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Full Length Article

Phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of *Lophira procera* A. Chev. (Ochnaceae) medicinal plant from GabonNgoua-Meye-Misso Rick-Leonid^{a,b,*}, Sima-Obiang Cédric^{a,b}, Ndong Jean De La Croix^c, Ondo Joseph Privat^{a,b}, Ovono Abessolo Felix^d, Obame-Engonga Louis-Clément^{a,b}^a Laboratory of Research in Biochemistry (LAREBIO), University of Sciences and Technology of Masuku, Franceville, Gabon^b Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM), University of Sciences and Technology of Masuku, Franceville, Gabon^c Ear, Nose and Throat Laboratory, Department of Otolaryngology-Head and Neck Surgery, Emory School of Medicine, Atlanta, USA^d Laboratory of Biochemistry, Joint Unit of Biomedical Research, University of Health Sciences Libreville, Gabon

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ABSTRACT

The objective of this study was to perform phytochemical screening, to determine the content of phenolic compound, to evaluate antioxidant, anti-inflammatory and antiangiogenic activities of ethanol, water-ethanol and water extracts of *Lophira procera*. Antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdenum assay, anti-inflammatory activity by proteins denaturation inhibition and membranes stabilization test and antiangiogenic activity by chicken chorioallantoic membrane (CAM) method. The results showed that this plant is rich in saponins, polyphenols, tannins, total flavonoids, proanthocyanidins and coumarins. Extracts presented a strong antioxidant activity (IC_{50} values of $5.452 \pm 0.119 \mu\text{g/mL}$ and $6.346 \pm 0.544 \mu\text{g/mL}$ and respective AAI of 9.173 ± 0.203 and 7.919 ± 0.711). Excellent anti-inflammatory activity was also observed ($IC_{50} = 16.952 \pm 1.897$ and $IC_{50} = 2.3172 \pm 0.066 \mu\text{g/mL}$ for inhibition of protein denaturation and membrane stabilization respectively). Finally, extracts manifested a very good anti-angiogenic activity (with inhibitions ranging from $57.142 \pm 0.124\%$ to 100%). These biological activities are certainly due to high content of phenolic compound. This is the first study to report the phytochemical screening, the content of phenolic compound, the antioxidant, anti-inflammatory and antiangiogenic activities of extract derived from *Lophira procera*. The use of this plant in traditional medicine against ulcers, breast cancer, kidney and dental pain is therefore justified and its potential as a candidate for bioactive therapeutic molecule.

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1. Introduction

Lophira procera is a plant of family ochnaceae. its vernacular name (Fang) is *Akoga* and its trade names are *Azobe* and *Bongossi*. It is a giant tree of the damp forest, one of the largest in the African virgin forest, easily recognizable by its slender barrel, fairly light brown and its large oblong, erect leaves and tufts at the ends of the branches at the top. This tree is very widespread in Gabon [1] (Fig. 1). In traditional Gabonese medicine, this plant is used in the treatment of several pathologies. The decoction of barks from this plant is used in lotions against the evil of the kidneys; by an

anal route against rheumatism and lumbago, this decoction is also used against chronic gonorrhea, sterility, sexual asthenia, ulcers, rheumatoid arthritis and breast cancers. traditional therapists and phytotherapists define cancer as an accumulation of hard clods in the body and plants that reduce these clods are considered anticancer.

Cancers are among the leading causes of morbidity and mortality in the world. In 2012, there were approximately 14 million new cases and 8.2 million deaths related to the disease. The number of new cases is expected to increase by about 70% over the next two decades [2]. Cancer, or malignant tumor, is characterized by a rapid proliferation of abnormal cells which, beyond their usual delimitation, can invade adjacent parts of the organism and then swarm into other organs. It was recognized in 1941 that cancers develop from “subliminal neoplastic states” caused by viral or chemical carcinogenic agents that induce somatic changes [3,4]. Today, these states correspond to “initiation”, involve DNA alterations,

* Corresponding author at: Department of Chemistry and Biochemistry, Faculty of Science, University of Science and Technology of Masuku, P.O. Box 61, Franceville, Gabon.

E-mail addresses: ngouamartel@gmail.com, ngouamartel@univ-masuku.ga (R.-L. Ngoua-Meye-Misso).



Fig. 1. *Lophira procera* A. Chev. (Ochnaceae). Synonym *Lophira alata* Banks ex CF Gaertn. Photo taken in a forest of Douala, a village in the town Mitzi/Gabon (Ngoua-Meye-Misso, 2016).

are irreversible and can persist in a tissue until a second type of stimulation called “promotion” occurs [5]. Several promoters directly or indirectly induce cell proliferation, recruit inflammatory cells, increase the production of reactive oxygen species (ROS) leading to oxidative damage to DNA and reduce DNA repair, resulting in replication of DNA and proliferation of cells that have lost normal growth control [6]. Studies have shown that ROS are activators of pro-angiogenic substances such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMP), the goal of which is to induce angiogenesis [6,7].

Angiogenesis is a process of formation of new vessels from arterial vascularization created by endothelial cells. It is essential for the continuous growth of the tumor because it supplies the tumor with nutrients and oxygen, and eliminates cellular waste, which can be toxic to cancer cells [8]. Inflammatory cells and soluble factors are present in all tumors. Signs of “burning” inflammation that include tissue remodeling, angiogenesis and other wound healing characteristics are usually used by pathologists as morphological indices of invasive cancer. Recent evidence demonstrates that these stromal processes play a fundamental role in the development and progression of cancer and, at least in some cases, can predict the clinical behavior of cancer better than the characteristics of neoplastic cells themselves [9]. There are biomolecules present in plants that can neutralize ROS [10,11], prevent inflammation and inhibit tumor angiogenesis [11,12] to finally kill the tumor cells. Also, the plants have been at the origin of many active molecules having shown their effectiveness in the treatment of different cancers, such as breast, ovary and lung treat taxol (paclitaxel) which comes from the bark of Pacific yew (*Taxus brevifolia*).

The aim of this study is to evaluate the antioxidant, anti-inflammatory and antiangiogenic activities of extracts of this plant after carrying out a phytochemical screening.

2. Materials and methods

2.1. Plant material

The stem barks of *Lophira procera* were collected 23 August 2016 in Mitzi (Woleu-Ntem, Northern of Gabon) (Fig. 1). They

were identified at National Herbarium of IPHAMETRA, Libreville (Gabon). Voucher specimen has been deposited in the Herbarium of IPHAMETRA and at Laboratory of Biochemistry Research (LARE-BIO), Department of Chemistry-Biochemistry, Faculty of Sciences of USTM in Franceville.

2.2. Preparation of plant extract

Barks were dried at ambient temperature of the Laboratory (20–30 °C) and protected from light for several days. After drying, barks were crushed using a grinder (Laboratory Blender, Torrington, CT, USA). This powder was used for extractions by maceration method. Briefly, 200 g of powder was mixed with 2000 mL of solvent (water, water-ethanol (50/50, v/v) and ethanol). After 72 h, the obtained extract was filtered using Whatman N 1 filter paper. Ethanol and water-ethanol extracts were concentrated under reduced pressure at rotavapor (Büchi, Labortechnik, Switzerland) at 40 and 60 °C respectively. Water extract was lyophilized using a lyophilizer (Alpha 1–2 LDplus, Germany). All crude extracts obtained were stored at 4 °C until analysis.

2.3. Phytochemical screening

Each extract was then tested for the presence of flavonoids, coumarins, tannins, total phenolic, saponosids, cardiac glycosides, reducing sugar, sterols and triterpenes, oses and holosides, anthracenics, anthocyanins, alkaloids and anthracenosids as described elsewhere [13].

2.4. Total phenolic content

The Folin-Ciocalteu Method [14] with minor modifications was used to determine the total phenolic contents of the different extracts using gallic acid as standard. The absorbance was measured at 735 nm using a Spectrophotometer (Thermo Scientific, Evolution 60S, USA). Results were expressed as gallic acid equivalent per gram of lyophilized sample (average of the triplicate analysis).

2.5. Total flavonoid content

The aluminum chloride (AlCl_3) colorimetric assay method [15] was used to determine total flavonoid contents, using quercetin as a standard [16]. The absorbance was measured at 415 nm and total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis).

2.6. Tannins content

Tannins content was determined according the reference method of European community [17]. The absorbance was measured at 525 nm and tannic acid was used as standard.

2.7. Proanthocyanidins (PAs) content

The proanthocyanidins content was determined according the method described by Prigent [18]. The absorbances were read at 550 nm. Apple procyanidins were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

2.8. Antioxidant activity

2.8.1. DPPH test

The method described by Scherer and Godoy [19], based on the DPPH radical test, was used to determine the Antioxidant Activity Index (AAI). Briefly, DPPH solution was prepared by dissolving 10 mg of DPPH powder in 100 mL ethanol. Graded concentrations of extracts ranging from 3.125 to 100 $\mu\text{g/mL}$ obtained by twofold dilutions were prepared and 100 μL of each dilution were mixed with 100 μL of the working solution of DPPH. Absorbencies were measured at 517 nm after 20 min incubation at room temperature in the dark. Ascorbic acid (vitamin C) and Butylated hydroxytoluene (BHT) were used as references. The ability to scavenge DPPH radical (RSA) was calculated by the following equation: $\% \text{ RSA} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

A = Absorbance at 517 nm. The IC_{50} (concentration providing 50% inhibition) of extracts and standards was determinate using regression curves in the linear range of concentrations. The AAI was then calculated as follows: $\text{AAI} = [\text{DPPH}]_f (\mu\text{g.mL}^{-1}) / \text{IC}_{50} (\mu\text{g.mL}^{-1})$, $[\text{DPPH}]_f$ is the final concentration of DPPH. We considered criteria of Scherer and Godoy (2009) according to which plant extracts show poor antioxidant activity when $\text{AAI} < 0.5$, moderate antioxidant activity when AAI between 0.5 and 1.0, strong antioxidant activity when AAI between 1.0 and 2.0, and very strong when $\text{AAI} > 2.0$.

2.8.2. Total antioxidant capacity

The assay was based on the reduction of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex in acid pH [20]. A total volume of 0.3 mL extract dissolved in methanol was added to 3 mL of reagent solution (0.6 mol/L sulphuric acid, 28 mmol/L sodium phosphate and 4 mmol/L ammonium molybdate). The mixtures were incubated at 70 °C for 90 min then cooled to room temperature. The absorbance was measured at 695 nm. The total antioxidant activity was expressed as the number of equivalence of ascorbic acid, BHT and quercetin.

2.9. In vitro evaluation of anti-inflammatory effect

2.9.1. Test anti-dénaturation of protéins

Protein denaturation methods have been used [21,22] with slight modifications. Briefly, 0.1 mL of albumin from fresh chicken eggs was mixed with 1.9 mL of phosphate buffered saline (PBS, pH 6.4) and 1 mL of varying concentrations of the aqueous extract so that the final concentrations become 31.25, 62.5, 125, 250 and

500 $\mu\text{g/mL}$. A similar volume of distilled double water served as a negative control. Then, the mixtures were incubated at 37 °C in an incubator (Ecocell, LSIS-B2V/EC55, Germany) for 20 min and then heated at 70 °C for 5 min. After cooling, the absorbances were measured at 660 nm on the spectrophotometer (Evolution 60S, USA). Diclofenac sodium in the final concentrations of (156.25, 312.5, 625, 1250 and 2500 $\mu\text{g/mL}$) was used as a reference drug and similarly treated for the determination of absorbance. $\% \text{ Inhibition} = [(Abs_{\text{sample}} - Abs_{\text{control}}) / Abs_{\text{control}}] \times 100$, Abs = absorbance. The concentration of the extract for a 50% inhibition (IC_{50}) was determined by the dose response curve.

2.10. In vivo evaluation of antiangiogenic activity

Antiangiogenic activity was evaluated using chick chorioallantoic membrane (CAM) Model according to previously reported method [23,24] with minors modifications. Briefly fertilized chicken eggs were purchased from a local poultry farm, were sterilized with ethanol (70%) and incubated at 37 °C in incubator (Ecocell, LSIS-B2V/EC55, Germany), with 80% relative humidity. On day 2 of post incubation, 3 mL of albumin were withdrawn. A square window of 1 cm^2 was opened in the egg shell at the opposite to blunt edge and sealed with an adhesive tape. The eggs were returned for further incubation. At the 8th day, the experimental groups were divided into 5 of each containing 50 numbers of eggs. Group 1, 2, 3 and 4 were treated with water extracts. Sterile discs (diameter: 10 mm) of Whatman N° 1 soaked of 10 μL of the water extract at concentrations ranging from 62.5, 125, 250 to 500 $\mu\text{g/mL}$ was applied to the CAM. In parallel Group 5 treated with phosphate buffered saline (PBS) alone as negative control, a paper disc Whatman N° 1 soaked of 40 μL PBS at pH 7.4 was placed on the CAM of egg. The treated CAM samples were incubated for 48 h. The experiments have been repeated at least four times, and the results were reproducible (see experimental part for details).

After 48 h of incubation at 37 °C and 80% relative humidity, a volume of 10 μL of formaldehyde at 4% was applied to the CAM. 5 min later, the CAM was cut around the disc and all (disc and CAM) was placed in a Petri. Then the photographs were taken with a Nikon digital camera D5100 (made in Thailand) and the images were subsequently analyzed with the software Image J. The numbers of vessel branch points contained in a circular region (equal to the area of each filter disc) were counted manually. The percentage of vascularization (density) is measured relative to a normal control vascularization. The ability to inhibition angiogenesis (AIA) was calculated by the following equation: $\% \text{ AIA} = [(Nb_{\text{vcontrol}} - Nb_{\text{vsample}}) / Nb_{\text{vcontrol}}] \times 100$. Nb_{v} = Number of blood vessel branch points.

2.11. Statistical analysis

The data were expressed as the mean \pm standard deviation (SD) of triplicate independent experiments and analyzed using one-way analysis of variance (ANOVA) and Student's *t*-test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

Phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts. In view of the results in Table 1, it appears that extracts contain abundant saponosids, polyphenols, sterols, triterpenes, oses, holosides, tannins, alkaloids, total flavonoids, proanthocyanidins, coumarins, reducing sugar and gitoxins.

Table 1
Results of the preliminary phytochemical screening.

Chemical Groups		Wat	Wat-eth	Eth
Saponosids		+++	+	–
Polyphenols		+++	+++	+++
Sterols and triterpenes		+++	+++	+++
Oses and holosides		+++	+++	+++
Tannins	Gallics	++	++	++
	Catechics	+++	+++	+++
Alkaloids		+++	+++	+++
Cyanidins	Flavons	++	+	+
	Flavanons	–	–	–
	Flavonols	–	–	–
	Flavanonols	–	–	–
Total flavonoids		+++	+++	–
Anthocyan		–	+++	–
Proanthocyanidins		+++	+++	+++
Anthracenic		+	+	+
Coumarins		+++	+++	+++
Cardiac glycosides	Digitoxins	–	–	+++
	Digitoxigenins	–	–	–
	Gitoxins	+++	+++	–
	Gitoxigenins	–	–	–
Reducing sugar		+++	+++	+++

+++ = Very abundant; ++ = Abundant; + = not abundant; – = Not Detected.
Wat = water; Eth = ethanol; Wat-Eth = water-ethanol.

3.2. Total phenolic contents

The contents of total phenolic, total flavonoids, total tannins and total proanthocyanidins of extracts from *Lophira procera* are presented in Table 2. The contents of total phenolic in terms of gallic acid equivalent (standard curve equation: $Y = 0.0012X - 0.0004$, $R^2 = 0.998$), ranged from 3446.444 ± 8.388 to 4205.333 ± 6.666 mg GAE/g of extract and were abundant in all extracts.

3.3. Total flavonoid contents

Total flavonoids (standard curve equation: $Y = 0.0032X + 0.0077$, $R^2 = 1$) ranged from 730.093 ± 1.362 to 644.364 ± 1.262 mg QE/g of extract. There were abundant in water extracts than water-ethanol and ethanol extracts.

3.4. Tannins contents

Levels of tannins were expressed in terms of tannic acid equivalent (TAE). The equation of the right-hand side of the

proportioning of the total tannins by the reference method of European Community (1994) gave $Y = 0.0009X + 0.2088$ with $R^2 = 1$. Total tannins are ranged from 3025.777 ± 2.222 to 3298.370 ± 1.697 mg TAE/g of extract and were abundant in water extracts.

3.5. Proanthocyanidins contents

Levels of proanthocyanidins were expressed in terms of apple proanthocyanidins equivalent (APE). The equation of the right-hand side of the proportioning of the proanthocyanidins by the HCl-Butanol method gave $Y = 0.0006 X + 0.0024$ with $R^2 = 0.999$. Among extracts, proanthocyanidin contents had ranged between 4589.888 ± 7.515 and 5178.777 ± 2.545 mg APE/g of extract. The proanthocyanidins are very abundant in water-ethanol extract.

3.6. Antioxidant activity

3.6.1. DPPH test

The method described by Scherer and Godoy [19], based on the DPPH radical test, was used to determine the Antioxidant Activity Index (AAI). The results showed that the extracts had an antioxidant capacity similar to standard products (Table 3). The IC_{50} value was defined as the concentration of the sample that inhibited 50% of the DPPH. The antioxidant activities of the ethanol and water-ethanol extract were superior to those of the water extract with IC_{50} values of 5452 ± 0.119 μ g/mL and 6346 ± 0.544 μ g/mL and respective AAI of 9173 ± 0.203 and 7919 ± 0.711 . This antioxidant capacity was close to the ability to inhibit BHT positive controls ($IC_{50} = 6.59 \pm 0.301$ μ g/mL, AAI = 7.58 ± 0.567) and vitamin C ($IC_{50} = 7.12 \pm 0.605$ μ g/mL, AAI = 7.02 ± 0.678).

3.6.2. Total antioxidant capacity

Antioxidant capacity of the *Lophira procera* extracts was confirmed with the phosphomolybdenum test which was a quantitative assay [25]. because the antioxidant activity was expressed as vitamin C (VtCE), quercetin (QE) and BHT (BHTE) equivalent, the method was based on the reduction of Mo (VI) to Mo (V) by the extracts resulting in the formation of a Mo (V) complex of green phosphate at acid pH. according to the results, the water-ethanol and ethanol extracts had a capacity greater than that of the water extract. Water-ethanol extract had the highest values with (117.686 ± 0.202) mg/g VtCE, $(77.2$

Table 2
Results of phenolic compounds dosage.

Phenolic compounds	Water	Water-ethanol	Ethanol
TPC* (mgGAE/g of extract)	4205.333 ± 6.666	3446.444 ± 8.388	3373.111 ± 5.091
TFC (mgEQ/g of extract)	730.093 ± 1.362	688.010 ± 1.831	644.364 ± 1.262
TTC (mgTAE/g of extract)	3025.777 ± 2.222	3163.555 ± 1.111	3298.370 ± 1.697
TPC (mgAPE/g of extract)	4589.888 ± 7.515	5091.000 ± 6.009	5178.777 ± 2.545

TPC* = Total phenolic content; TFC = Total flavonoid content; TTC = Total Tannins Content; TPC = Total Proanthocyanidins Content.

Table 3
Results of antioxidant activity of extracts from *Lophira procera*.

Samples	Antioxidant activity DPPH assay		Total antioxidant capacities		
	IC_{50} (μ g/mL)	IAA	VtCE (mg)/g dry extract	QE (mg)/g dry extract	BHTE (mg)/g dry extract
Eth ext	5.452 ± 0.119	9.173 ± 0.203	85.673 ± 0.727	52.462 ± 0.562	350.515 ± 3.502
Wat-Eth ext	6.346 ± 0.544	7.919 ± 0.711	117.686 ± 0.202	77.231 ± 0.156	504.758 ± 0.974
Wat ext	11.521 ± 0.463	4.344 ± 0.178	50.264 ± 0.499	25.066 ± 0.386	179.909 ± 2.405
BHT	6.59 ± 0.301	7.58 ± 0.567			
Vitamin C	7.12 ± 0.605	7.02 ± 0.678			

Wat ext = water extract; Eth ext = ethanol extract; Wat-Eth extract = water-ethanol extract.
VtCE = Vitamin C equivalent; QE = Quercetin equivalent; BHTE = BHT equivalent.

31 ± 0.156) mg/g QE, (504.758 ± 0.974) mg/g BHTE (mg) dry extract followed by ethanol extract with (85.673 ± 0.727) mg/g VtCE, (52.462 ± 0.562) mg/g QE and (350.515 ± 3.502) mg/g BHTE (mg)/g dry extract (Table 3).

3.7. Anti-inflammatory effect

3.7.1. Test anti-denaturation of proteins

The Anti-inflammatory activity is summarized in Table 4. These results showed inhibition of protein denaturation (albumin) dependent on the concentration of aqueous extract. The aqueous extract was used with concentrations ranging from 31.25 to 500 µg/mL, the percentage inhibition increased with the concentration ranging from 230 ± 0.531% to 4360 ± 1.078%. Diclofenac sodium, used at concentrations ranging from 156.25 to 2500 µg/mL, was used as a reference drug which also showed inhibition of concentration-dependent protein denaturation. The effect of diclofenac sodium was found to be rather low compared to that of the aqueous extract tested.

3.8. Antiangiogenic activity

The antiangiogenic potential of the extracts was assessed in vivo with the chicken chorioallantoic membrane (CAM) the eighth embryonic day. The fertilized eggs were treated with aqueous extracts (62.5–500 µg/mL). The degree of vessel training on CAM was scored 48 h later. The density of vessels represents the percentage of vascularization of the analysis zone. It is inversely proportional to the degree of inhibition. In the presence of phosphate buffer saline (PBS) taken as control, the target zone has a percentage of vascularization of 100%, corresponding to a normal vascularization or control with a number of vessels equal to 7 (Fig. 2b). Analysis of the images revealed two zones on the CAM: a vascularized zone (absence of extract) and an avascularized zone (Fig. 2a). The inhibition of the formation of blood vessels is increased with the concentration of the extract. In the presence of 62.5 µg/mL, 125 µg/mL, 250 µg/mL and 500 µg/mL extract, the inhibition percentage was 57.142 ± 0.124%, 71.428 ± 0.106%, 85.714 ± 0.029% and 100% (Fig. 2). The proportion of eggs exhibiting antiangiogenic effects is summarized in Table 5.

4. Discussion

It is clearly established that free radicals or ROS are involved in the inflammatory and angiogenic process and that inflammation and angiogenesis are among the main phenomena responsible for growth and tumor invasion [5]. The present study consisted of phytochemical screening, antioxidant, anti-inflammatory and antiangiogenic activities of extracts of *Lophira procera*, a Gabonese medicinal plant used in traditional medicine to treat ulcers, kidney, dental pain, and breast cancer.

All extracts of this plant showed strong antioxidant activities marked by the inhibition of the DPPH radical and confirmed with reduction of Mo (VI) to Mo (V) by the extracts resulting in the formation of a Mo (V) complex of green phosphate. Ethanol and water-ethanol extract had the highest activity substantially equal to that of vitamin C and BHT. Extracts of this plant can be precursor of potential antioxidant drugs protecting the organism of the free radicals which are at the origin of serious pathologies like the cancers.

In addition to the antioxidant effect, by inhibition of protein denaturation (albumin), water extract shows a very strong anti-inflammatory activity compared to that of the reference molecule, diclofenac sodium. This was confirmed by comparing IC₅₀ values. It is a well known fact that denaturation of tissue proteins leads to inflammatory and arthritic diseases [26]. The production of autoantigens in inflammatory diseases may be due to the denaturation of proteins in vivo. Non-steroidal anti-inflammatory drugs (NSAIDs) may not only inhibit the synthesis of pro-inflammatory prostaglandins but also inhibit protein denaturation [27]. They prevent the denaturation of thermally treated albumin at physiological pH (pH 6.2–6.5) [22]. Therefore, according to the results of this study, the water extract of *Lophira procera* is able to control the production of autoantigen by inhibiting the denaturation of proteins and can prevent the release of lytic enzymes and active mediators of inflammation by the stabilization of lysosomal membranes. Its use in traditional medicine against renal and dental pain can therefore be justified.

Since the idea of tumor angiogenesis was suggested by Folkman [8], the blocking of tumor-induced angiogenesis was considered an attractive anticancer strategy because anti-angiogenic agents can be used independently of cancer types. The aqueous extract also showed strong antiangiogenic activity by the inhibition of blood vessel formations on chick embryo chorioallantoic membrane (CAM). Inhibition was dose dependent. In the range of doses tested, no dead embryos were recorded, indicating that the antiangiogenic effect observed was not due to the toxicity of the plant. This extract shows a stronger anti-angiogenic activity than the aqueous extracts of *Oncoba welwitschii*, *Tetrorchidium oppositifolium*, which showed a percentage inhibition of 83.334% at 500 µg/mL [14]. The CAM is an extraembryonic membrane whose main function is to ensure the exchange of gases and nutrients. Because of its large vascularity and ease of use, CAM is a popular research tool widely used to study glioma angiogenesis, growth, invasiveness and screening of anti-tumor drugs [28]. Therefore, according to the results, the aqueous extract of *Lophira procera* may have good inhibitory activity on tumor growth by blocking angiogenesis. Thus the use of *Lophira procera* in traditional medicine against breast cancer can be justified.

According to the phytochemical screening, *Lophira procera* is rich in phenolic compounds including total polyphenols, total flavonoids, tannins and proanthocyanidins. It has been reported that there is a correlation between total polyphenol content

Table 4
Effect of *Lophira procera* A. Chev. and diclofenac sodium against protein denaturation.

	Aq ext. of <i>L. procera</i>		Diclofenac sodium	
	Conc. (µg/mL)	% Inhibition	Conc. (µg/mL)	% Inhibition
	500	4360 ± 1.078	2500	2410 ± 1.508
	250	2170 ± 1.891	1250	1160 ± 0.970
	125	1040 ± 0.901	625	490 ± 0.609
	62.5	590 ± 0.709	312.5	200 ± 0.048
	31.25	230 ± 0.531	156.25	20 ± 0.002
Equation	Y = 8.746X–16.667		Y = 1.018X–130.830	
R ²	0.999		0.999	
IC ₅₀ (µg/mL)	16.952 ± 1.897		177.819 ± 0.374	

Aq ext = aqueous extract; *L. procera* = *Lophira procera*; Conc. = concentration.

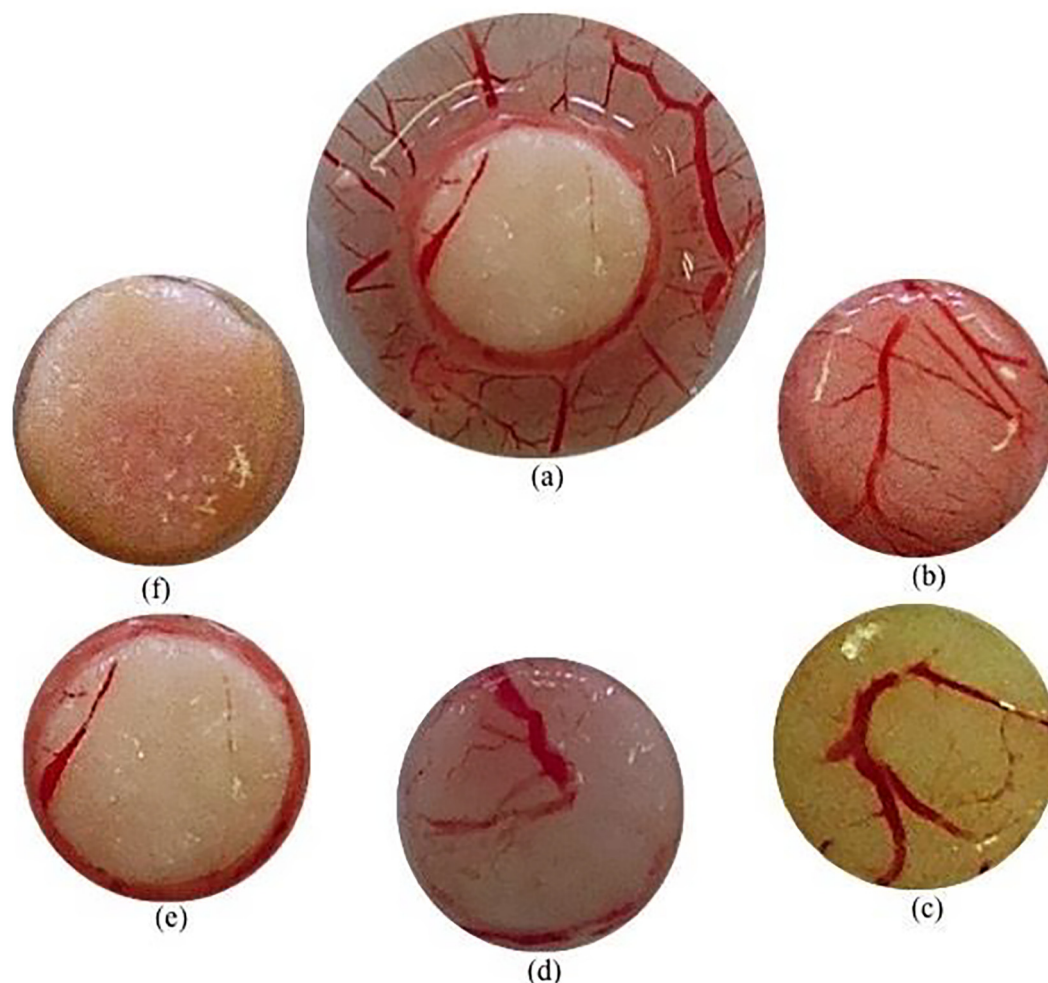


Fig. 2. Inhibition of blood-vessels formation on the CAM by the aqueous extract of *Lophira procera*. The CAM of an 8 days old chick embryo was separately exposed to PBS (control). Extracts were introduced on top of the CAMs. After 48 h of incubation, the CAM tissue directly beneath each filter disc was resected, and digital images of the CAM sections were captured. (a) shows two zones: a vascularized zone around the disc and another avascularized zone, where the disc imbibed with extract has been deposited; (b) control (PBS); (c) water extracted at 62.5 µg/mL; (d) water extracted at 125 µg/mL; (e) water extracted at 250 µg/mL; and (f) water extracted at 500 µg/mL.

Table 5
Antiangiogenic effect of water extract from *Lophira procera*.

	Dose µg/mL per eggs	Tested eggs (n)	Embryos status after 48 h	Mean vessels numbers	Inhibition (%)
PBS (control)		4	Living	7 ± 0.096	0
<i>Lophira procera</i>	62.5	4	Living	3 ± 0.070	57.142 ± 0.124
	125	4	Living	2 ± 0.007	71.428 ± 0.106
	250	4	Living	1 ± 0.005	85.714 ± 0.029
	500	4	Living	0	100

Table 5 shows the effect of aqueous extract on neovascularization of CAM. The extract, taken at different concentrations, has an inhibitory action and the percentage of inhibition increases with the dose. The statue of embryo after 48 h gives information on the toxicity or not of the extract. If the embryo dies, the extract is toxic otherwise the extract is non-toxic. Here there was no dead embryo so up to 500 µg/mL, aqueous extract of *Lophira procera* A. Chev is not toxic.

and antioxidant activity [29]. The present invention relates to a method for the preparation of antiangiogenesis agents [30,31].

5. Conclusion

In conclusion, this is the first study carried out on *Lophira procera*. It has been shown that water, water-ethanol and ethanol extracts have a strong antioxidant activity marked by the inhibition of DPPH radical and by reduction of Mo (VI) to Mo (V), a strong anti-inflammatory activity manifest by the inhibition of protein denaturation and a strong antiangiogenic activity marked by the inhibition of vascularization in CAM. The study also identified the

different chemical groups present in this plant. These biological activities are due to the high content of phenolic compounds in this plant. The use of this plant in traditional medicine as anti-ulcers, anti-breast cancer, anti-pain is therefore justified. The isolation of the phenolic compounds responsible for these activities will be the subject of next study.

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