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Chemical Screening, Antioxidant Potential and Antiangiogenic Effect of Microporus Xanthopus (fr.) Kuntze, Ganoderma Orbiforme (fr.) Ryvarden and Polyporus Fasciculatus (pat) lloyd, Medicinal Mushrooms from Gabon

Orango-Bourdette Juliette-Ornely[1,2], Eyi-Ndong Hugues Calixte[3], Ndong-Atome Guy Roger[1,2], Ngoua Meye Misso Rick-Léonid[1,2], Sima-Obiang Cédric[1,2], Ondo Joseph-Privat[1,2], Obame-Engonga Louis-Clément[1,2*]

1. *Laboratory of Research in Biochemistry (LAREBIO), University of Sciences and Technology of Masuku, Franceville, Gabon.*
2. *Laboratory of Natural Substances and Organometallic Synthesis (LASNSOM), University of Sciences and Technology of Masuku, Franceville, Gabon.*
3. *Institute of Technological Research (IRT), CENAREST-Libreville, Gabon.*

ABSTRACT

The objective of the present study was investigation of the chemical screening, antioxidant potential and antiangiogenic effect of *Microporus xanthopus*, *Ganoderma orbiforme* and *Polyporus fasciculatus*, medicinal mushrooms from Gabon. Theantioxidant activities of water, water-ethanol and ethanol extracts of three fungi were tested by measuring the trapping power of DPPH radical. The anti-angiogenic activity was evaluated for some water extracts by chicken chorioallantoic membrane method (CAM). The results obtained for DPPH trapping test showed that the various extracts tested had antioxidant activities going from low to very high. The greatest activities were found in *Microporus xanthopus* with IAAs of 3.88 and 4.13, respectively, for water and water-ethanol extracts. *Polyporus fasciculatus* fungi exhibited strong antioxidant activities. The moderate activity was found in the extract of *Ganoderma orbiforme*. The water extracts tested for their anti-angiogenic activity acted by decreasing the density and/or number of blood vessels of the CAM with inhibition percentages of 66.67%, 55.45% and 37%, for *Ganoderma orbiforme*, *Microporus xanthopus* and *Polyporus fasciculatus*, respectively. Thus, angiogenesis can be neutralized by the antioxidant molecules contained in the fungi, but these molecules are not the only ones to possess these potentialities. Harvested mushrooms could be potential agents for the fight against cancer.

Keywords: Cancer, antioxidant and anti-angiogenic activities, phenolic compounds, CAM, mushrooms.

*Corresponding Author Email obamengonga2@gmail.com
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INTRODUCTION

The cell is the basic functional and structural unit of living organisms. It contains genetic information and various biological elements that allow it to be autonomous, viable and able to multiply. Cell multiplication is a finely regulated process. Indeed, the mechanisms of cell growth are usually clear and the cells obey. However, in certain situations, following a set of mutations, some cells escape this regulation and turn into cancer cells. Cancer is one of the leading causes of morbidity and mortality worldwide [1]. In 2012, there were approximately 14 million new cases and 8.2 million global deaths related to the disease [2]. In Gabon, there were 110,000 new cases in 2008 [3]. It is estimated that the number of cancers per year is expected to increase by 70% over the next two decades [4]. As a result, the fight against cancer represents a major public health challenge. Despite the multiple faces of this disease, the research work of the last thirty years made it possible to define points common to the various cancers. Cancer cells differ from normal cells by several criteria, including an overproduction of free radicals (ROS), which is responsible for the activation of tumor angiogenesis. Angiogenesis is the biological process by which new vessels are formed from preexisting vessels. Its involvement in tumor growth was first stated by Folkmanin 1971. The hypothesis of the latter states that tumor growth depends on angiogenesis and that inhibition of angiogenesis could have therapeutic effects [5]. Similarly, in the last two decades, reactive oxygen species (ROS) have been presented in many studies as a key component of carcinogenesis. Indeed, they would intervene in all stages of carcinogenesis including transformation, promotion, progression, angiogenesis and tumor metastases [6, 7]. Their effects can be neutralized or prevented by molecules called antioxidants. Antioxidants of natural origin mainly belong to the group of phenolic compounds. These are found in all natural substances like mushrooms. Used for millennia in traditional Japanese and Chinese medicine, mushrooms are presented as living beings with nutritional values and a large number of therapeutic properties. A total of 126 medicinal properties are attributed to them, including antioxidant, anti-cancer, antitumor, antiviral, antibacterial, immunomodulatory, hepatoprotective and antidiabetic effects [8]. However, in Africa, especially in Gabon, mushrooms are only known for their food use and toxicity. Their therapeutic potential is known only by a minority of peoples. Thus, because of the importance of ROS and angiogenesis in carcinogenesis, in this work, the antioxidant and anti-angiogenic potentialities of three (3) fungi of Gabon were tested, from a perspective of research of new molecules.

MATERIALS AND METHOD

Fungal material

Microporus xanthopus, *Ganoderma orbiforme* and *Polyporus fasciculatus* were harvested at Monda and Malibé II forest in August 2016. The specimens were authenticated by Dr. Eyi Ndong Hugues (Biochemist-Mycologist) and deposited at Institute of Technological Research (IRT) CENAREST-Libreville and at Biochemistry Research Laboratory (LAREBIO), Faculty of Science, USTM-Franceville. The collected mushrooms were described macroscopically, then the identification of the collections was made by a microscopic study (Olympus CX31 with sampling tube) of the dry matter, with as a means of observation of the red Congo in ammonia or the solution of Melzer [9]. The reference works used are essentially "Illustrated flora of mushrooms from Central Africa", "Iconographic flora of Congo mushrooms", "Fungus Flora from tropical Africa", especially for the genera *Volvariella*, *Cantharellus*, *Lentinus*, for edible mushrooms from dense Central African forests taxonomy and identification and guide to edible fungi of Benin.

Preparation of fungal extract

Water-ethanol (50/50 v/v), ethanol and water extractswere prepared from dry powder. 50 g of powder from each sample were soaked with 500 mL of the appropriate solvent mixture and left under shaking conditions at room temperature (25°C) for 24 h. Water extract was prepared by maceration mixing 50 g of powder to 500 mL of distilled water. The mixture was boiled for 72 h. Each extract was filtered using Whatman N°1 filter paper and solvents were completely removed at low pressure with a rotary evaporator (Büchi, Labortechnik, Switzerland). The extracts were then concentrated, freeze-dried and stored at 4°C until analysis.

Chemical 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, anthocyans, alkaloids and anthracenosids as described elsewhere [10].

Total Phenolic Content

The total phenolic contents of the different extracts were determined according to the Folin-Ciocalteu Method [11] with minor modifications as described by Sima et *al*. [12] using gallic acid as standard. The absorbance was measured at 735 nm using a multiwell plate reader (μQuant Bio-Tek Instrument, Inc, USA). All analyses were done in triplicate and results (average of triplicate analysis) were expressed as gallic acid equivalent per gram of lyophilized sample.

Total Flavonoid Content

Total flavonoid contents were determined by the aluminum chloride ($AlCl_3$) colorimetric assay

method [13] adapted to 96 well-plate, using quercetin as a standard [14]. The total flavonoid contents were expressed as quercetin equivalents in milligrams per gram sample (average of the triplicate analysis).

Tannins content

The reference method of European community was used to measure total amount of tannins (1994) [15]. Tannic acid was used like standard.

Proanthocyanidins (PAs) content

The method consists on the hydrolysis of proanthocyanidins in a hot acid-alcohol medium into anthocyanidins. This method allows taking into account all the units of flavans-3-ols constituting the polymers [16]. The assay is performed by mixing 50 µL of the extract with 700 µL of 30% HCl-butanol solution (v/v). The mixture was put in tightly closed 1.5 mL Eppendorf tube and vortexed for 1 min. Subsequently, the tube was heated at 100°C for 2 h and after cooling, 200 µL aliquots were put in triplicate into a 96-well plate and the absorbance were read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as aforementioned were used as a standard. Results were expressed as apple procyanidins equivalent (APE).

Antioxidant Activity Index

The Antioxidant Activity Index (AAI) was assessed according to the method described by Scherer and Godoy in 2009 [17]. This method is based on the DPPH radical test. Briefly, the working reagent was prepared by dissolving 10 mg of DPPH in 100 mL ethanol. Graded concentrations of extracts ranging from 0.781 to 100 µg/mL obtained by two-fold dilutions were prepared and 100 µL of each dilution were mixed with 100 µL of the working solution of DPPH in a 96-well plate. Absorbencies were measured at 517 nm after 15 min incubation at room temperature in the dark. Ascorbic acid (Vitamin C) and Butylated Hydroxyanisole (BHA) were used as references. The ability to scavenge DPPH radical was calculated by the following equation: %RSA = [(A control – A sample)/A control] x 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: AAI = [DPPH] (µg/mL) / IC_{50} (µg/mL).

[DPPH] is the final concentration of DPPH. We considered criteria of Scherer and Godoy (2009) according to which plant extracts show poor antioxidant activity when AAI < 0.5, moderate antioxidant activity when AAI is between 0.5 and 1.0, strong antioxidant activity when AAI is between 1.0 and 2.0, and very strong when AAI > 2.0.

Evaluation of Antiangiogenic Activity

Chick Chorioallantoic Membrane Model (CAM): In this assay, the antiangiogenic efficacy of

water extracts of *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus,* was evaluated according to previously reported methods [18, 19]. Fertilized chicken eggs were purchased from a local poultry farm, were sterilized with 70° ethanol and incubated at 37°C in an egg incubator (Lab. Incubator, Digisystem Laboratory Instruments inc.), with 60-65% relative humidity. On day 3 of post incubation, 2 to 3 mL of albumin were withdrawn, using a 21 gauge needle, through a small opening at the large blunt edge of the egg to minimize adhesion of the shell membrane with CAM. 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 to prevent dehydration. Then the adhesive tape is replaced after every 24 hours in order to remove water drops deposited. The eggs were returned for further incubation. At the 8^{th} day, the experimental groups were divided into 3 of each containing 30 numbers of eggs. Group 1 and 2 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 0.25 to 0.50 mg/mL was applied to the CAM. In parallel Group 3 treated with phosphate buffered saline (PBS) alone as control, a paper disc Whatman N°1 soaked of 10 µL PBS at pH 7 was placed on the CAM of egg. On the 9^{th} day, a volume of 10 µL of para-formaldehyde was applied to the CAM. 10 min later, the CAM was cut around the disk using a small pair of sharp scissors and all disc (CAM) was placed in a Petri dish containing agarose gel 1.6%. Then the photos were taken with a cannon digital camera of 16 × 5.0 Mega Pixel (made in China) and the images were subsequently analyzed with the software Image J. The percentage of vascularization (density) is measured relative to a normal control vascularization.

Statistical Analysis

The data were expressed as the mean ± standard deviation (SD) of three independent experiments and analyzed using one-way analysis of variance and Student's t-test. p-values of <0.05 were considered to be statistically significant.

RESULTS AND DISCUSSION

Results

The three medicinal mushrooms studied *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus* are represented in figure 1.

Figure 1: Identification of fungi

a) *Microporus xanthopus*, b) *Ganoderma orbiforme*, c) *Polyporus fasciculatus*,

Selecting the proper extraction method is a very important parameter for obtaining extracts with acceptable yields. The selection of a proper solvent may affect the quantity and quality of the resulting extracts. Various organic compounds including phenolics and flavonoids have solubility in ethanol and water.

Chemical screening

The 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,

Table 1: Chemical groups detected in *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus* fungal extracts

Chemical Groups		*Microporus xanthopus*			*Ganoderma orbiforme*			*Polyporus fasciculatus*		
		Aq	**Eth-Aq**	**Eth**	**Aq**	**Eth-Aq**	**Eth**	**Aq**	**Eth-Aq**	**Eth**
Saponosids		++	-	-	+++	-	-	++	-	-
Polyphenols		+++	+++	+++	+++	+++	+++	+++	+++	+++
Sterols and triterpenes		-	-	-	++	++	++	-	-	-
Oses and holosides		-	-	-	-	+++	+++	-	-	-
	Gallics	-	+	+	+	++	++	+++	+++	++
Tannins	Catechics	-	-	-	-	-	-	++	+++	+++
Alkaloids		++	++	++	+++	++	++	+++	+++	+++
	Flavons	-	-	-	-	-	+++	++	+++	++
Cyanidins	Flavanons	+	+++	++	+++	+++	+++	-	-	-
	Flavonols	-	-	-	-	-	-	-	-	-
	Flavanonols	-	-	-	-	-	-	-	-	-
Total flavonoids		+	+++	+	++	+++	+++	++	+++	+
Anthocyans		-	+	-	++	++	+++	++	+++	++
Proanthocyanidins		+	+++	-	++	++	+++	+	+++	++
Anthracenics		+	++	+	+++	+++	+++	++	+++	++
Coumarins		++	++	++	+++	++	++	+++	+++	+++
	Digitoxine	-	-	-	-	-	-	-	-	-
Cardiac glycosides	Digitoxigenine	-	-	-	-	-	-	-	-	-
	Gitoxine	-	-	-	-	-	-	-	-	-
	Gitoxigenine	++	+++	-	-	-	-	++	+++	++
Reducingsugar		-	-	-	+++	+++	+++	++	+++	++

+++ = Very abundant; ++= Abundant; + = Not abundant; - = Not detected, Aq = Aqueous; Eth-Aq = Ethanol-aqueous; Eth = Ethanol.

it appears that three fungi studied *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus,* contain abundant coumarins, polyphenols, alkaloids and reducing sugars. Chemical screening of *Microporus xanthopus* extracts show that all the extracts of this fungus are abundant in total polyphenols but moderately abundant in alkaloids, and in coumarins. Gitoxigenins, flavone, proanthocyanidines and total flavonoids are abundant in water-ethanol extract. *Ganoderma orbiforme* screening show that the extracts are all abundant in reducing sugars, total polyphenols, anthracenosidesand total flavonoids (flavones and flavones being abundant in the water and water-ethanol extracts). They are moderately abundant in sterols, triterpenes and gallic tannins. The aqueous extract is rich in digitoxins and saponosides; moderately abundant in proanthocyanidins. The water-ethanol extract is rich in oses, holosides and moderately abundant in digitoxins and anthracenosides. The ethanol extract is rich in oses, holosides and proanthocyanidins. *Polyporus fasciculatus* extracts are abundant in alkaloids, gallic tannins, total polyphenols and coumarins. Reducing sugar, flavone and gitoxigenins are moderately abundant in water and ethanol extracts but abundant in water-ethanol extract.

Total Phenolic, Total Flavonoid, Tannins and Proanthocyanidins contents

The contents of total phenolic, total flavonoids, total tannins and total proanthocyanidins of extracts from *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus,* are presented in table 2.

Table 2. Total phenolic content (TPC), Total flavonoid content (TFC), Total Tannins Content (TTC) and Total Proanthocyanidins Content (TPC) of extracts from *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus.*

Extracts		Total phenolic content (TPC) (mgGAE/100 g of drug)	Total flavonoid content (TFC) (mgEQ/100 g of drug)	Total tannins content (TTC) (mgEAT/100 g of drug)	Total proanthocyanidins content (TPC) (mgAPE/100 g ofdrug)
Microporus xanthopus	Aq	5356.67±0	160.37±7	0	936.27±40
	Eth-Aq	34080±47	4442.25±170	0	64911.76±624
	Eth	3882.50±348	623.62±2	0	0
Ganoderma orbiforme	Aq	5496.33±78	609.70±1	18.09±2	201.96±41
	Eth-Aq	7006.33±424	2079.60±91	1470.09±404	500.98±62
	Eth	5940.85±139	301.57±0	654.93±82	431.01±13
Polyporus fasciculatus	Aq	9168.57±620	699.03±77	581.08±188	372.55±198
	Eth-Aq	3870.26±69	1425.75±0	2272.20±74	4797.13±474
	Eth	3077.50±77	212.25±5	286±63	139.70±98

Aq = Aqueous; Eth-Aq = Ethanol-aqueous; Eth = Ethanol.

The contents of total phenolic in terms of gallic acid equivalent (standard curve equation: Y=0.0012X-0.0004, R^2=0.9902; [20] ranged from 3077.5±77 to 9168.57±620mg GAE/100 g of drug. Total flavonoids (standard curve equation: Y=0.0032X+0.0077, R^2= 1) ranged from 160.37±7 to 4442.25±170 mg EQ/100 g of drug. 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 18.09±2 to 2272.20±74 mg EQ/100 g of drug. There were abundant in water extracts than water-ethanol and ethanol extracts. 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 HCl-Butanol method gave Y=0.0006 X+0.0024 with R^2 =0.9869 [20]. Among extracts, proanthocyanidin contents had ranged between 201.96±41 to 64911.76±624 mg APE/100 g of drug. The hydro-ethanolic extract of *Microporus xanthopus* has the highest levels of total polyphenols, total flavonoids and proanthocyanidin. The tannins are absent from the 3 extracts. The water extract has a higher phenolic content than ethanol extract. The results of the determination of *Ganoderma orbiforme* phenolic compounds show that the various extracts derived from this fungus are rich in polyphenol. All the same, their content is much higher in the water-ethanol extract. All extracts of *Polyporus fasciculatus* are abundant in total polyphenols. The aqueous extract is rich in gallic tannins and moderately abundant in catechin tannins. These results are inverses for ethanol extract. However, water-ethanol extract is rich in these two compounds.

Antiradical activity

The free radical-scavenging activities of various fungal extracts were evaluated at their initial concentration. All extracts show free radical scavenging activity (Table 3).

Table 3. Antioxidant Activity Index (AAI) of fungal extracts by DPPH free radical scavenging method.

Plants extracts		Equations	R^2	IC_{50}(µg/mL)	AAI	Activity
Microporus	Aq	Y=1602.6X+29.386	0.9571	12.86	3.88	Verystrong
xanthopus	Eth-Aq	Y=1679.9X+29.682	0.9857	12.09	4.13	Verystrong
	Eth	Y=273.34X +40.268	0.9833	35.60	1.40	Strong
Ganoderma	Aq	Y= 165.23X +33.163	0.9999	101.90	0.50	Moderate
orbiforme	Eth-Aq	Y= 224.26X +27.069	0.9987	102.25	0.50	Moderate
	Eth	Y= 164.82X +25.784	0.9912	146.92	0.34	Poor
Polyporus	Aq	Y= 289.27X +41.502	0.996	29.38	1.70	Strong
fasciculatus	Eth-Aq	Y= 145.35X +46.021	0.9974	27.37	1.83	Strong
	Eth	Y= 210.82X +35.200	0.9114	70.20	0.71	Moderate
Vitamin C		Y = 14.559X - 0.613	0.9990	3.48	11.32	Verystrong
BHT		Y = 5.659X + 11.513	0.9960	6.30	7.85	Verystrong

Aq = Aqueous; Eth-Aq = Ethanol-aqueous; Eth = Ethanol, BHT= Butylated hydroxy toluene: Positive control.

The crude extract of *Microporus xanthopus*, *Ganoderma orbiforme* and *Polyporus fasciculatus* show best DPPH free radical-scavenging activities (IC_{50} values ranged from 12.90 to 35.60 µg/mL for *Microporusxanthopus*,101.90 to 146.92 µg/mL for *Ganoderma orbiforme* and 27.37

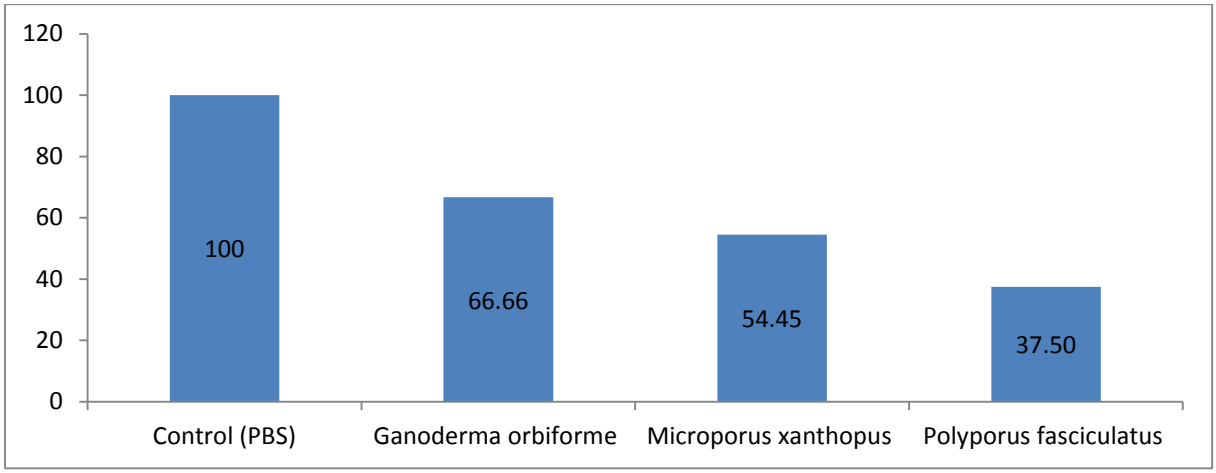
to 70.20 µg/mL for *Polyporus fasciculatus*. As it can be seen, AAI of extracts ranged from 0.34 to 4.13 and can be compared to AAI of Vitamin C and BHT (AAI are 11.320 and 7.850, respectively). The water and water-ethanol extracts of *Microporus xanthopus* showed a very strong activity (AAI values of 3.88 and 4.13, respectively).The ethanol extract of *Microporus xanthopus* and water, water-ethanol extracts of *Polyporus fasciculatus* showed a strong activity (AAI values of 1.40; 1.70 and 1.88, respectively).The ethanol extract of *Polyporus fasciculatus* showed a moderate activity (AAI values of 0.71). The moderate activity was found in water and water-ethanol extracts of *Ganoderma orbiforme*.

Antiangiogenic Activity

The antiangiogenic potential of the extracts was evaluated *in vivo* with the chicken chorioallantoic membrane (CAM) the eighth embryonic day. The fertilized eggs were treated with aqueous extracts (0.25-0.5 mg/mL). The degree of vessel formation on CAM was scored 1 day later. The vessel density is the percentage of blood supply to the analysis area. It is inversely proportional to the degree of inhibition; plus the value of the density, the lower the degree of inhibition of angiogenesis is strong. In the presence of phosphate buffered saline buffer (PBS) used as a control, the target area has a vascularization percentage of 100%, corresponding to a normal vasculature (Figure 1).Image analysis revealed that the degree of blood vessel formation in the presence of the extract was decreased compared with the normal vasculature (in the absence of the extract), and the avascularised area has been increased in a manner dependent on the concentration of the extract (Figure 1 (a)).The activity of the aqueous extract of *Ganoderma orbiforme* was evaluated at a concentration of 5 mg/mL. On the one hand, Figure 2 (b) shows that *Ganoderma orbiforme* prevented the formation of new vessels, and decreased the density and number of vessels in the grafted area with whattman paper soaked with extract.

(a)

Density (%)



(b)

Figure 2: Inhibitory effects of water extracts of *Microporus xanthopus*, *Ganoderma orbiforme* and *Polyporus fasciculatus* on angiogenesis.

(a) The CAM of an 8 days old chick embryo was separately exposed to PBS (control). Extracts were introduced on top of the CAMs. After 24 h of incubation, the CAM tissue directly beneath each filter disk was resected, and digital images of the CAM sections were captured.

(b) The bar graph represents the number of branches after action of extracts. Photographs were imported into an image software program to visualize the new vessel branch points. Data are shown as the mean ± SD: $p<0.05$ compared with untreated control. None of the tested concentrations (0.25 mg/mL) of the water extract display statistically significant differences with respect to each other.

Indeed, the CAM presented non-contiguous vessels that did not appear in the grafted area. On the other hand, the comparison with PBS shows that the number of vessels was reduced to 2 in the presence of *Ganoderma orbiforme*. That is a percentage inhibition of 66.67%. The CAM treated with the aqueous extract of *Polyporus fasciculatus* at 250 µg/mL exhibited less dense and fewer vessels than those present in the CAM treated with PBS. Analysis of the vascularization of the CAM after treatment with *Microporus xanthopus* at 2 mg/mL showed a decrease in density and number of vessels with a percentage inhibition of 55.45%. *Polyporus fasciculatus* induced a percentage inhibition of 37.50%.The status of embryos after action on vascularization extracts information on toxicity (Table 4).

Table 4: Antiangiogenic effect of *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus*

	Dose µg/mL per eggs	Tested eggs (n)	Embryos status after 24h	Antiangiogenic effect	Vessels nombers	Density (%)
PBS (control)		6	living	-	24	100
Microporus xanthopus	250	6	living	+++	24	55.45
Ganoderma orbiforme	250	6	living	+++	24	66.66
Polyporus fasciculatus	250	6	living	+++	24	37.50

DISCUSSION

Microporus xanthopus, Ganoderma orbiforme and *Polyporus fasciculatus,* contain abundant coumarins, polyphenols, alkaloids and reducing sugars. The results of the determination of total phenolic, total flavonoids, total tannins and total proanthocyanidins show that the various extracts derived from these fungi are rich in polyphenol. Proanthocyanidins have been reported to exhibit many beneficial health effects such as antioxidant and anti-carcinogenic effects. Thus, Ba et *al*., in 2012 [21], have reported that taken together, the proanthocyanidins could be used as a potential natural cancer chemopreventive agent through Nrf2/ARE mediated phase II detoxifying/antioxidant enzymes induction via p38 and PI3K/Akt pathway. Phenolic substances have been suggested to play a preventive role in the development of chronic diseases such as cancer and heart disease.Several researchers have shown that polyphenols are highly active compounds against inflammatory diseases, cardiovascular, neuro-degenerative (Parkinson's and Alzheimer's disease), are powerful antioxidants and antivirals agents [22].

The crude extract of *Microporus xanthopus, Ganoderma orbiforme* and *Polyporus fasciculatus* show best DPPH free radical-scavenging activities. The results of *Ganoderma orbiforme*, however, differ from those obtained by Mbayo et *al*. in 2015 [23] in the evaluation of antioxidant activity with DPPH as a free radical, after an ascending thin layer chromatography. In this work, the various authors have shown that *Ganoderma orbiforme* possess a strong antioxidant activity. On the other hand, this result is surprising and unexpected because of its amount of phenolic compounds. The latter have beneficial biological effects to scavenge free radicals [24], important property underlying their various biological and pharmacological activities [25].A number of studies conducted on plant and fungal samples in order to evaluate their antioxidant efficacy have confirmed that organic extracts rich in total phenolic compounds exert potent antioxidant activities [26, 27, 28]. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [29], decrease the risk of heart diseases by inhibiting the

oxidation of low-density lipoproteins. They are also known to possess antibacterial, antiviral, antiangiogenic and anticarcinogenic properties [12, 14, 28, 30].

The anti-angiogenic effect of water extracts of these fungi on the chicken embryo CAM revealed that these extracts had anti-angiogenic properties. No embryo death was recorded in the concentration range tested indicating that the observed anti-angiogenic activity is not due to the toxicity of the extracts.Inhibition was dose dependent. Studies by Song et *al*. in2004 and Jang et *al*. in 2010 [31, 32] have shown that *Ganoderma orbiforme* extracts suppress angiogenesis in human cancer cells. These extracts have anti-angiogenic activity much greater than that of mannose 6-phosphate with a 38% densityat 60 mg/mL and of the reference molecules, sunitinib (Sutent™) [33]. Furthermore, a combination of four compounds called SMYGT (*Sargassum fusiforme, Laminaria japonica, Ostreagiga* sand *Prunella vulgaris*) at 0.4 mg/mL, has a 10% density, so an antiangiogenic activity less than what offer the water extract of *Ganoderma orbiforme*, *Microporus xanthopus* and *Polyporus fasciculatus* [19]. These aqueous extract showed strong antiangiogenic activity by not only,the inhibition of blood vessel formations on chick embryo chorioallantoic membrane (CAM), but also reducing and/or canceling of vessel's diameters. 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* and *Tetrorchidium oppositifolium*, which showed a percentage inhibition of 83.334% at 500 g/mL [34]. Therefore, according to the results, the aqueous extract of *Lophira procera* may have good inhibitory activity on tumor growth by blocking angiogenesis [28]. The antiangiogenic activity of *Scyphocephalium ochocoa* (IC_{50} = 1.153 ± 0.089 µg/mL) is lower than that of the reference drug, sorafenib (IC_{50} = 0.197 ± 0.062 µg/mL)[35].

CONCLUSION

The works of Folkman (1961) made it possible to elucidate the link between cancer and angiogenesis. Thus, it has emerged that anti-angiogenic therapy is a highly effective strategy that represents a treatment, not curative, but that supersedes conventional treatments used in cancer therapy. In addition, as antioxidant molecules protect cellular components from oxidative damage, the consumption of antioxidant-rich substances can help prevent cancer and other chronic diseases. As a result, this work consisted of testing the antioxidant and anti-angiogenic potential of *Microporus xanthopus*, *Polyporus fasciculatus* and *Ganoderma orbiforme* from Gabon. The results of the phytochemical screening supported by the determination of the phenolic compounds of the water, water-ethanol and ethanol extracts of these fungi showed that all the fungi contained phenolic compounds and the phenolic

compound contents of the extracts were proportional to the antioxidant activity. Although some had mild or moderate activities, other extracts had stronger activities. The anti-angiogenic activity of the aqueous extracts of these fungi on the chicken embryo CAM revealed that these extracts had anti-angiogenic potentialities. These fungi could thus be potential sources of myco-drugs.

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CONFLICT OF INTEREST

The authors declare that there are no competing interests. All the authors read and approved the final version.

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