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Phytochemical Screening, Antioxidant and Antiangiogenic activities of *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* Medicinal Mushrooms from Gabon

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Abstract The oxidation process is essential for living organisms for energy production. However, it has been shown that free radicals generated in large quantities can activate proangiogenic factors and be involved in many human diseases such as cancers. Thus, in this work, the antioxidant activities of water, water-ethanol and ethanol extracts of three fungi of Gabon were tested by measuring the trapping power of the DPPH radical. The anti-angiogenic activity was evaluated for some aqueous extracts by chicken chorioallantoic membrane (CAM) method. The results obtained for the DPPH trapping test showed that the various extracts tested had antioxidant activities going from low to very high. *Daedaleopsis nitida*, *Pycnoporus sanguineus* and *Phellinus gilvus* fungi exhibited very strong antioxidant activities. The aqueous 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 66.66%, 54.55% and 37% for *Phellinus gilvus, Daedaleopsis nitida* and *Pycnoporus sanguineus*, 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 activity, anti-angiogenic activity, phenolic compounds, CAM, fungi

Introduction

Fungi are among the most popular non-timber forest products, mainly due to the marketing of edible mushrooms. Several species are sought after in international markets for gastronomy such as chanterelles. The total number of fungi species on Earth has been estimated at 140 000, of which 22 000 have been identified. More than 2000 species of fungi are listed as edible. The number of fungi known to possess pharmacological properties of interest has been estimated at about 700 species [1]. Nearly 200 species of fungi are recognized for their medicinal value [2]. Only 10% of these identified species have been the subject of in-depth studies on their biological properties [3]. However, there is a significant lack of knowledge of the pharmacological properties of the majority of macroscopic fungi

recorded in Congo Basin. A total of 126 medicinal properties are attributed to the fungi, including antidiabetic, anticancer, antitumor, immunomodulatory, antiviral, antibacterial, hepatoprotective, antioxidant and antiangiogenic effects [4]. Angiogenesis is a complex process that requires the involvement of many types of factors. These factors are proteins that stimulate it (pro-angiogenic factors) or inhibit it (anti-angiogenic factors). In adults, these factors are in equilibrium and produce a dynamic state of rest. The rupture of this equilibrium in favor of pro-angiogenic factors is essential for the initiation of angiogenesis. In physiological or pathological condition, angiogenesis is initiated by tissue demands for oxygen and nutrients, resulting in a hypoxia/reoxygenation cycle, which, in turn promotes the formation of reactive oxygen species (ROS) [5]. Thereby, tumor angiogenesis corresponds to the appearance of new vessels intended to meet the metabolic needs of the tumor. Indeed, the expansion of a tumor beyond 2 mm requires the development of a blood circulation. This circulation is necessary to provide the tumor with oxygen and nutrients necessary for its growth, but also to promote the spread of metastases in the bloodstream [6]. The hypothesis of the involvement of angiogenesis in the cancer process was first described by Folkman in1971. In his postulate, he states that tumor growth depends on angiogenesis and that inhibition of angiogenesis could have a therapeutic effect [7]. 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 [8, 9]. Thus, because of the importance of ROS and angiogenesis in carcinogenesis, in this work, the antioxidant and anti-angiogenic potentialities of three (3) medicinal Mushrooms of Gabon were tested, from a perspective of research of new molecules for the fight against cancer. *Daedaleopsis nitida*, *Pycnoporus sanguineus* and *Phellinus gilvus* (Figure 1) are used in traditional medicine to treat a number of diseases. *Daedaleopsis nitida* is used to treat diabetes and arterial hypertension; *Pycnoporus sanguineus* is used for wound healing and sexually transmitted diseases; *Phellinus gilvus* is used in traditional medicine to fight fever, scabies, dry cough, hemorrhoid. These three mushrooms are often associated in traditional medicine of Fang people of Gabon to fight against breast cancer.

Material and methods

Fungal Material

The macro fungi *Daedaleopsis nitida*, *Pycnoporus sanguineus* and *Phellinus gilvus* (Figure 1) were harvested in classified forests of Monda and Malibé 2, 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, University of Sciences and Technology of Masuku, Franceville, Gabon.

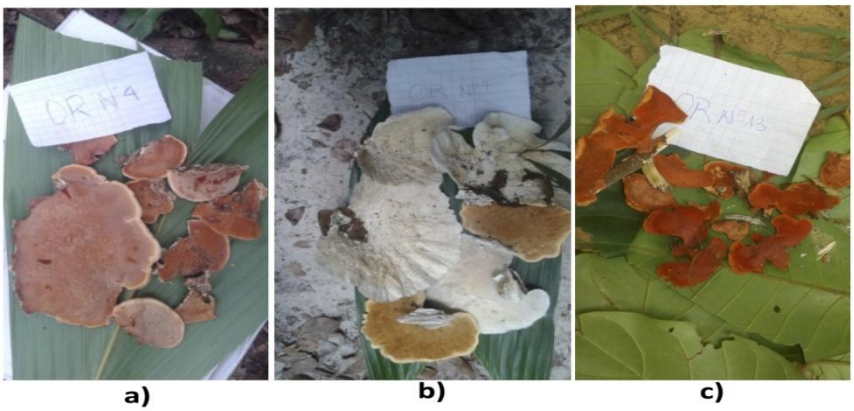

Figure 1: Identification of fungi: a)Phellinus gilvus, b) Daedaleopsis nitida, c) Pycnoporus sanguineus

These three mushrooms were harvested from a cape forest in Libreville-Gabon and the photos were taken with a 16 × 5.0 Mega Pixel camera (made in China).

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 [10]. 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*, 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) extract, ethanol extract and water extract were 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. 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.

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, anthocyans, alkaloids and anthracenosids as described elsewhere [11].

Total Phenolic Content

The total phenolic contents of the different extracts were determined according to the Folin-Ciocalteu Method [12]with minor modifications as described by Obame [13] 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 aluminum chloride ($AlCl_3$) colorimetric assay method [14] adapted to 96 well-plate, using quercetin as a standard [15].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 [16].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 [17]. 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. 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 [18]. 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: $\%RSA = [(A_{control} - A_{sample}) / A_{control}] \times 100$.

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: $AAI = [DPPH]_f(\mu g/mL) / IC_{50}$ ($\mu g/mL$).

$[DPPH]_f$ is the final concentration of DPPH. We considered criteria of Scherer and Godoy (2009) according to which plant extracts show poor antioxidant activity when 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 AAI > 2.0.

Evaluation of Antiangiogenic Activity

Chick Chorioallantoic Membrane (CAM) Model: in this assay, the antiangiogenic efficacy of the water extracts of *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus,* was evaluated according to previously reported methods[19, 20]. 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 h 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 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

Daedaleopsis nitida, Pycnoporus sanguineus and *Phellinus gilvus,* three medicinal mushrooms studied, are represented in figure 1. 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.

Phytochemical Screening

The phytochemical screening of the extracts was first performed to detect the major chemical groups occurring in the extracts (Table 1). It appears that *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* three fungi studied, contain abundant coumarins, polyphenols and reducing sugars. The results of the phytochemical screening of *Daedaleopsis nitida* show that the ethanol and water-ethanol extracts of *Daedaleopsis nitida* are rich in coumarin and total polyphenols, moderately abundant in gitoxigenins, flavonoids (flavones) and reducing sugars. *Pycnoporus sanguineus* extracts are rich in reducing sugars, flavones, digitoxigenins and coumarins; they are moderately rich in

alkaloids. Polyphenols, sterols, triterpenes and anthracenosides are moderately abundant in water and ethanol extracts. Proanthocyanidins are abundant in water-ethanol and ethanol extracts; moderately abundant in aqueous extract.The phytochemical screening of *Phellinus gilvus* extracts showed that all extracts are rich in coumarins, anthracenosides, total polyphenols and moderately abundant in total flavonoids. The reducing sugars and gitoxigenins are moderately abundant in water and ethanol extracts but abundant in water-ethanol extract which is also rich in gallic tannins. The phenolic compounds are an important property underlying their various biological and pharmacological activities.

Table 1: Chemical groups detected in *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* fungal extracts

Chemical Groups		***Daedaleopsis nitida***			***Pycnoporus sanguineus***			***Phellinus gilvus***		
		Aq	**Eth-Aq**	**Eth**	**Aq**	**Eth-Aq**	**Eth**	**Aq**	**Eth-Aq**	**Eth**
Saponosids		+++	-	-	+++	-	-	+++	-	-
Polyphenols		+++	+++	++	++	++	-	+++	+++	+++
Sterols and triterpenes		-	-	-	++	+++	++	+	++	+
Oses and holosides		-	-	-	+	++	-	+	+++	+++
Tannins	Gallics	-	-	-	+	+	+	-	+++	++
	Catechics	-	-	++	-	-	-	-	-	-
Alkaloids		+	+	+	++	++	++	+	+	+
Cyanidins	Flavons	++	++	-	+++	+++	+++	++	+++	-
	Flavanons	-	-	-	-	-	-	-	-	++
	Flavonols	-	-	-	-	-	-	-	-	-
	Flavanonols	-	-	-	-	-	-	-	-	-
Total flavonoids		++	++	+	++	++	-	++	++	++
Anthocyans		++	+	+	+	++	+++	+	+	+
Proanthocyanidins		++	+	+	++	+++	+++	-	+	+
Anthracenics		++	++	-	++	+++	++	++	++	++
Coumarins		++	+++	+++	+++	+++	+++	+++	+++	+++
Cardiac glycosides	Digitoxine	-	-	-	-	-	-	-	-	-
	Digitoxigenine	-	-	-	+++	+++	++	-	-	-
	Gitoxine	++	++	++	-	-	-	-	-	-
	Gitoxigenine	-	-	-	-	-	-	++	+++	++
Reducing sugar		++	++	++	+++	+++	+++	++	+++	++

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

Total phenolic, Total flavonoid, Tannins and proanthocyanidins contents

The contents of total phenolic, total flavonoids, total tannins and total proanthocyanidins of extracts from *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus,* 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= 0.9902[21] ranged from 640.16±3.70 to 5170.91±3.13 mg GAE/100 g of drug. Total flavonoids (standard curve equation: Y = 0.0032X + 0.0077, R^2= 1) ranged from 99.53±4.00 to 3043.50 ±2.90mg 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 13.78±1.90 to 1344.83±6.24mg 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 [22]. Among extracts, proanthocyanidin contents had ranged between TPC=18.72±2.60 and 2718.14±8.21 mg APE/100 g of drug. The determination of the phenolic compounds of *Daedaleopsis nitida* shows that the tannins are absent from the aqueous and water-ethanol extracts but present in a significant amount in ethanol extract. The aqueous extract has the highest content of total polyphenols and proanthocyanidins but a total flavonoid content intermediate between that of water-ethanol and ethanol extracts. The ethanol extract has higher total tannin and proanthocyanidin contents than those of water-ethanol but lower total polyphenol and flavonoid contents. The content of phenolic compounds in *Pycnoporus sanguineus* extracts is greater in water extract than in water-ethanol extract, which contains contents greater than those present in ethanol extract. For *Phyllinus gilvus*, the total tannins, total flavonoids and proanthocyanidins contents were high in water-ethanol extract but low in the other two extracts. Polyphenols for their part have high levels. This content varies in a decreasing manner from water extract to ethanol extract.

Table 2: Total phenolic content (TPC), Total flavonoid content (TFC), Total tannins content (TTC) and Total proanthocyanidins content (TPC) of extracts from *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus*

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 of drug)**
Daedaleopsis nitida	Aq	2536.00 ± 2.45	303.65 ± 1.50	Nd	815.69 ± 2.80
	Eth-Aq	1783.11 ± 3.10	352.90 ± 1.77	Nd	33.33 ± 1.40
	Eth	979.22 ± 4.70	99.53 ± 4.00	974.04 ± 5.79	66.90 ± 1.60
Phellinus gilvus	Aq	5170.91 ± 3.13	482.91 ± 3.08	13.78 ± 1.98	18.72 ± 2.60
	Eth-Aq	3684.53 ± 3.80	706.59 ± 2.11	1344.83 ± 6.24	143.53 ± 2.61
	Eth	2475.26 ± 1.23	308.72 ± 1.09	251.39 ± 2.06	65.01 ± 3.17
Pycnoporus sanguineus	Aq	18490 ± 7.07	3043.5 ± 2.90	Nd	2718.14 ± 8.21
	Eth-Aq	2869.39 ± 1.82	1677.48 ± 1.67	303.11 ± 1.00	1199.64 ± 1.39
	Eth	640.16 ± 3.70	107.49 ± 3.89	127.23 ± 7.30	164.04 ± 2.98

Aq = Aqueous; Eth-Aq = Ethanol-aqueous; Eth = Ethanol; Nd = Non determined

Antiradical activity

The free radical-scavenging activities of various extracts were evaluated at their initial concentration. All extracts show free radical scavenging activity (Table 3). *Pycnoporus sanguineus* exhibited very high antioxidant activity in its aqueous extract (IAA = 3.13); moderate activity in its water-ethanol extract (IAA = 0.90) and low activity in its ethanol extract (IAA = 0.22). Studies by Borderes et *al*. [23] also showed that *Pycnoporus sanguineus* has a promising activity in terms of DPPH trapping. The water extract of *Daedaleopsis nitida* exhibited very high antioxidant activity (IAA = 2.18) while water-ethanol and ethanol extracts had moderate antioxidant capacities; with LPNs of 0.50 and 0.60 respectively. The aqueous extract of *Phellinus gilvus* had moderate antioxidant activity (IAA= 0.58) and the water-ethanol and ethanol got low antioxidant activities (IAA equal to 0.27 and 0.36, respectively). A study done by Seephonkai et *al*. [24] and Yang et *al*. [25] showed that water, ethanol (50%, 80%, pure) and ethyl acetate extract of *Phellinus gilvus* got an antioxidant property. Phenolic compounds have beneficial biological effects to scavenge free radicals [26]. Phenolic extracts have been reported to retard lipid oxidation in oils and fatty foods [27, 28], 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 [15, 21, 22, 29].

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

Plants extracts		Equations	R^2	IC_{50}(µg/mL)	AAI	Activity
Daedaleopsisfn itida	Aq	Y= 304.92X + 43.004	0.955	22.96	2.18	Verystrong
	Eth-Aq	Y= 81.715X + 41.894	0.976	99.20	0.50	Moderate
	Eth	Y= 73.168X + 43.954	0.983	82.63	0.60	Moderate

Phellinusgilvus	Aq	Y= 155.78X + 36.62	0.985	85.88	0.58	Moderate
	Eth-Aq	Y= 91.762X + 32.884	0.989	186.52	0.27	Poor
	Eth	Y= 99.279X + 36.436	0.987	136.62	0.36	Poor
Pycnoporussan guineus	Aq	Y= 1112.9X + 32.23	0.996	15.97	3.13	Verystrong
	Eth-Aq	Y= 156.13X + 41.38	0.986	55.19	0.90	Moderate
	Eth	Y= 47.779X + 39.16	0.999	226.88	0.22	Poor
Vitamin C		Y = 14.559X - 0.613	0.999	3.48	11.32	Verystrong
BHT		Y = 5.659X + 11.513	0.996	6.30	7.85	Verystrong

Aq = Aqueous; Eth-Aq = Ethanol-aqueous; Eth = Ethanol, BHT= Butylatedhydroxytoluene: Positive control.

Antiangiogenic activity

The antiangiogenic potential of the extracts was evaluated *in ovo* with the chicken chorioallantoic membrane (CAM) the eighth embryonic day. The fertilized eggs were treated with aqueous extracts (250 µg/mL). The degree of vessel formation on CAM was scored 1 day later. The vessel density is the percentage of blood vessels 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 2). 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 presence of PBS), and the avascularised area has been increased in a manner dependent on the concentration of the extract (Figure 2).

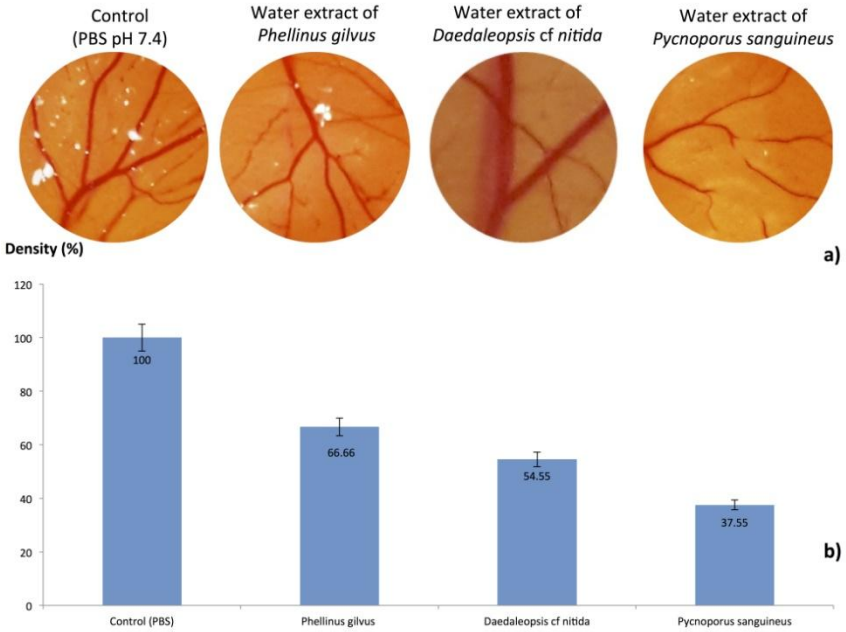

Figure 2: Inhibitory effects of water extracts of Daedaleopsis nitida, Pycnoporus sanguineus and Phellinus gilvus fungal extracts on angiogenesis

(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.

Analysis of the vascularization of the CAM after treatment with *Daedaleopsis nitida* at 250 µg/mL showed a decrease in density and number of vessels with a percentage inhibition of 54.55%. The aqueous extract of

Daedaleopsis nitida showed an anti-angiogenic potential. The photograph of the CAM treated with *Pycnoporus sanguineus* (250µg/mL) shows that the vessels of the latter saw their density decrease. Indeed, for a concentration of 250 µg /mL, the aqueous extract of *Pycnoporus sanguineus* acted by inhibiting the formation of angiogenesis, thus the formation of new vessels on the one hand, and on the other hand by narrowing the diameter pre-existing vessels, tending to make them disappear. The aqueous extract of *Pycnoporus sanguineus* showed inhibitory activity on angiogenesis with a 37% inhibition percentage. In the presence of water extract of *Phellinus gilvus*, the vascularization of the CAM was almost nonexistent. *Phellinus gilvus* reduced both the density and the number of blood vessels in the CAM with a 66.66% inhibition percentage. In spite of the fact that water extract of this fungus has a low antioxidant activity, it has an anti-angiogenic potential. This could result in the fact that free radicals are not the only promoters of angiogenesis. The status of embryos after action on vascularization extracts information on toxicity (Table 4). Recent studies show that plants with high antioxidant activity, also have a strong anti-angiogenic activity [19, 21, 22, 29].

Table 4: Antiangiogenic effect of *Daedaleopsis nitida*, *Phellinus gilvus* and *Pycnoporus sanguineus* fungal extracts

	Dose µg/mL per eggs	Tested eggs (n)	Embryos status after 24 h	Antiangiogenic effect	Vessels nombers	Density (%)	P value
PBS (Control)		6	living	-	24	100±2.00	
Daedaleopsisfnitida	250	6	living	+++	13	54.55±1.34	0.076
Phellinusgilvus	250	6	living	+++	16	66.66±1.56	
Pycnoporussanguineus	250	6	living	+++	9	37.55±2.43	

Conclusion

It provides the first convincing and integrated evidence that extracts of *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* have health-protecting effects as confirmed by their potent antioxidant effects in terms of their reducing power and with a significant capacity to scavenge DPPH. Their fungal extracts were found to be strong sources of total phenolic compounds, total flavonoids and total proanthocyanidines. The phyto-constituents identified in extracts were found to be biologically active, thus confirming their role in preventing severe infections arising from oxidative stress caused by the over production of free radicals. In addition, *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* displayed inhibitory activities towards CAM angiogenesis *in vivo*. Hence, *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* can be used as easily accessible sources of natural antioxidants for potential preventative therapies against angiogenesis related diseases. This study confirm the multiple uses of *Daedaleopsis nitida, Pycnoporus sanguineus* and *Phellinus gilvus* for the treatment of many infectious diseases and place them as candidate for further investigations for enhanced traditional drug utilizable as complementary and alternative medicines development and new active compounds discovery.

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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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