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CHEMICAL COMPOSITION AND ANTICANCER ACTIVITY OF DALDINIA CONCENTRICA (XYLARIACEAE)

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ABSTRACT

The mycochemical study of hexanic (EH), ethyl acetate (EE), and ethanolic extracts (EET) of *Daldinia concentrica* by thin layer chromatography revealed the presence of terpenes, sterols and coumarins. The presence of flavonoids and quinones has been mentioned only in the ethyl acetate and ethanolic extracts. In vitro bioassays performed on the CA 431 cell line, showed the anticancer activity of the extracts with a greater efficiency for the ethanolic extract (IC50 = 0.03 mg / mL), which seems to depend on the coexiting conjugated secondary metabolites detected.

KEYWORDS: *Daldinia concentrica*, chemical composition, anticancer activity.

INTRODUCTION

Daldinia concentrica is a non-edible fungus from the Ivorian pharmacopoeia that belongs to the family Xylariaceae. It is widespread in humid areas where it is most often found on dead woods.[1] Used as a fire initiator, *D. concentrica* helps relieve the pain caused by cramps.[2] It is also used in the treatment of umbilical hernias.[3] Several pharmacological properties are conceded to it, including neuroprotective,[4] antibacterial, antifungal, antimicrobial and nematocidal effects.[5] The chemical investigations carried out on *D. concentrica* revealed the presence of biomolecules, where the structures of some of them have been elucidated. These

include 6,8-dihydroxy-3-methyl-3,4-dihydroisocoumarin, (22R)-Hydroxylanosta-7,9(11),24-trien-3-one, Ergosterol,[6] 2,6-dihydroxy-butyrophenone, 8-methoxy-1-naphthol and 2-hydroxy-5-methylchromone.[7] However, most of these works have been done on Asian species, while those of Africa in general and Côte d'Ivoire in particular remain unknown, despite their recurrent use in traditional medicine.

The present work is a contribution to the valuation of *Daldinia concentrica*, a mushroom of the Ivorian pharmacopoeia, by studying its chemical composition and evaluating its anticancer potential.

MATERIALS AND METHODS

Plant material

The plant material consists of *Daldinia concentrica* harvested in May 2016 after a mycotherapeutic survey carried out in Adzopé (city of south-east of Côte d'Ivoire located at 6.11 latitude and -3.86 longitude), from naturotherapists then identified at the National Center of Floristics (CNF) located at the University Felix Houphouet-Boigny (Abidjan / Cocody). It was sheared and then dried under air conditioning (18 °C) for one week. The powders obtained, after spraying with an HB1902 mixer, were stored in glass jars.

Preparation of extracts

10 g of *Daldinia concentrica* powder were respectively macerated in 100 mL of hexane, ethyl acetate and 96% ethanol for 24 h at room temperature with constant stirring. The extracts obtained (hexane (EH), ethyl acetate (EE) and ethanol (EET)) were used for studies of the chemical composition and anticancer potential.

Phytochemical Screening on CCM: Phytochemical screening was performed using TLC.[8-9] The solvent systems used as developing are

- Hexane extract (EH): Hexane / ethyl acetate (10 / 1.7, v / v).
- Ethyl acetate (EE) and ethanolic (EET) extracts: Methanol / Ethyl acetate / Acetic acid (5: 5: 1.5, v / v / v).

The revealers applied for the demonstration of secondary metabolites are shown in Table 1.

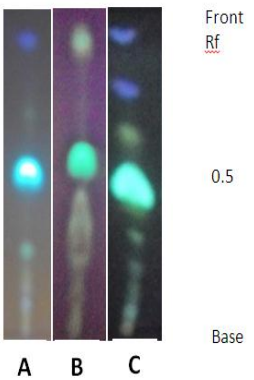
Table 1: Revealers used to detect secondary metabolites.

Secondary metabolite	Revealers
Sterols et terpenes	Libermann-Bürchard
Flavonoïds	Neu reagent, NH_3 from NH_4OH, Godin reagent
Coumarins	KOH (5%), NH_3 from NH_4OH, Dragendorff
Tannins and Phenolic compound	$FeCl_3$ (2%)
alkaloids	Dragendorff
Quinones	NH_3 from NH_4OH

Anticancer activity of the extracts: The cancerous cellular lines of the carcinoma vulvar squamous CA 431 of the University Paris 13 were used to evaluate the cytotoxic activity at different concentrations (0.001, 0.01, 0.1 and 1 mg / mL) of the extracts EH, EE and EET. They were cultured in Dulbecco's modified Eagle medium (DMEM) (Sigma) supplemented with 10% (v/v) fetal calf serum (FCS), 2 mM L-glutamine and 1% (v/v) of Penicillin Streptomycin at 37 °C in an atmosphere humidified with 5% CO_2. The survival of the cells was evaluated using an MTT colorimetric test (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide),[10] modified by Carmichael and collaborators.[11] Viability percentage (PV) was calculated from the following equation: PV = 100 × (Sample Abs / Sample Abs).

RESULTS AND DISCUSSION

Chemical Composition: The chromatographic profiles (FIG. 1) and the frontal ratios of the hexanic extracts of *Daldinia concentrica* and those of the ethyl acetate and ethanol extracts were carried out **(Table 1).**



Mushroom	*Daldinia concentrica*
Fig 1A	Hexanic extract Developing: Hexane / ethyl acetate 10 / 1.7; v / v visualization UV 366 nm
Fig1B	Hexanic extract Developing: Hexane / ethyl acetate 10 / 1.7; v / v Revealer sulfuric vanillin UV 366 nm
Fig1C	Hexanic extract Developing: Hexane / ethyl acetate 10 / 1.7; v / v Revealer : NH_3 at UV 366 nm

Figure 1: Chromatographic fingerprints of the hexanic extract of *Daldinia concentrica.*

With regard to the figure 1, we observe several spots of different colors which translate the presence of the different mycocomposés. Indeed, the sterols appear in green under UV / 366

nm with the reagent Libermann-Bürchard.[12] Thus, the spots of Rf = 0.50; 0.87 of the hexanic extract are sterols (Table 1). These compounds were also identified in ethyl acetate and ethanol extracts at Rfs = 0.70 and 0.95. The terpenes are revealed with sulfuric vanillin in the visible and UV / 366 nm in purple[13] at spots of Rfs = 0.04; 0.15; 0.35 in the hexane extract. As for ethyl acetate and ethanol extracts, these molecules were visualized at different frontal ratios. In addition, the methanol solution of KOH 5% (v / v) was used to demonstrate the coumarins, which are yellow under UV at 366 nm.[14]

Table 2: Compounds identified in the hexane extract of *Daldinia concentrica.*

		Without revealing [a]		Libermann Bürchard [b]		Sulfuric vanillin[c]		KOH à 5% [d]		Possible compounds
	Rf	visible	UV366 nm	visible	UV366nm	visible	UV366nm	visible	UV366 nm	
Hexanic Extract	0.04	-	-	-	-	purple	purple	-	-	Terpene [c]
	0.15	-	blue	-	bleu	purple	purple	-	blue	Terpene [c]
	0.35	-	blue	-	bleu	purple	purple	-	blue	Terpene [c]
	0.40	-	-	-		-	-	yellow	yellow	Coumarin[d]
	0.50	-	blue	green	green	-	blue	green	green	Sterol [b]
	0.61	-	yellow	-	-	-	-	yellow	yellow	Coumarin [d]
	0.71	-	-	-	-	-	-	-	blue	Not identified
	0.87	-	bleu	green	green	-	blue	-	blue	Sterol [b]

They were identified as well in the hexane extract (Rfs = 0.40; 0.61) as in the ethyl acetate and ethanol extracts (Rfs = 0.60; 0.80). On the other hand, quinones and flavonoids have been revealed only in ethyl acetate and ethanol extracts. In fact, the ammonia vapors reveal the quinones in spots of yellow, green and blue colors[15] and the Neu reagent highlights the flavonoids in yellow and brown spots in the visible and these colors intensify or sometimes change under UV / 366 nm.[14] On these bases, the spots of Rfs = 0.55; 0.65 of the ethyl acetate and ethanol extracts are quinones and those of Rfs = 0.32; 0.42 are flavonoids. The presence of these secondary metabolites in *D. concentrica* may justify its use in traditional medicine. However, we found the absence of tannins and alkaloids in all extracts of *D. concentrica*.

Comparing our results with those of Tao and collaborators we noted a similarity in the presence of coumarins, terpenes and sterols.[16] In addition no presence of quinones and flavonoids has been reported in Asian species. This difference could be explained by soil fertility, which is one of the factors in the variation in the chemical composition of plants.[17-18]

Anticancer activity of extracts

Figure 2 represents the percentages of viability (PV) of strain CA 431 with respect to the various extracts of *Daldinia concentrica.*

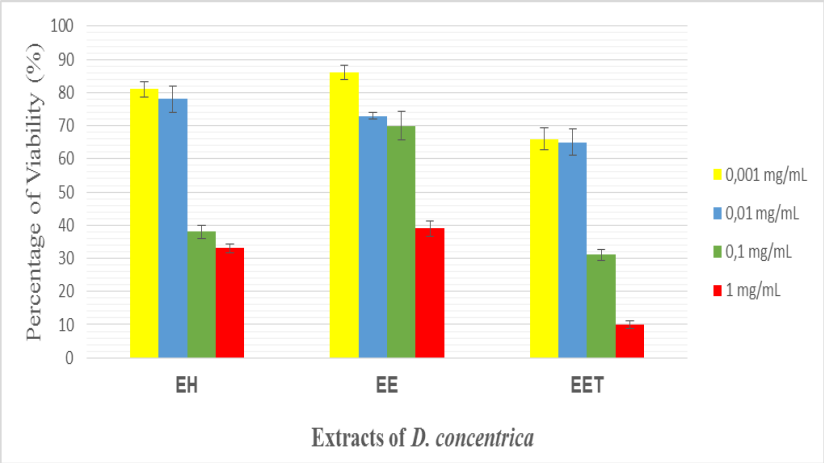


Figure 2: Percentages of viabilities of the CA 431 cancer strain.

The analysis of the histograms in figure 2 indicates viability percentages (PV) ranging from 81 ± 2.28 (0.001 mg/mL) to 33 ± 1.4 (1 mg/mL) for the hexanic extract (EH); 86 ± 2.23 (0.001 mg/mL) at 39 ± 2.27 (1 mg/mL) for the ethyl acetate extract (EE) and 66 ± 3.31 (0.001 mg/mL) at 10 ± 1.2 (1 mg/mL) for ethanol extract (EET). We observe a variation of cell viability percentages as a function of the concentration of the extracts. Also, let us note significant differences between percentages of viability. Moreover, we notice a moderate activity compared to hexanic and ethyl acetate extracts, unlike the ethanolic extract, which has a better cytotoxic activity towards CA 431 cells. Figure 3 shows the cellular variability of the different extracts of *Daldinia concentrica* as a function of the logarithmic concentrations.

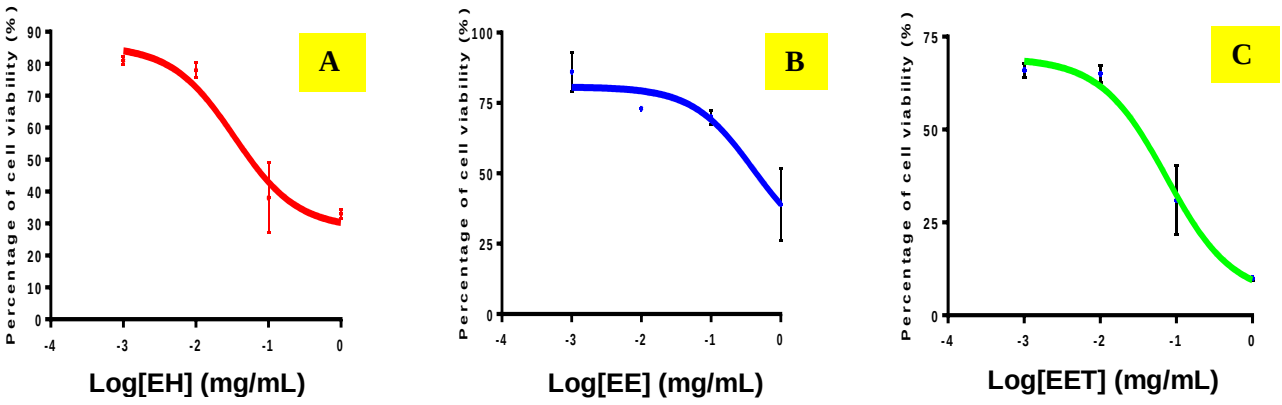


Figure. 3: Cellular Variability of EH (A), EE (B), EET (C) extracts as a function of log concentration.

The recorded IC50 values are 0.051; 0.46 and 0.03 mg/mL respectively for hexanic (EH), ethyl acetate (EE) and ethanolic (EET) extracts. All these values are less than 0.5 mg/mL. This reflects a notable activity in all of the different extracts. In addition, EET is most effective with an IC50 equal to 0.03 mg/mL. This observed activity is due to the combined action of coexisting secondary metabolites detected. Indeed, some studies have indicated the involvement of flavonoids, terpenes and coumarins in the fight against cancer. This is the case of Matsubara and al., where in their investigation showed the anti-angiogenic activity of quercetin and its derivatives.[19] Thus, this potential gives the latter, the ability to prevent the supply of tumors in blood, which could lead to their apoptosis. Flavonoids also have the potential to prevent and stop gastric, cervical, breast, prostate and uterine cancers.[20] With respect to terpenes, a study conducted by Sheeja et al., mentioned the anticancer character of phytol, a diterpene on human breast cells (MCF-7).[21] In addition, potent anti-cancer activity on human tumor lines (KB, DLD-1, NCI-661 and Hela) was detected in vernolide-A, a sesquiterpene.[22] Finally, some studies have reported the anticancer nature of coumarins on prostate and breast cells.[23-24]

CONCLUSION

The present study is a contribution to the valorization of the superior mushrooms of the Ivorian pharmacopoeia, by chemical and biological approaches. For this purpose, the mycochemical screening carried out made it possible to demonstrate the presence of terpenes, sterols, coumarins, flavonoids and quinones in the different extracts of *Daldinia concentrica*. In addition, the pharmacological studies indicated an anticancer potential in vitro of extracts on the CA 431 cancer cell line, with a greater efficiency of the ethanolic extract, with regard to its low IC50 = 0.03 mg/mL. Terpenes, flavonoids and coumarins could be the origin of this property. *Daldinia concentrica* is a therapeutic source. It could be the subject of sustainable exploitation through more in-depth chemical studies such as the determination of its mycocomposites.

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