical tests and *in vitro* evaluation of antioxidant and anti-inflammatory activities of *Ximenia americana L.* (olacaceae) extracts

II.1 MATERIALS AND METHODS

II.1.1. Plant material

Stem and root barks of *Ximenia americana L.* were collected in November 2021 in the vicinity of the village Biri sous-prefecture of Tapol in the western Logone province of Chad.

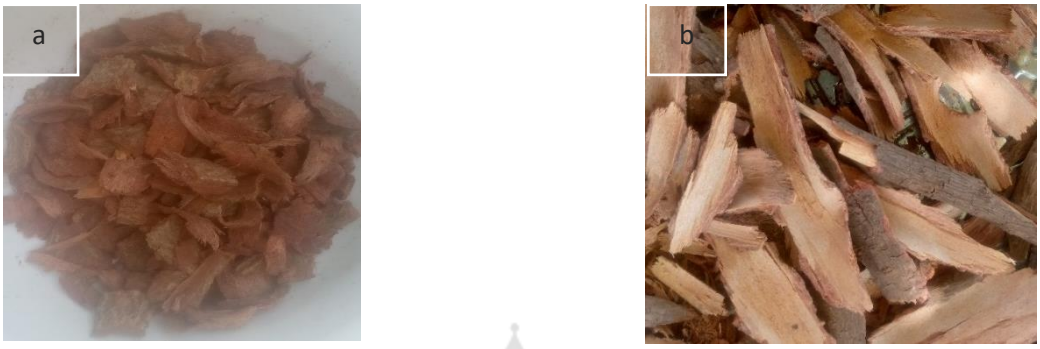


Figure 1 : a) Stem bark and b) root bark of *Ximenia americana L.*

II.1.2 Methodologies

II.1.2.1 Extraction of phenolic compounds

The different types of extracts were prepared from the powders of the stem and root barks of *Ximenia americana L.*

Different extraction methods are used including maceration with different organic solvents, decoction, infusion and reflux heating. Methanol and infusion allowed the extraction of more phenolic compounds. The extracts obtained were filtered and the filtrates were kept for phytochemical tests and determination of total polyphenols, total flavonoids and biological contents *in vitro*.

II.1.2.2 Phytochemical screening

Reactions in tubes

The chemical tests of characterization of the main chemical groups were carried out on the extracts of the bark of the stems and the bark of the roots of *Ximenia americana* L. The detection of the main chemical groups in the extracts was carried out following the protocols described and used at the Department of Chemistry of the University of N'Djamena (Yaya, 2014).

The characterization tests were carried out on compounds such as tannins, flavonoids, alkaloids, saponins, sterols and trapezoids with the main biological and pharmacological properties.

Thin Layer Chromatography (TLC)

In order to confirm the results of the tube reactions, phytochemical screening was performed by Thin Layer Chromatography (TLC) on ALUGRAM Xtra SIL G/UV254 plates according to the methods described by Hildebert Wagner and Sabine Bladt (1996).

After migration, the plates are observed under UV lamp at 254 nm and 366 nm. The development is done by specific chemical developers.

The frontal ratio (FR) for each task was calculated according to the following formula:

$$Rf = \frac{\text{Distance traveled by the substance}}{\text{Distance traveled by the solvent}}$$

II.1.2.3 Quantitative analyses of *Ximenia americana L.*

- Determination of total polyphenols

The content of total phenols was determined using the method based on the reduction in alkaline medium of the phosphotungistic and phosphomolibdic mixture of Folin-Ciocalteu reagent. To 100 µL of the extract, 500 µL of Folin-Ciocalteu reagent (10%) was added. After 04 min, 400 µL of sodium carbonate (7.5%) is added to it. The mixture is incubated at room temperature for 02 hours. The absorbance is measured at 765 nm. The concentration of total polyphenols is calculated from the regression equation of the calibration range established with gallic acid and

is expressed as mg of gallic acid equivalent per gram of extract (mg GAE /g extract). All experiments were performed in triplicate (Hadouchie et al., 2016).

- Determination of flavonoids

The flavonoid contents of the extracts are estimated by the aluminium trichloride ($AlCl_3$) method. It is based on the formation of a flavonoid - aluminium complex. The quantification of flavonoids was done using a calibration curve performed by a standard (quercetin) at different concentrations under the same conditions as the extracts. Results are expressed as mg quercetin equivalent per gram (mg EQ/g) of dry material.

1 mL of 2% aluminum trichloride ($AlCl_3$) is added to 1 mL of the sample. The mixture is allowed to react for 15 min at room temperature and protected from light. The reading is taken at 430 nm. All experiments were performed in triplicate (Talbi et al., 2015).

II.1.2.4. Evaluation of biological activities

Evaluation of the antioxidant activity

DPPH assay

Many methods are used to evaluate the antioxidant activity of extracts. Most of these methods are based on the staining or decoloration of a reagent in the reaction medium. The DPPH assay is used to evaluate the antioxidant activity of *Ximenia americana* L. extracts. This method is based on the reduction of the stable radical species DPPH- in the presence of a hydrogen-donating antioxidant (HA), resulting in the formation of a non-radical form. In the presence of the free radical scavengers, the purple-colored DPPH- reduces to the yellow-colored DPPH. The reduction of the DPPH free radical can be monitored by UV visible spectrometry, measuring the decrease in absorbance at 517 nm (Athamena et al., 2010).

The reaction medium was made by mixing 0.16 mL of DPPH solution of concentration 0.04 mg/mL (10 mg/250 mL methanol) with 8 mL of extracts at concentrations between 0, 312 mg/mL and 10 mg/mL. The absorbance reading was taken at 517 nm after 30 min of incubation in the dark. The percentage of DPPH radical inhibition was calculated according to the following équation:

$$\text{DPPH inhibition}(\%) = [\left(\frac{\text{Absorbance Assay}}{\text{Absorbance Blank}}\right) - 1] \times 100$$

Each point represents the average of 3 replicates. IC_{50} values which are the concentrations of plant or quercetin extracts producing 50% DPPH radical inhibition are determined (Nabila et al., 2012).

Analysis of the antioxidant power by reduction of the ferric ion FRAP (Ferric Reducing Antioxidant Power)

The reducing power of an extract is associated with its antioxidant power. The iron reducing activity of extracts is determined according to the method described by Oyaizu and repeated by Nabila et al. in 2012 with a slight modification. It is based on the chemical reduction reaction of Fe^{3+} to Fe^{2+} present in the $K_3Fe(CN)_6$ complex. This reducing ability can serve as a significant indicator of the potential antioxidant activity of a compound. The absorbance of the reaction medium is determined at a wavelength of 700 nm using a UV - Visible spectrophotometer. Ascorbic acid is used as standard.

Procedure

One milliliter of extracts of different concentrations (from 0.018 to 2.5 mg /mL) is mixed with 2.5 mL of a 0.2 M phosphate buffer solution (PH = 7) and 2.5 mL of a 1% potassium ferricyanide $K_3Fe(CN)_6$ solution. The whole mixture is heated in a water bath at 50 ˚C for 20 min. Then 2.5 mL of 10% trichloroacetic acid is added to stop the reaction. The tubes are centrifuged at 3000 rom for 10 min.

An aliquot (2.5 mL) of the supernatant is diluted with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ aqueous solution. The absorbance of the reaction medium is read at 700 nm against a blank having undergone the same preparation, replacing the extract with distilled water. The activity of the extracts is compared to that of controls, two standard antioxidants, ascorbic acid and gallic acid, whose absorbance was measured under the same conditions as the extracts, but at lower concentrations. An increase in absorbance corresponds to an increase in the reducing power of the extracts tested (Singleton and Rossi, 1965).

II.1.2.5. Anti-inflammatory activity *in vitro*

Method of denaturation of albumin

The test was performed by adopting the method described by Kumari et al (2015). One reaction vessel for each mixture consisting of 200 µL of egg albumin, 1400 µL of phosphate buffered saline and 1000 µL of the test extract (100 ; 300 ; 1000 mg/mL). Distilled water instead of extract was used as a negative control. Then, the mixtures were incubated at 37 °C for 15 minutes and then heated to 70 °C for 5 minutes. After cooling, the absorbance was measured at 660 nm.

Diclofenac sodium was used as a positive control under the same operating conditions. The experiment was performed in triplicate. The percentage inhibition of protein denaturation was calculated as follows:

$$\%\ \text{Denaturation inhibition} = \left(\frac{D}{C} - 1\right) \times 100$$

D: absorbance reading of the test sample; C: absorbance reading without test sample (negative control).

Statistical analysis

The statistical study was performed by Excel software at the 5% probability level. All experiments were performed in triplicate. Results are expressed as mean ± standard deviation. Values of $p < 0.05$ are considered statistically significant.

II.2 RESULTS AND DISCUSSION

II.2.1 Qualitative analyses of extracts

II.2.1.1 Phytochemical tests.

The results of the phytochemical screening on the extracts of stem bark and root bark of *Ximenia americana L.* are presented in Table 3. The detection methods consist of precipitation or staining with specific reagents. These results provide insight into likely biological activities. These tests

revealed the presence of several compounds such as total polyphenols, total flavonoids, gall tannins, saponins, polyterpenes and alkaloids.

Table 3: Results of the tests for the identification of chemical compounds in the aqueous extracts of *Ximenia americana L.*

Chemical families	**Observation**	**Results**	
		Stem bark extract	Root bark extract
Flavonoids	Orange coloration : flavones	+	+
Tannins	Blue-black : gallic tannins	+	+
	Red coloring : catechic tannins	-	+
Saponosides	Height of persistent moss (MI = 2 cm)	+	+
Sterols and terpenoids	Purple coloration turning to green	+	+
Alkaloids	Precipitate, flocculation	+	+
Reducing compounds	Brick red precipitate	+	+

+ : presence, - : absence, MI : moss index

The flavonoid detection test gave an orange coloration indicating the presence of flavones in both extracts of *Ximenia americana L.* These results reveal on the one hand the presence of gall tannins in the studied barks and on the other hand the presence of catechic tannins in the extract of the bark of the roots and the absence of catechic tannins in the extract of the bark of the stems of *Ximenia americana L.* The formation of foam after shaking the infusion and its persistence after 15 min of rest with a height of 2 cm shows the relative richness of the extracts of the bark of roots and stems of *Ximenia americana* in saponins. The test also revealed the presence of terpenes in the extracts of the plant studied. The presence of reducing compounds and alkaloids is also detected in both extracts of *Ximenia americana L.*

The results of the typically qualitative primary phytochemical study show that the extracts from the stem and root barks of *Ximenia americana L.* contain total polyphenols, total tannins and total flavonoids. Work by Soro and colleagues on the aqueous extract of *Ximenia americana*

stem barks collected in Korhogo in northern Côte d'Ivoire, showed the presence of the same families of compounds, except for the tests for catechic tannins which were positive instead of gall tannins (Soro et al., 2015).

2.1.2 Thin layer chromatography

Thin layer chromatography (TLC) is a qualitative method to confirm the results of tube characterization reactions. Extracts were separated using several specific solvent systems. The identification of the compounds was based on the observation of the color of the spots resulting from the separation after the revelation by the specific reagents or under the UV lamp at 254 nm and 366 nm on the one hand, and on the other hand by the comparison of the Rf of the samples with those of the standards used (quercetin or gallic acid). All the results of this analysis are recorded in table 4.

Table 4: TLC results of DCM extracts of stem and root barks of *Ximenia americana L.*

Eluting system	**Extract**	Nber of spots	**Revelators**			**FR**
			UV		**Chemical reagents**	
			254 nm	**366 nm**		
DCM/MeOH/H_2O (6/14/1)	Quercetin	1	quenting	Yellow	$FeAl_3$: Yellow	0.77
	DCM Stem bark extract	4	quenting	Reddish	$FeAl_3$: Red	0.55
			quenting	Brown	$FeAl_3$: Grey	0.66
			quenting	Pale blue	$FeAl_3$: Pale blue	0.72
			quenting	Yellowish	$FeAl_3$: Yellowish	0.76
	DCM Root bark extract	4	quenting	Reddish	$FeAl_3$: Red	0.55
			quenting	Brown	$FeAl_3$: Grey	0.66
			quenting	Pale blue	$FeAl_3$: Pale blue	0.72
			quenting	Yellowish	$FeAl_3$: Yellowish	0.76

DCM: dichloromethane; MeOH: methanol; Nber: number; FR: frontal reference

The gray color spot shows the presence of tannins. The yellow colors obtained at the frontal references 0.76 indicate the presence of total flavonoids. By calculation of the frontal ratios of

the extracts and their comparison with those of the control, both extracts contain quercetin. TLC plate observation under the UV lamp at 366 nm gave spots of different colors (pale yellow, blue, grayish yellow, gray, brown, green and red). These colors correspond to the different classes of secondary metabolites such as flavanols, phenolic acid, terpenes etc. The quenting observed at 254 nm would indicate the presence of double bonds (Wagner and Bladt, 1996).

II.2.2. Quantitative analysis of *Ximenia americana L.* extracts

II.2.2.1. Establishment of calibration curves

The equations and correlation coefficients of the calibration curves of the different standards are recorded in table 5 and the calibration curves are represented in figures 2 and 3.

Table 5: Calibration curves

Calibration curves	Standard	Equations	Correlation Coefficients
FCR	Gallic acid	Y= 0.0128x+ 0.1646	R^2 = 0.9987
TFC	Quercetin	Y = 0.0417x + 0.0202	R^2 = 0.9921

FCR: Folin-Ciocalteu reagent; TFC: Total flavonoid content

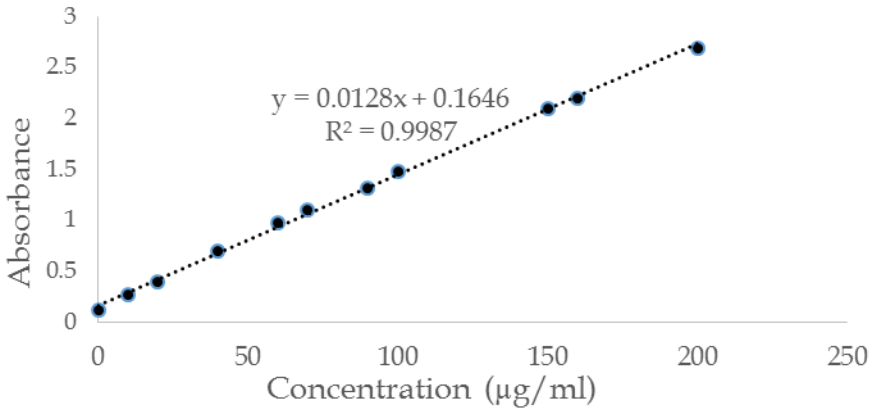


Figure 2: Calibration curve of gallic acid

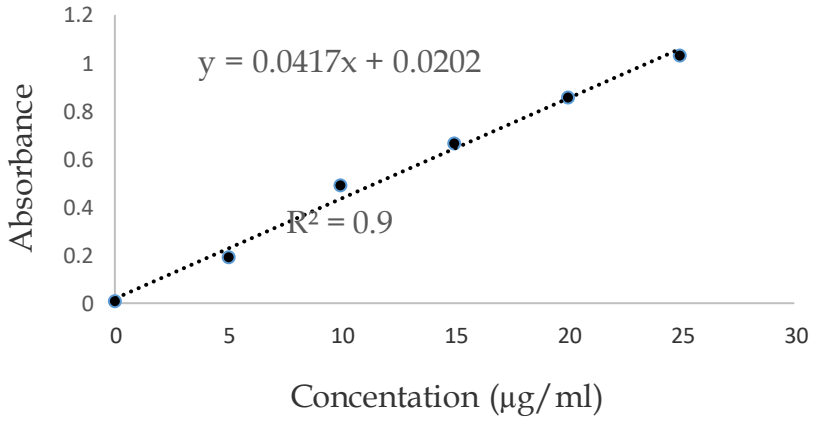


Figure 3: Calibration curve of quercetin

II.2.2.2 Contents of total polyphenols and total flavonoids

The results of the quantification of total polyphenols and total flavonoids are designed in table 6.

Table 6: Contents of total polyphenols (TPC), total flavonoids (TFC)

	Methanolic extract		**Aqueous extract**	
	Root bark	Stem bark	Root bark	Stem bark
TPC (mg GAE/g).	131 ± 0.0011^{b}	176 ± 0.004^{a}	108 ± 0.0013^{c}	128 ± 0.0031^{b}
TFC (mg QE/g)	38.33 ± 0.0057^{e}	50.00 ± 0.0002^{d}	37.33 ± 0.0017^{e}	49.66 ± 0.001^{d}

Values with the same superscript letters in the columns are not significantly different ($p < 0.05$) according to Duncan's multiple comparison test.

Total polyphenol content (TPC)

The polyphenol contents of the extracts were determined by the Folin - Ciocalteu method. The results are expressed in milligram of gallic acid equivalent per gram (mg GAE/g) of dry material. Extracts of root and stem barks do not have the same total polyphenol content. This variation in content is a function of several factors.

Using this method, the results reported in Table 6 shows that the methanolic extract of stem bark is richer in total polyphenols (176 mg GAE/g) than the methanolic extract of root bark with a

total polyphenol content of 131 mg GAE/g. For the aqueous extracts, the results reveal that the stem bark extract has a high content of total polyphenols (128 mg GAE/g) compared to the root bark extract (108 mg GAE/g).

The different extraction methods reveal a significant difference for the two methanolic extracts and for the two aqueous extracts. On the one hand, these results show a significant difference between the methanolic extract of stem barks (176 mg GAE/g) and the aqueous extract of root barks (108 mg GAE/g). On the other hand, a non-significant difference between the methanolic extract of root bark (131 mg GAE/g) and the aqueous extract of stem bark (128 mg GAE/g). Methanol extracted significantly more polyphenols from root and stem barks of *Ximenia americana L*. than water. However, several authors report that there is a significant difference in phenolic content within the same species depending on its origin and habitat. This variability is probably related to severe environmental conditions such as soil type, salinity, low precipitation and water stress which may induce the synthesis of phenolic compounds as a response to oxidative stress generated by the formation of reactive oxygen species.

In our case, the samples are collected from a plant that grows in a forest with high rainfall, which explains the high content of polyphenols.

Total flavonoid content (TFC)

The total flavonoid contents measured by the aluminum trichloride method are reported in Table 6. Statistical study of the total flavonoid contents obtained by the different extraction methods reveals a non-significant difference ($p < 0.05$) for the two stem bark extracts which is equivalent to 50.00 mg QE/g for the MeOH extract compared to 49.66 mg QE/g for the aqueous extract. For the root bark extracts, we have 38.33 mg QE/g for the MeOH extract and 37.33 mg QE/g for the aqueous extract. The difference is still not significant.

In view of the literature data, we note that the total flavonoid content varies for the same plant depending on the region of origin, the solvent and the extraction method used.

II.2.2.3. Evaluation of biological activities

Antioxidant activity: DPPH test

The profiles of the antiradical activity obtained by the method using the stable radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) are reported in figure 4. The antioxidants react with DPPH and neutralize the radical. The color of the reaction mixture changes from purple to yellow. The intensity of the discoloration measures the potentiality of antioxidant scavenging activity. Both plant extracts show significantly lower free radical scavenging activities than the reference product (quercetin). At concentrations below 0.5 mg/ml, the inhibition increases very quickly to stabilize or remain approximately constant at concentrations above this value. This is what we see with quercetin. These results were obtained by others who found very high inhibition as a function of concentrations (Valdiléia et al., 2016).

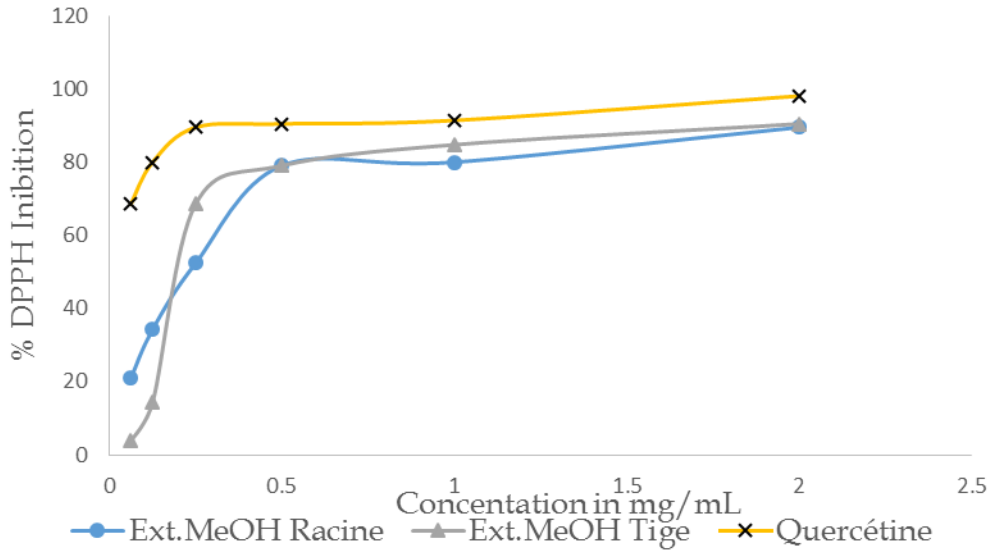


Figure 4 : Variation in DPPH inhibition as a function of quercetin concentration and methanolic extracts of *Ximenia americana L.* root and stem barks.

Analysis of the antioxidant power by reduction of the ferric ion FRAP

The results of this activity are shown in Figure 5. The absorbances of the extracts increase with the concentration; which is the proof of an activity. Indeed, an increase in absorbance corresponds to an increase in the reducing power of the tested extracts. The bark extracts studied

show lower antioxidant activities than the reference molecules used. The measurements show that some extracts used at high concentrations have an activity equivalent to those of the controls (MeOH extract of stem bark and gallic acid). This fact attests that the extracts contain substances that can reduce the ferric ion to ferrous ion.

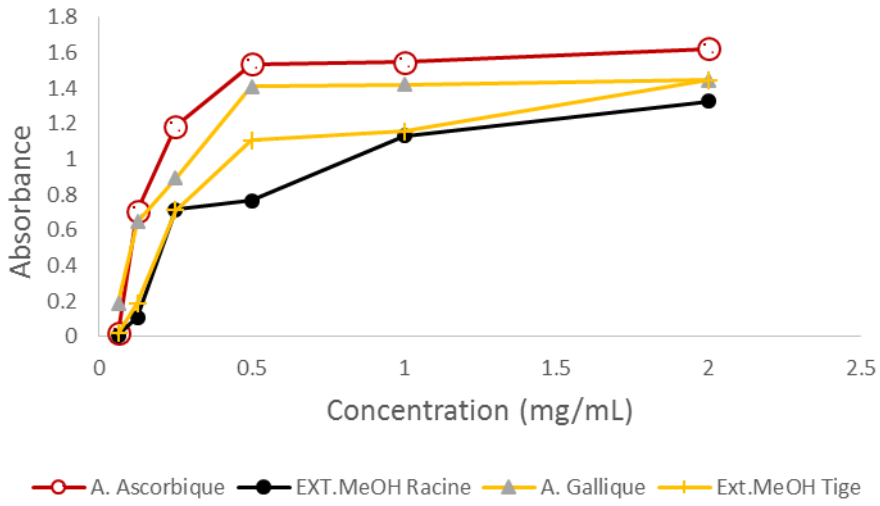


Figure 5: FRAP activity of MeOH extracts from the root bark of *X. americana* in relation to ascorbic acid and gallic acid.

We note a high antioxidant activity of *Ximenia Americana* extracts, correlated with a high polyphenol content. Previous studies performed on the different organs of this species have also shown a high antioxidant activity (Soro et al., 2015 ; Valdiléia et al., 2016).

Both quantitative and qualitative analyses performed in this work, show that methanol extracts phenolic compounds better. These extracted compounds manifest a good antioxidant activity. This confirmation was also made by other researchers attributing the antioxidant activity to phenolic compounds and other secondary metabolites found in the plant (Maikai et al., 2010).

Anti-inflammatory activity *in vitro*

The anti-inflammatory activity of the plant was evaluated *in vitro* by the inhibition test of protein denaturation (egg albumin) induced by heat treatment. This test was done as a preliminary test to verify the presence of anti-inflammatory properties. The maximum percentages of protein

denaturation inhibition by the extract and the positive control (diclofenac sodium) are presented in Table 7 and Figure 6.

The IC_{50} values were obtained from the graph plotted as a function of percentage of DPPH trapping inhibition and sample concentration are presented in Table 7.

Table 7: Protein (albumin) denaturation activity of extracts from stem and root barks of *X. americana* and diclofenac sodium

Inhibitors	**% of inhibition**		
	100 µg/ml	300 µg/ml	1000 µg/ml
Diclofenac Sodium	22.85 ± 0.002	34.76 ± 0.0008	76.66 ± 0.0013
MeOH roots extract	19.52 ± 0.001	31.42 ± 0.0014	71.90 ± 0.0012
MeOH stems extract	20.00 ± 0.0002	32.85 ± 0.0001	72.85 ± 0.0014

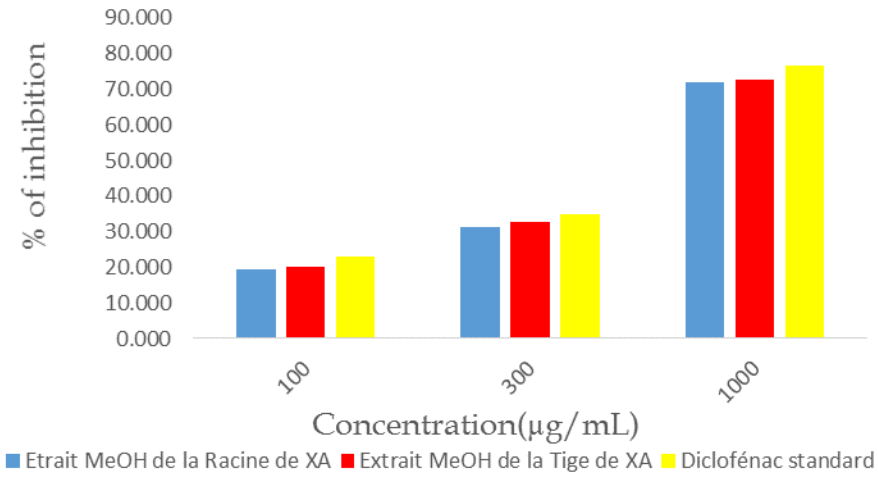


Figure 6: Methanolic effects of *Ximenia americana* stem and root extracts and diclofenac sodium on egg albumin protein denaturation.

The different concentrations of MeOH extracts of *Ximenia americana* stems and roots gave the percentages of inhibition of protein denaturation not significant ($p< 0.05$) ranging from 19.52% to 71.90% for MeOH extracts of root barks and 20% to 72.85% for methanolic extracts of stems. Diclofenac was used as a standard to compare its anti-inflammatory activity to that of the extracts. A percentage of inhibition of 76.66% is obtained with this standard. This percentage is

close to that of the extracts studied. The results obtained with the reference molecule, diclofenac sodium are almost consistent with the results of Hadjer and Somia (2019). The production of autoantigen in some arthritic diseases may be due to protein denaturation, membrane lysis and proteinase action (Hasana et al., 2016).

The anti-inflammatory activity of methanolic extracts of *Ximenia americana* may be due to one constituent or the synergistic effect of several phytochemical constituents such as flavonoids and tannins present in it. Some flavonoids have potent inhibitory activity against a variety of enzymes such as protein kinase C, protein tyrosine kinase and phospholipase A2. The enzyme phospholipase A2 is known to be responsible for the formation of inflammatory mediators such as prostaglandins and leukotrienes which, by attracting leukocytes to the site of inflammation, would result in tissue damage probably through the release of free radicals. Phospholipase A2 hydrolyzes phospholipids in the cell membrane to arachidonic acid, which is very rapidly metabolized by cyclooxygenase to prostaglandins which are major components responsible for pain inflammation (Parvin, et al., 2015). According to the literature, free radicals can harm surrounding tissues, initiating lipid peroxidation that leads to membrane destruction. Damaged tissue elicits an inflammatory response through the production of mediators. Free radical scavengers may be beneficial in the treatment of inflammatory disorders (Sen et al., 2010). Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of an external stress or chemical compound such as a strong acid or base, concentrated inorganic salt, organic solvent, or heat. Most proteins lose their biological function when denatured. Denaturation of tissue proteins is one of the causes of inflammatory diseases. The mechanism of denaturation probably involves electrostatic alteration of hydrogen and disulfide bridge (Mishra et al., 2011). Diclofenac sodium (anti-inflammatory drug) and hydroalcoholic extract showed a dose-dependent ability to inhibit thermally induced protein denaturation.

These experimental results support the traditional use of this plant for the treatment of various ailments, especially against inflammation. To the best of our knowledge, no results on the evaluation of in vitro anti-inflammatory activity by the protein denaturation inhibition assay have been reported by other authors on *Ximenia americana L.* to be able to compare our results, but

many studies have reported the anti-inflammatory and analgesic effect of alkaloids and essential oils of *Ximenia americana L.* (Soro et al., 2015 and Aminata, 2004).

CONCLUSION

The objective of our work was to adopt a scientific basis for the validation of some biological properties attributed to *Ximenia americana L.*, selected on the basis of its traditional use in Chad. Methanol and water were found to be ideal solvents for the extraction of phenolic compounds, due to their ability to extract polar and non-polar molecules. Phytochemical analysis showed on the one hand that methanolic extracts are rich in polyphenols and flavonoids. On the other hand, all the extracts have an antioxidant activity by trapping free radicals and protecting macromolecules against oxidation. The different extracts of *Ximenia americana L.* stem and root barks studied revealed an effective inhibition of the thermal denaturation of albumin compared to diclofenac. Therefore, they have anti-inflammatory activity.

This plant is a promising source of antioxidant and anti-inflammatory agents. This study showed that the plant has good antioxidant and anti-inflammatory activity, which justifies its use in complementary (traditional) medicine.

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