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MYCOCHEMICAL ANALYSIS AND PREDICTION OF *PLEUROTUS TUBER-REGIUM'S* (PLEUROTACEAE) PHARMACOLOGICAL ACTIVITIES, A FOOD AND MEDICINAL FUNGI FROM GABON

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ABSTRACT: *Pharmaceutical activities of a fungus depend on its bioactive compounds composition. Pleurotus tuber-regium (paleotropical species) is a fungus used in Gabon and throughout tropical Africa for its culinary and medicinal properties. The aim of this study was to predict the therapeutic potential of this species, in particular of its carpophore and its sclerotia, based on the main chemical groups highlighted during the chemical screening of aqueous, hydro-ethanolic and ethanolic extracts. Chemical screening revealed that the three extracts (aqueous, hydro-ethanolic and ethanolic) prepared from the carpophore are rich in total polyphenols, alkaloids, coumarins and proanthocyanidins. Aqueous and hydro-ethanolic extracts are moderately rich in tannins and coumarins while the ethanolic extract is very rich in reducing sugars. About the sclerotia, the three extracts are rich in total polyphenols, alkaloids, reducing sugars and proanthocyanidins. Aqueous and hydro-ethanolic extracts are moderately rich in tannins, total flavonoids and coumarins. The dosage of phenolic compounds carried out on aqueous and hydro-ethanolic extracts confirmed the richness of this fungus in total polyphenols and proanthocyanidins, as well as its deficiency in flavonoids and tannins. The chemical groups thus identified in the carpophore and the sclerotium of P. tuber-regium allow to predict its antioxidant, antiallergic, antiplasmodial, anesthetic, analgesic, anticancer, vasodilator, anti-inflammatory and ant-mutagenic activities.*

KEYWORDS: Chemical Screening, Bioactive Compounds, Therapeutic Potential, Pleurotus Tuber-Regium, Gabon.

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INTRODUCTION

Pleurotus tuber-regium (Pleurotaceae) is a species of paleotropical fungus food (Eyi-Ndong, 2009; Eyi-Ndong *et al*., 2011; De Kesel *et al*., 2002, 2018) and medicinal (Eyi-Ndong *et al*., 2020; Mengue-Eyi, 2012; Walleyn & Rammeloo, 1994) used in Gabon. This fungus, particularly its sclerotia, got great interest to the populations of tropical Africa. In traditional medicine, *P. tuber-regium* is used, in various dosages, to treat common diseases in several African countries (Walleyn & Rammeloo, 1994). The populations of northern Gabon unanimously use it to treat diseases such as influenza, cough, hernia, scabies, ear infections, genito-anal diseases and navel infection in infants (Eyi-Ndong *et al.*, 2020; Mengue-Eyi, 2012).

In Nigeria, it is used for abdominal pain, constipation, headache, fever, chest pain, smallpox, boils, asthma, control of high blood pressure, nervous disorders and for fetal development during pregnancy (Walleyn & Rammelo, 1994). In Madagascar, Heim (1935) reported that this fungus is not only used against headaches but also, according to popular beliefs, it protects against certain poisons such as the sap of *Cerbera venenifera* (Poir.) Steud. (Apocynaceae). He also observed an important use of sclerotia in witchcraft; pregnant women were allowed to enter into the hut where there was a human corpse only after ingesting powder from the sclerotia of *P. tuber-regium*.

Regarding its relative importance and degree of popularity, the study of Eyi-Ndong *et al*. (2020) among the Fang and Baka ethnic groups found that *P. tuber-regium* is important and popular for both the Fang and the Baka in northern Gabon, with high informant consensus.

This work is part of the knowledge of fungal biodiversity and the enhancement of the traditional knowledge of rural populations in tropical Africa. The objective is to identify the different chemical groups responsible for biological activities in the carpophore and sclerotia of this fungus in order to better understand the reasons for the renewed interest of this fungus in tropical Africa.

MATERIALS AND METHODS

Harvest and Identification of *P. tuber-regium*

The mushroom studied was collected in a former plantation in Malibe 2 (Libreville-Gabon). The specimens collected were photographed, described macroscopically, then dried at 60°C for 24 hours using a travel dryer. After drying, the mushrooms were described microscopically using an Olympus BX51 microscope equipped with a drawing tube. Microscopic observations of spores, basidia, cystidia, pileus coating and stipe coating were made in Congo Ammoniacal Red. In practice, the structures observed were drawn, their dimensions were measured and compared with the descriptions appearing in reference works (mainly “Illustrated Flora of Central African Mushrooms”, “Iconographic Flora of Congo Mushrooms”, “Fungus Flora of Tropical Africa”, Eyi-Ndong *et al*. (2011) and the protologues published in various mycological journals). For the description of the spores, the symbol Q = L / l represents the length to width ratio of the spores. Measurements of spores, basidia and cystidia were made using the software, Olympus Soft Imaging Solutions GmbH; this software was installed in a computer connected to the microscope. The average value of Q retained for each species was calculated on a sample of at least twenty spores (Eyi-Ndond *et al.*, 2011).

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Chemical Analysis

Preparation of Mushroom Extract

The extracts of the carpophore and the sclerotia were prepared separately. A water-ethanol extract (50/50 v / v), an ethanol extract (90°) and a water extract were prepared from the dry powder of the carpophore and the sclerotia of *P. tuber-regium*. 500 mL of each solvent or mixture of solvents were pour into Erlenmeyer containing 50 g of mushroom powder and left stirring at room temperature (25°C) for 24 hours. Each extract was filtered using Whatman No. 1 filter paper and the solvents were completely removed at low pressure with a rotary evaporator (Büchi, Labortechnik, Switzerland). The extracts were then concentrated, lyophilized and stored at 4°C until analysis.

Qualitative Analysis

Chemical screening was carried out on each extract in order to highlight the different main chemical groups (Ciulei, 1964). The extracts of *P. tuber-regium* were analyzed for their classes of bioactive compounds using standard procedures with small modifications (Ciulei, 1964). The extracts were tested qualitatively for the presence of chemical constituents such as tannins, terpenes, saponins, flavonoids, cardiac glycosides, coumarins, alkaloids, anthraquinones and reducing sugar. For gallic tannins, 2 mL of 1% ferric chloride solution was added to 2 mL of the filtrate (Stiasny's test). Dark-greenish coloration indicated their presence. For catechic tannins, 2 mL of a solution of hydrochloric n-butanol was added to 2 mL of filtrate, and then heated in a water bath for 5 to 10 minutes (Bate-Smith's test). Intense red coloration indicated the presence of the catechin tannins. For total flavonoids and anthocyanins, 1 mL of the NaOH was added to 2 mL of the filtrate; then, 1 mL of sulfuric acid was added to the mixture. A dark color after adding acid indicated the presence of flavonoids; the color changing to purple after addition of NaOH indicated the presence of anthocyanins. To test the cyanidin presence, to 2 mL of the filtrate was added hydrochloric alcohol and some magnesium strips. A rose-orange effervescence showed the presence of flavones; a rose-purplish color indicated the presence of flavanones and red denoted the presence of flavanols. The Folin's test was applied to determine polyphenol contents. 1 mL of the Folin reagent was added to 2 mL of the filtrate. After 5 minutes of incubation at 25°C, 1 mL NaOH was added. Dark green coloration indicated the presence of polyphenols. For coumarins, 2 mL of filtrate combined with 2 mL of NH_4OHthen, looking at UV lamps (366 nm). The fluorescence presence indicated the presence of coumarins. A rose-pink color of the solution after adding 2 mL NH_4OH solution into 2 mL of the filtrate (Borntrager's test) indicated the presence of anthraquinones. For alkaloids, some drops of sulfuric Dragendorff's reagent were added to 2 mL of the filtrate.

Orange precipitate formed showed the presence of alkaloids. To determine cardiac glycosides and terpenes, tests such as Salkowski's and Lieberman's test were applied; 2 mL of concentrated H_2SO_4 were added to 2 mL of filtrate. A reddish-brown ring indicated the presence of steroid, an aglycone part of the cardiac glycoside (Salkowski's test). Another part of the filtrate (2 mL) was added with 2 mL of acetic anhydride and cooled well in ice, and concentrated H_2SO_4 (2 mL) was carefully added. A color change from blue to green indicated the presence of terpenes (Lieberman's test). Saponins were determined through a frothing test. The filtrate was vigorously shaken. Frothing which persisted on warming for about 15 minutes indicated the presence of saponins. For reducing sugars, equal volume of Fehling’s A and Fehling’s B reagents were taken in equal quantities.

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Total Phenolic Content

The total phenolic content of the water-ethanol and the ethanol extract was determined according to the Folin-Ciocalteu method (Singleton *et al*., 1999) using gallic acid as standard (Obame-Engonga *et al*., 2017). Absorbance was measured at 735 nm using a multi-well plate reader (μQuant Bio-Tek Instrument Inc., USA). All analyses were carried out in triplicate and the results (mean of the analysis in triplicate) were expressed in gallic acid equivalent per gram of lyophilized sample (GAE / mg).

Total Flavonoid Content

The total flavonoid content was determined by colorimetric aluminum chloride ($AlCl_3$) assay method (Quettier-Deleu *et al*., 2000) adapted to a 96-well plate, using quercetin as standard (Sima *et al*., 2016). Total flavonoid content was expressed in quercetin equivalents in milligrams per gram sample (EQ)/ mg of extract (analysis average in triplicate).

Tannin Content

The reference method by Sima-Obiang was used to determine the tannin content (Sima-Obiang *et al*., 2017). Absorbance was measured at 525 nm and tannic acid was used as a standard. The tannin contents were expressed in mg of tannic acid equivalent (TAE)/100 g of extract.

Proanthocyanidin Content

The method consists of proanthocyanidins hydrolysis in a hot acid-alcohol medium into anthocyanidins. This method makes it possible to take into account all the units of flavan-3-ols constituting the polymers (Sima-Obiang *et al*., 2017). The assay was carried out by mixing 50 μL of the extract with 700 μL of HCl-butanol solution at 30% (v / v). The mixture was placed in a hermetically sealed 1.5 mL Eppendorf tube and vortexed for 1 minute. The tube was then heated for 2 hours at 100°C. After cooling, 200 μL aliquots were placed into a 96-well plate and the absorbance was read at 550 nm. Apple procyanidins (DP ≈ 7.4) treated as mentioned above were used as the standard. The results were expressed in apple procyanidin equivalents (APE).

Statistical Analysis

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. Values of $p < 0.05$ were considered to be statistically significant.

RÉSULTATS ET DISCUSSION

Studied Mushroom

The species studied was identified as *P. tuber-regium* This saprotrophic species grows in dense humid forests, in open forests, or in plantations. *P. tuber-regium* was harvested in a plantation of Malibé 2 at 172 m above sea level. The geographical coordinates are 0° 34′0″N and 9° 27′0″.

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Chemical Groups Content

The results of the chemical screening of *P. tuber-regium* extracts (Figure 1) are shown in Table 1 for the carpophore, and in Table 2 for the sclerotia. The choice of solvents is justified by the fact that various organic compounds, including phenolics and flavonoids, have good solubility in them.

Figure 1. Fruit bodies of *P. tuber-regium* Photograph by Hugues Calixte Eyi Ndong

Table 1: Chemical groups of the carpophore of *P. tuber-regium* extracts

Chemical group	Extracts		
	Aqueous	Hydroalcoholic	Ethanolic (90°)
Saponosids	+	+	-
Sterols and triterpenes	++	++	++
Alkaloids	++	++	+++
Gallic tannins	+	-	-
Catechin tannins	+	+	-
Anthraquinones	++	+	-
Reducing sugars	+++	-	-
Total Polyphenols	+++	++	++
Total flavonoids	+	+	-
Flavonol	-	-	-
Flavone	+	-	-
Flavanone	+	-	-
Coumarins	+++	++	+
Digitoxin	++	-	-
Digitoxigenin	++	++	++
Gitoxin	-	+	+
Gitoxigenin	+	-	-
Osse and holosides	+++	++	+++
Anthocyanins	-	-	-
Proanthocyanidins	+++	-	+++

+++, very abundant; ++, abundant; +, not abundant; −, not detected.

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Table 2. Chemical groups of the sclerotum of *P. tuber-regium* extracts.

Chemical group	Extracts		
	Aqueous	Hydroalcoholic	Ethanolic (90°)
Saponosids	+	-	-
Sterols and triterpenes	+	+	+
Alkaloids	++	++	+++
Gallic tannins	+	+	-
Catechin tannins	-	-	-
Anthraquinones	-	-	-
Reducing sugars	++	++	++
Total Polyphenols	++	++	++
Total flavonoids	+	+	-
Flavonol	-	-	-
Flavone	-	-	-
Flavanone	-	-	-
Coumarins	++	++	-
Digitoxin	-	-	-
Digitoxigenin	++	++	++
Gitoxin	+	+	+
Gitoxigenin	-	-	-
Osse and holosides	+	++	+++
Anthocyanins	-	-	-
Proanthocyanidins	+	+++	+++

+++, very abundant; ++, abundant; +, not abundant; −, not detected.

The choice of the extraction method is a very important parameter to obtain extracts with good yields. The results of our study show that the chosen solvent defines the quantity and quality of the compounds contained in the treated extract. Table 1 shows that the three extracts (aqueous, hydro-ethanolic and ethanolic) prepared from the carpophore of *P. tuber-regium* are abundant in total polyphenols, alkaloids, sterols and triterpene, and digitoxigenin. Proanthocyanidines and oses and holosides are very abundant in aqueous and ethanolic extracts but the proanthocyanidines are respectively absent and abundant in the carpophore and the sclerotum hydro-ethanolic extracts. The aqueous extract is very rich in reducing sugars. Regarding the sclerotia (Table 2), the three extracts prepared from the sclerotia are rich in total polyphenols, alkaloids, reducing sugars and digitoxigenin. Tannins and flavonoids are rare while proanthocyanidins are very abundant in hydro-ethanolic and ethanolic extracts.

The abundance of polyphenols suggests *P. tuber-regium* antioxidant (Bruneton, 2009), antiallergic (Karou *et al*., 2007) and antiplasmodial (Casano *et al*., 2010) properties. Alkaloids give it anesthetic, analgesic, anticancer and vasodilator properties (Bruneton, 2009). It also has some anti-bacterial, anti-plasmodial and anti-inflammatory virtues due to its moderate content of saponosides (Bruneton, 2009). As for the coumarins detected, they give it anti-carcinogenic and ant-mutagenic properties (Ferguson, 2001).

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Phenolic groups content

The phenolic groups content of *P. tuber-regium* are shown in Table 3.

Table 3. Phenolic compounds content of de *P. tuber-regium* extracts

Part of the fungus	Extraits	Total phenolic content (GAE/mg)	Total flavonoid content (EQ/mg)	Total tannin content (TAE/mg)	Total proanthocyanidin content (APE/mg)
sclerotum	AQ	1148,93 ± 1230,27	337,23 ± 0,88	187,65 ± 54,75	1086 ± 21,18
	HE	1904,56 ± 1087,79	236,23 ± 33,26	400,31 ± 57,74	979,47 ± 360,39
Carpophore	AQ	1742,81 ± 454,05	325,15 ± 29,27	393,46 ± 49,63	834,36 ± 281,26
	HE	1129,56 ± 254,82	130,84 ± 40,36	398,28 ± 47,43	705,11 ± 363,1

AQ : Aqueous ; HE : Hydroethanolic

The dosage of phenolic compounds carried out on the aqueous and hydro-ethanolic extracts (Table 3) confirmed the richness of this fungus in total polyphenols and proanthocyanidins, as well as its deficiency in flavonoids and tannins. These data confirm the anticarcinogenic (Fantini *et al*., 2015; Ramos, 2008), antioxidant, anti-allergic and antiplasmodial properties of the fungus studied.

The pharmacological properties of *P. tuber-regium* thus identified confirm the legendary importance of this species in certain countries of tropical Africa such as Gabon (Mengue-Eyi, 2014; Eyi-Ndong *et al*., 2020), Nigeria (Walleyn & Rammeloo, 1994; Wakefield, 1914) and Zanzibar (Hennings, 1895). This essentially therapeutic interest of *P. tuber-regium* is certainly linked to the physicochemical composition of its sclerotia. According to Walleyn and Rammeloo (1994), a chromatographic analysis of sclerotia in Nigeria revealed the presence of essential elements for good health, such as carbohydrates—glucose (fuel of cells and main actor in blood sugar regulation), fructose, mannose, galactose, maltose, and sucrose—inositol (group B vitamin involved in growth control and preventing the proliferation of cancer cells), cholesterol, palmitic acid, oleic acid and stearic acid. Also, analysis of the ash revealed the presence of 0.271% potassium (which directs the hydration of the body's cells) and 0.0039% sodium (which maintains the acid-base balance of the blood and the isotonicity of the cells).

CONCLUSION

The present study based on a chemical screening allowed not only the highlighting of numerous bioactive compounds contained in the studied sample, but also the prediction of the therapeutic properties of *P. tuber-regium*. The results of the chemical screening supported by the determination of the phenolic compounds of these extracts showed that it contains phenolic compounds in varying amounts depending on the solvents used. The presence of these compounds makes it possible to predict anesthetic, analgesic, anticancer, antiallergic, antibacterial, anti-inflammatory, antimutagenic, antiplasmodial, vasodilator and especially

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antioxidant properties, which makes this fungus a potential remedy for common diseases such as malaria, bacterial infections, various inflammations and even some forms of cancer.

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