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Phytochemical Characterization and *In Vitro* Antioxidant, Anticancer, and Antibacterial Effects of Endophytic *Fusarium Tricinctum* Isolated from Root *Aristolochia Paucinervis*

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Abstract: In the present study, the qualitative and quantitative (polyphenols and flavonoids) content present in endophytic *Fusarium tricinctum* isolated from the root of *Aristolochia paucinervis* has been evaluated. The *in vitro* antioxidant, antiproliferative, and antibacterial activities of the organic extracts of *F. tricinctum* were performed using DPPH assay, MTT assay against two cancer cell lines, and agar well diffusion against *Escherichia coli*, *Pseudomonas aeruginosa*, *Rhodococcus equi*, and *Staphylococcus aureus*, respectively. Preliminary phytochemical screening for aqueous and ethanolic extracts revealed the presence of tannins, flavonoids, saponosides, sterols, and triterpenes. The highest polyphenol and flavonoid concentrations (115.52 mg GAE (gallic acid equivalent)/g and 68.21 mg QE (quercetin equivalent)/g extract, respectively) were observed for *F. tricinctum* methanol extract. In parallel, the highest antioxidant capacity of DPPH (IC_{50} = 1.32 mg/mL) was observed for the same extract (methanolic extract). The antibacterial activity showed sensitive antibacterial action against *Rhodococcus* (GK1) at 10 mg/mL (1.9 mm). Moreover, the anticancer activity showed high cytotoxic activity of dichloromethane and methanol extracts against VERO tumor cell lines (IC_{50} = 62.5 ± 36.13 µg/mL, IC_{50} = 31.25 ± 74.43 µg/mL, respectively). The finding of this work showed that dichloromethane, methanol, and hexane extracts of endophytic *F. tricinctum* could constitute a new source of antibacterial, antioxidant, and anticancer properties. However, further investigations should be conducted to identify and isolate the main compounds of endophytic *F. tricinctum* and investigate their biological properties.

Keywords: *Fusarium tricinctum*; preliminary screening; phenolic content; antioxidant; antibacterial; anticancer.

1. Introduction

Natural products are used traditionally to treat diseases including diabetes, Alzheimer's, lithiasis, cancer, diabetes, and parasitic diseases [1-6]. Currently, 13% of naturally derived products used in pharmaceutical companies originate from microorganisms, such as endophytic fungi [7]. These last produce secondary metabolites and present a promising source of novel bioactive compounds [8,9]. Several investigators reported the biological activity of extracts and bioactive compounds from endophytic fungi, offering promising potential for new drug discovery [10-12].

Endophytic fungi are microorganisms that colonize inter- and intracellular compartments of plant tissues with no adverse effect on the host [13] and protect the host against biotic and abiotic stresses [14]. This protection is due to the production of complexity and structure diversity of their secondary metabolites. Recently, there has been an increasing interest in using endophytic fungi as a source of novel natural bioactive metabolites for exploitation in the agriculture industry, medicine, pharmaceuticals, and cosmetics [15-18]. Indeed, various studies showed pharmacological proprieties of endophytic fungi such as anticancer, antiviral, insecticidal, antidiabetic, antibacterial, antiangiogenic, and antioxidant activities [14,19-23] due to the presence of natural compounds discovered from fungi species such as ambuic acid, torreyanic acid, colutellin A, and cryptocin [15,24]. For example, taxol® was a natural bioactive compound that proved its effect as a blockbuster anticancer drug [15]. These pharmacological findings have been used as a primer to continue their exploitation in research and the isolation of natural bioactive compounds to improve human health.

The genus Fusarium colonizes a large variety of plant species [25] and contains more than 70 species, among them Fusarium tricinctum. These lasts are distributed around cosmopolite climates, which contaminate cereals [26] and postharvest fruit roots [27]. Several authors identified bioactive secondary metabolites such as enniatins A1, B, and B1 [28], L-Asparaginase [29], (-)-citreoisocoumarinol [30] fusartricin, and fusarielins [31] from this endophytic fungi. In the present study, total phenolic and flavonoid contents of endophytic Fusarium tricinctum extracts were determined, and their in vitro antioxidant, anticancer, and antibacterial activities were evaluated.

2. Materials and Methods

2.1. Isolation and identification of endophytic F. tricinctum.

Fresh roots of *Aristolochia paucinervis* (Aristolochiaceae) were collected in January 2006 from the mountain of Beni-Mellal, Morocco. Professor A. Boulli from Sciences and Techniques Faculty (Beni-Mellal, Morocco) identified the plante. Voucher specimens have been deposited at the University Mohammed V Agdal, Faculty of Sciences, Rabat, Morocco. The endophytic fungi were isolated from root *A. paucinervis* following the method described by Wätjen et al. [28].

Taxonomic identification of endophytic isolates was carried out using standard identification keys on morphological characters of fungal culture, colony appearance, hyphae, and the characters of the reproductive structure of an endophytic fungus [28].

2.2. Preparation of organic extract of F. tricinctum.

F. tricinctum extracts were obtained by their maceration, separately, in hexane, dichloromethane, and methanol solvents for three days at room temperature and stirring at 70 revolutions/minute. The solvent was then evaporated under reduced pressure; the organic extracts were collected, stored at -12°C until further use, and diluted in methanol to obtain a series of solution concentrations.

2.3. Qualitative analysis.

A preliminary qualitative phytochemical analysis was carried out to identify the phytochemical constituents of plants in methanol and aqueous extracts. The following phytochemicals were tested: starch, saponosides, tannins, flavonoids, anthocyans, coumarins, sterols, and triterpenes using standard methods as reported in the literature [32,33].

2.4. Determination of phenolic content.

2.4.1. Determination of total phenolic content.

The assessment of total polyphenols was performed according to the method of Singleton et al. [34] using the Folin-Ciocalteu as a reagent. On this base, the extracts are diluted to a concentration of 1 mg/mL. An amount of 100 µL of the diluted extract is placed in test tubes, and 500 µL of Folin-Ciocalteu reagent diluted 10 times in distilled water is added. After incubation for 1h at room temperature, 2 mL of sodium carbonate (Na_2CO_3) to 2% is added. The tubes are then shaken and placed in the dark for 30 minutes at room temperature. The same steps were followed to establish a reference range (0 to 100 µg/mL) prepared from an aqueous stock solution of gallic acid (0.5 g/L). The absorbance is measured using a UV-visible spectrophotometer at a wavelength of 760 nm [34]. The absorbance values of each concentration enabled us to plot the calibration curve of gallic acid. The results are expressed in mg of gallic acid equivalents per gram of dry extract (GAE mg/g of dry extract). All manipulations are performed in triplicate.

2.4.2. Determination of total flavonoid content.

Flavonoid contents are measured using aluminum trichloride (AlCl3) as a reagent. 1mL of the extract of F. tricinctum is mixed with 1 mL of the solution of aluminum trichloride (2%) and 50 µL of acetic acid. The tubes are then gently mixed and incubated in the dark for 40 minutes at room temperature. Under the same conditions, a stock solution of quercetin mass concentration 0.2 g/L was prepared in methanol. From this stock solution, a standard range of concentrations from 0 to 25 mg/mL was prepared. The absorbance was measured in the same spectrophotometer at a wavelength of 415 nm [34]. The obtained absorbance values enabled us to plot the calibration curve of quercetin. The results are expressed in mg of quercetin equivalent per g of dry extract (mg QE/g of dry extract). All manipulations are performed in three trials.

2.5. Antioxidant activity.

The antioxidant activity was determined using the Kubola and Siriamornpun methods, with some modifications. Briefly, 1.8 mL of 0.1 mM DPPH solution is prepared in methanol

and added into 0.2 mL tubes containing extract (methanol or ethanol) at increasing concentrations (0.31 mg/mL - 10 mg/mL for *F. tricinctum* extracts) and are placed away from light at room temperature after agitation by vortex. After 30 min, the absorbance is measured at 517 nm [35]. Ascorbic acid (vitamin C) was used as a positive control, while methanol was used as a negative control. The scavenging activity of DPPH radical is calculated using the following formula (1) :

$$A\% = \frac{(Abs\ control - Abs\ sample)}{Abscontrol} \times 100 \qquad (1)$$

Where Abs control: Absorbance without antioxidants (containing all reagents except the test sample); Abs sample: absorbance with the test sample.

IC_{50} is the concentration of the test sample required to reduce 50% of DPPH radical. IC_{50} was graphically calculated using linear regression plots graphs for percentage inhibition as a function of different concentrations of the tested fractions and standards.

2.6. Cell viability assays.

The *in vitro* cytotoxic effect of the various extracts was evaluated on RD: Embryonal Rhabdomyosarcoma cancerous cell lines (ATCC N°CCL-136) and Vero: Monkey kidney cancerous cell lines (ATCC N°CCL-81), which obtained from the National Institute of Health, Rabat-Morocco. PBMC (Peripheral Blood Mononuclear Cell) isolated and purified from human blood were used as a positive control. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) (GIBCO) supplemented with 10% heat-inactivated fetal calf serum and 1% Penicillin-Spreptomycin mixture. Cultures were maintained at 37°C in a 5% CO_2 and 100% relative humidity atmosphere. The effect of the isolated extracts on cell viability was assessed using the 3-(4,5-dimethylthiazol -2-yl)-2, 5 diphenyltetrazolium bromide (MTT) assay, which measures the metabolic activity of mitochondria [36]. The tests were conducted on the 96-well microplate. Before treatment with extracts, 100 µL medium DMEM (GIBCO) containing 3-4x106 cells/mL were placed in each well containing DMEM (GIBCO) and cultured at 37°C in 5% CO_2/humidified air for 24 h. After 24h incubation and attachment, cells were treated with crude extracts. Exactly from the stock solution (80 mg/mL), each extracted sample was applied in a series of 6 dilutions (final concentrations ranging from 12.5 µg/mL to 400 µg/mL) in Dimethyl sulfoxide (DMSO 1%). Test solution (100 µL) was added in decreasing concentrations in duplicate. Microplates were then incubated for 48 h at 37°C in air condition of 5% CO_2. After, 20 µL MTT solution (5 mg/mL) (SIGMA) was added to the wells containing cells. The cells were incubated for 4 - 5 h at 37°C in 5% CO_2. Tetrazolium salts are cleaved to formazen dye by cellular enzyme (only in the viable cells). A solubilization solution (Isopropanol/hydrochloric acid) is added to dissolve the insoluble purple formazen product into a coloration solution. The absorbance was measured at 545 nm using a microplate reader (Statfax 2100).

2.7. Antibacterial activity.

We used agar well diffusion methods to determine the antimicrobial activities of our extracts. Six species of bacteria were studied: 4 species of Gram-positive bacteria and two species of Gram-negative bacteria (Table 1). The test samples were first dissolved in dimethylsulfoxide (1%), which, thus, did not affect the microbial growth. Briefly, 8mL of medium agar was poured into sterile petri plates. After solidification, 100 µL of fresh cultures

of each bacterial (one microorganism per Petri dish) were swabbed on the respective plates. Then, 50 µL of extracts were placed in wells previously punched over the agar plates using a sterile Pasteur pipette at various concentrations (10 mg/mL; 20 mg/mL; 30 mg/mL; 40 mg/mL; 50 mg/mL). All Petri plates were then incubated at 37°C for 24 h. The diameters of inhibition zones were measured in millimeters. Moreover, commercially available antibiotics were used to compare with the antimicrobial activities of our plant extracts on all bacterial species studied. The antibiotic discs of Ampicillin were placed on the surface of the plates. DMSO 1% was used as a negative control. The plates were incubated at 37°C for 24h, and after incubation, the diameter of the inhibition zones was measured in mm and recorded [37].

Table 1. Bacterial species used and their origins.

Bacterial species	Origins
Escherichia coli	Food Microbiology Laboratory, UCL, Belgium (MBLA)
Pseudomonas aeruginosa IH	Rabat Institute of Hygiene
Staphylococcus aureus	German Collection of DSM Microorganisms
Rhodococcus equi (GK1, GK2, GK3)	Laboratory of Human Pathologies Biology, UM5, Rabat

3. Results and Discussion

3.1. Qualitative analysis.

Tannins, flavonoids, sterols, triterpenes, and saponosides were identified in *F. tricinctum*, while the absence of coumarins, anthocyans, and starch was reported in Table 2. To the best of our knowledge, this is the first qualitative report on the analysis of *F. tricinctum* extract from *A. paucinervis* roots in Morocco.

Table 2. Preliminary qualitative phytochemical analysis of *F. tricinctum* methanol and aqueous extracts.

Plants constituents	Methanol extract	Aqueous extract
Starch		-
Saponosides		++
Tannins	++	+
Flavonoids	++	
Coumarins	-	
Anthocyans	-	
Sterols	+	
Triterpenes	+	

+++: highly present, ++: moderately present, +: Low, -: absent.

3.2. Phenolic content.

The contents of total polyphenols were determined by colorimetric assay using the Folin-Ciocalteu reagent. The results are expressed in mg GAE/g extract by referring to the previously established calibration curve with gallic acid (polyphenol correlation: $R^2 = 0.998$). Total flavonoids are determined by the trichloride reagent aluminum, and the results are expressed in mg QE/g extract based on the established calibration curve with quercetin (flavonoid correlation: $R^2 = 0.985$). The total phenolic and flavonoid contents in the dichloromethane, methanol, and hexane extract from *F. tricinctum* are shown in Table 3.

Table 3. Total polyphenols and flavonoids content of *F. tricinctum* extracts.

	F. tricinctum	
	Polyphenols (mg of GAE/g of extract)	**Flavonoids (mg of QE/g of extract)**
Dichloromethane extract	98.24 ± 0.55	56.23 ± 0.71
Methanol extract	115.52 ± 1.68	68.21 ± 0.59
Hexane extract	66.87 ± 8.74	35.552 ± 0.64

GAE: Gallic acid equivalents; QE: Quercetin equivalent

The total phenolic content of various extracts of *F. tricinctum* from root parts varied widely between 35.55 to 115.52 mg GAE/g extract (Table 3). Methanolic extract demonstrated higher total polyphenols content (115.52 ± 1.68 mg GAE/g), followed by dichloromethane and hexane extracts which were 98.24 ± 0.55 mg GAE/g, and 66.87 ± 8.74 mg GAE/g, respectively. Results of flavonoids contents show that methanolic extract resulted in a high value (68.21 ± 0.59 mg QE/g) compared with dichloromethane and hexane extract (56.23 ± 0.71 mg QE/g and 35.552 ± 0.64 mg QE/g, respectively).

The dosage of polyphenol and flavonoid contents of the methanol extract revealed that *F. tricinctum* had a higher value compared to other studies. Recently, Mollaei et al. worked on the *F. tricinctum* isolated from the roots of *L. officinales*, and they reported the total phenolics and flavonoids content in the methanolic extract as 39.1 mg GAE/g and < 5 mg QE/g, respectively [38]. At the same time, Pan and co-workers evaluated the total phenolic content in different extracts, including petroleum ether, ethyl acetate, n-butyl alcohol, and ethanol of *F. tricinctum* isolated from *Fritillaria unibracteata* var. *wabuensis* (from Beijing, China) and reported a high total phenolic content in n-butyl alcohol [39]. These results revealed that the methanolic extract of *F. tricinctum* is a promising source for natural fungi source of polyphenols and flavonoid compounds.

3.3. Antioxidant activity

The antioxidant activity of fungal endophytes was increasing due to their role in helping plant hosts neutralize ROS and in decreasing diseases of human health, including Alzheimer's disease, diabetes, cardiovascular disorder, and cancer [39, 40]. In our study, antioxidant activity was performed using *in vitro* DPPH methods. The results are expressed in acid ascorbic equivalent. Figure 1 illustrates the DPPH radical scavenging activity of different extracts of *F. tricinctum* at various concentrations. An increase in the concentration of the fungal extract was associated with increase of scavenging activity. The IC_{50} value of methanol and dichloromethane extract was found to be 1.32 mg/mL and 8.26 mg/mL, respectively (Table 4). Acid ascorbic was used as positive control and presented a high IC_{50} (27.20 ± 0.17 µg/mL), which can be explained by the use of pure molecule compared to our extract. This result showed that *F. tricinctum* extracts contained a high amount of radical scavenging compounds with proton-donating ability, concluding the possibility of exploring this fungus as a source of antioxidant agents.

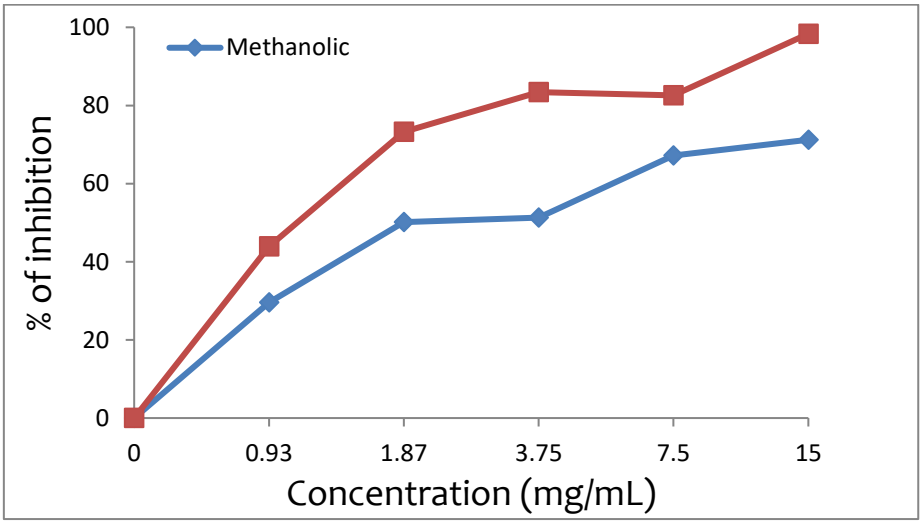

Figure 1. DPPH radical scavenging activity of extracts from *F. tricinctum* organic extract.

Table 4. Free radical (DPPH) scavenging activity of *F. tricinctum* organic extract.

	Extracts	IC_{50} (mg/mL)
F. tricinctum	Methanol	1.32
	Hexane	6.58
	Dichloromethane	8.26

A previous study studied the effect of various organic extracts of *F. tricinctum* to scavenge free radicals using a DPPH assay. In India, Vasundhara et al. showed a dose-dependent scavenging activity and reported an IC_{50} = 482 µg/mL using *F. tricinctum* ethyl acetate extract [14]. An *in vitro* investigation carried out by Pan et al. showed an IC_{50} = 696.12 µg/mL for n-butyl alcohol fraction of *F. tricinctum* collected from the bulbs of *Fritillaria unibracteata* var. *wabuensis* [39]. A recent Iranian study reported a high and significant DPPH scavenging activity of *F. tricinctum* methanolic extract isolated from the root of *Lithospermum officinale* with an IC_{50} = 29.9 µg/mL [38]. Numerous factors influence the potential of antioxidant activity and the reduction kinetic, in particular, the reaction conditions (time, Antioxidant/DPPH ratio, type of solvents, pH), the phenolic profile, and the extraction method. The antioxidant activity of fungus extract was related to the highly important charges of phenolic compounds, viz. polyphenols, and flavonoids. Indeed, recent research reported a highly significant correlation between phenolic compounds and antioxidant effects and with the synergy effect with other compounds [38, 23]. The mechanism of action of phenolic compounds was through hydroxyl groups, and for flavonoids was through scavenging or chelation [41].

3.4. Cytotoxicity effects.

Two tumor cell lines (RD and Vero), at different concentrations (15.12 µg/mL to 1000 µg/mL), have been used to test the cytotoxic potential of organic extracts from *F. tricinctum.* The MTT assay indicates that the extracts revealed different cytotoxic activities in the two cancer cell lines investigated. The antiproliferative effects of extracts have been compared to control (PBMC). Table 5 summarizes the obtained results. In general, Figures 2, 3, and 4 illustrated a dose-dependent decrease in the survival of the two cancerous cell lines. In our study, we concluded that hexane extract exhibited potent and selective antiproliferative activities against RD and VERO cell lines with IC_{50} = 125 ± 79.295 µg/mL and IC_{50} = 15.124 ± 75.39 µg/mL, respectively.

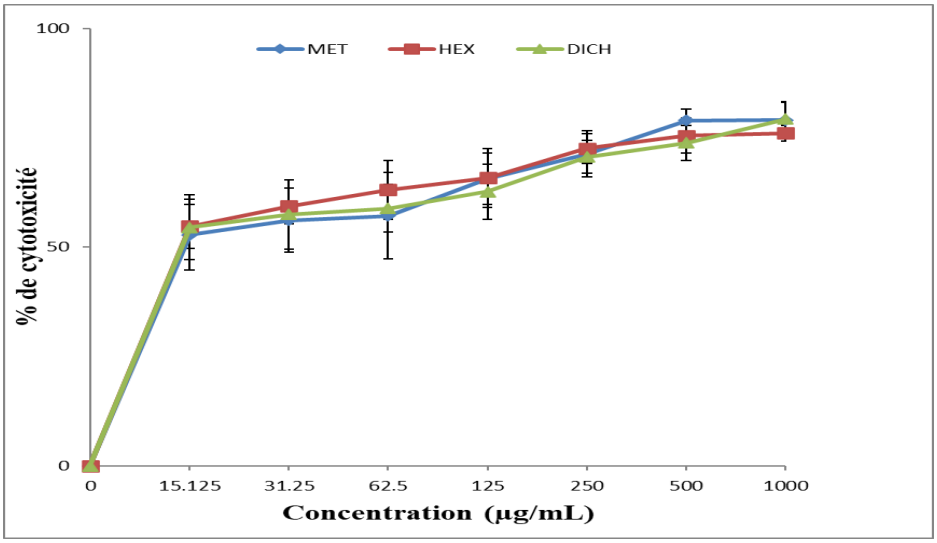

Figure 2. Effect of *F. tricinctum* extracts on the growth of VC cancer cell.

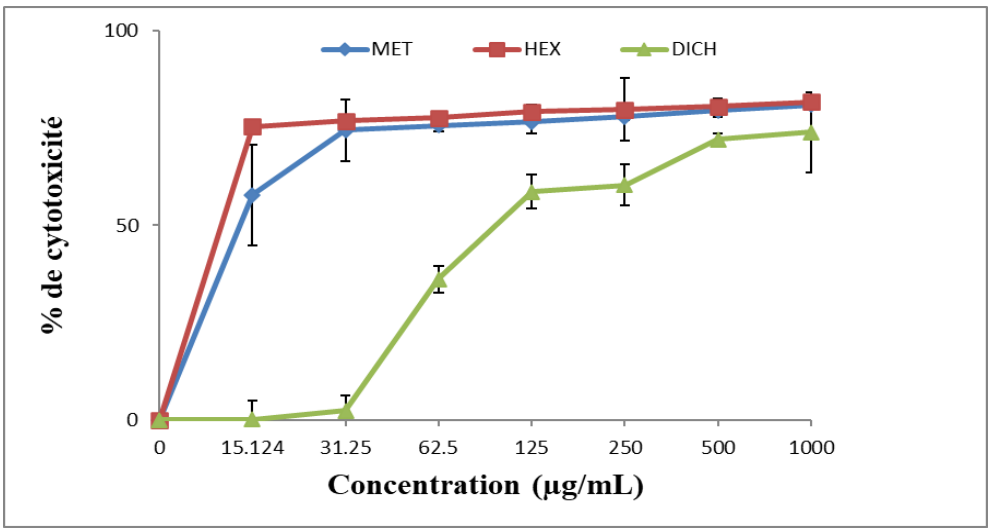

Figure 3. Effect of *F. tricinctum* extracts on the growth of RD cancer cell.

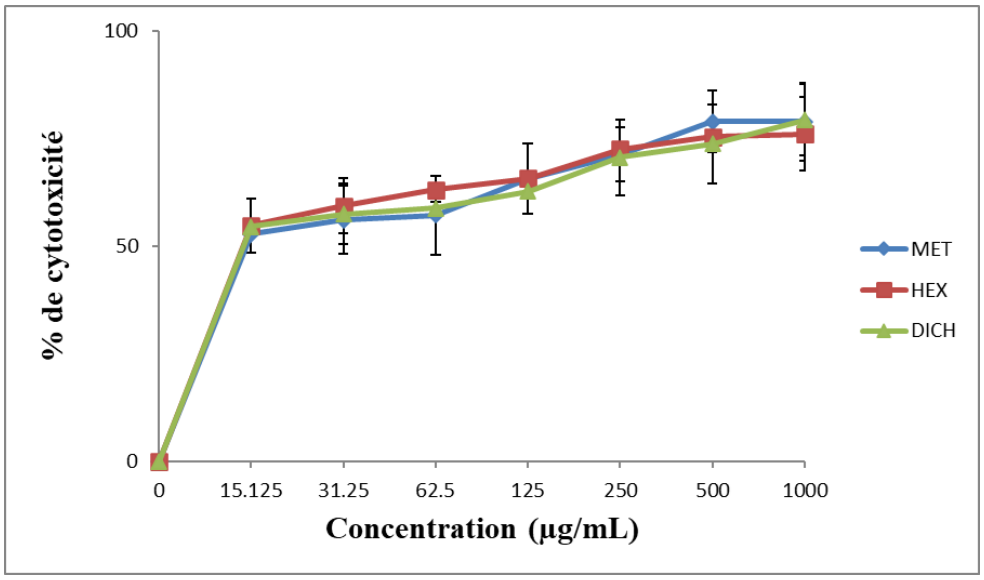

Figure 4. Effect of *F. tricinctum* extracts on the growth of PBMC cancer cell.

Table 5. Inhibition concentration (IC_{50} in µg/mL) values from *F. tricinctum* towards RD, VERO cancerous cell lines, and PBMC (control) as determined by the MTT assay.

		F. tricinctum		
		Hexane	**Dichloromethane**	**Methanol**
IC_{50} (µg/mL)	RD	125 ± 79.295	250 ± 60.365	250 ± 60.365
	PBMC	125 ± 65.685	125.62 ± 62.68	250 ± 70.61
	VERO	15.124 ± 75.39	62.5 ± 36.135	31.25 ± 74.43

In parallel, dichloromethane and methanol extracts present selective antiproliferative against VERO tumor cell lines (IC_{50} = 62.5 ± 36.135 µg/mL, IC_{50} = 31.25 ± 74.43 µg/mL, respectively), while no significant effect has been reported for RD tumor cell lines. The cytotoxic activity of hexane and methanol extracts showed low cytotoxicity against normal cells (PBMC) (IC_{50} = 125.62 ± 62.68 µg/mL and IC_{50} = 250 ± 70.61 µg/mL, respectively). The activity of *F. tricinctum* extracts against various cancer cell lines has been reported by several researchers [14,28]. An *in vitro* research carried out by Vasundhara et al. investigated the effect of *F. tricinctum* crude extract on other cancer cell lines, namely, the human breast cancer cell line (MCF-7) and human cervical cancer cell line (HeLa). From this research, they reported an IC_{50} = 225 ± 26 µg/ml and 220 ± 18 µg/ml for MCF-7 and HeLa, respectively, using *F.*

tricinctum crude extract isolated from the bark of the Himalayan yew [14]. Furthermore, bioactive compounds, enniatins A1, B, and B2, isolated from *F. tricinctum*, an endophyte of *A. paucinervis*, exhibited high toxicicity against H4IIE cells (IC_{50}= 1–2.5 μM), and moderate cytotoxic activity against Human hepatocarcinoma (HepG2) and C6 cells (IC_{50} =10-25 μM). The authors reported that enniatins increased caspase 3/7 activity as markers of apoptotic cell death [28]. From this research, *F. tricinctum* exhibited potent cytotoxic activity against cell lines due to the presence of different compounds that represent several chemical classes.

Various authors reported the effect of other endophytic *Fusarium sp*. In particular, those of *F. solani* isolated from *Datura metel* L. and *F. oxysporum* isolated from *Smallanthus sonchifolius* [42,43]. *F. oxysporum* has been used to evaluate their *in vitro* cytotoxic activity on Human tumor cell lines MDA-MB435 (melanoma), HCT-8 (colon), and SF295 (brain). At 50 μg/mL, *F. oxysporum* crude extract exhibited high activity against HCT-8 cells (84.82%) and moderate effect against SF295 cells (67.02%) [42]. In 2014, Kuriakose and co-workers (2014) studied the effect of *F. solani* ethyl acetate extract on HepG2, HeLa, MCF-7, human ovarian carcinoma (OVCAR-3), and human prostate cancer (PC-3). Ethyl acetate extract of *F. solani* was most effective against HeLa cell lines (50% cell inhibition using 2.5 μg/mL) [43]. Several signaling pathways have been reported, including induced stress in tumor cells, membrane potential modification, cell cycle arrest, and induced apoptosis [42-44].

3.5. Antibacterial activity.

Gram-positive and Gram-negative strains have been evaluated using *F. tricinctum* hexane, dichloromethane, and methanol extracts. Each extract has a different degree of growth inhibition using agar well diffusion methods, and the inhibition of bacteria growth was measured in millimeters (Table 6). The results of agar well diffusion showed a variation of inhibition diameters, which varies according to the bacteria and extract used.

At 10 mg/mL, *Rhodococcus* (GK1) showed high sensitivity against methanol extract with diameters inhibition of 1.9 mm, while hexane and dichloromethane exhibited an inhibition of 1.87, and 1.55 mm, respectively, at 50 mg/mL (Table 6). F. tricinctum hexane extract at 10 mg/mL exhibited moderate sensitivity against *S. aureus* with diameters inhibition 1.55 mm. At the same time, a low activity inhibition effect has been observed for *Rhodococcus* (GK3) and *P. aeruginosa*.

A recent study investigated the *in vitro* antibacterial activity of *Fusarium* sp. isolated from *Sceletium tortuosum* against *Enterococcus faecalis*, *Enterococcus gallinarum*, and *Bacillus cereus* [22]. Manganyi et al. reported the highest inhibition effect against *B. cereus*, *E. faecium*, and *E. coli* (9 mm, 9 mm, and 8mm, respectively). A previous study reported the strongest antimicrobial activity against *P. aeruginosa*, *S. aureus*, and *E. coli* using other endophytics belonging to the genus of *Fusarium* sp. [45,46]. From these researches, *Fusarium* sp. possessed bioactive compounds with a potential antibacterial activity that act in different mechanism pathways, including increasing cell membrane permeability and modifying gene expression [45,47].

Table 6. The antibacterial activity of *F. tricinctum* extracts against *Rhodococcus* sp., *S. aureus*, *E. coli*, and *P. aeruginosa* was determined by agar well diffusion (Ø mm).

	Extract	**Concentrations**	**Zone of inhibition (Ø mm)**					
			GK1 +	***GK2***	***GK3***	***S. aureus***	***E. coli***	***P. aeruginosa***
F. tricinctum		10 mg/mL	0	0	0	1.5	0.95	0
		20 mg/mL	0	0.55	0	1.6	0.85	0.65

Extract	Concentrations	Zone of inhibition (Ø mm)					
		GK1 +	*GK2*	*GK3*	*S. aureus*	*E. coli*	*P. aeruginosa*
Dichloromethane	30 mg/mL	1	0.6	0	1.25	0	0.95
	40 mg/mL	0.35	0.45	0	0.55	0	0
	50 mg/mL	1.55	0.85	0	1.65	0	0
Hexane	10 mg/mL	0.45	0.6	0.7	1.55	1.4	0.5
	20 mg/mL	0	1.6	0.2	1.05	1.2	0.85
	30 mg/mL	1.5	0.7	1	0.75		0
	40 mg/mL	0.95	0	0.36	1.2	0.2	0
	50 mg/mL	0.875	0	0	1.25	1.3	0
Methane	10 mg/mL	1.9	1.2	1.2	0.15	1.1	0.75
	20 mg/mL	1.75	1	0	1	1.05	0
	30 mg/mL	0.75	1.4	1.4	0.8	1.2	0
	40 mg/mL	0	0	0	0.8	0	0
	50 mg/mL	0	0	0	0.65	0	0

4. Conclusions

F. tricinctum dichloromethane and methanol extracts present selective antiproliferative against VERO tumor cell lines. In parallel, methanol extract showed a sensitive effect against *Rhodococcus* (GK1) (1.9 mm). This effect can be attributed to the high content of polyphenols and flavonoïds content in dichloromethane (98.24 mg GAE/g and 115.52 mg GAE/g) and methanol (68.21 mg QE/g and 56.23 mg QE/g) extracts. These findings suggested that *F. tricinctum* from *A. paucinervis* produced bioactive compounds that could serve as a potential source for the isolation of novel anticancer and antibacterial agents. However, further investigation should be conducted to isolate and identify the bioactive compound responsible for these activities, which can help identify new compounds of pharmaceutical importance.

Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: E.A.E.I, Y.B., T.M.; data collection: E.A.E.I, A.K.; analysis and interpretation of results: A.B., F.Z.; draft manuscript preparation: E.A.E.I, M.E.F. All authors reviewed the results and approved the final version of the manuscript.

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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