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Antioxidant and cytotoxic properties of *Combretum paniculatum* and *Fadogia agrestis* extracts on prostate and cervical cancers cell lines

Boukaré OUEDRAOGO[1,2], Bagora BAYALA[1,2,3*], Boukaré KABORÉ[4], Pengdwendé Fabienne Ingrid ZONGO[1,2], Estelle OUEDRAOGO[1,2], Liz Laetitia COULIBALY[1,2], Marc Donald Wilfried ADICO[1,2], Florencia Wendkuuni DJIGMA[1,2], Albert YONLI[2], Moumouni KOALA[4] and Jacques SIMPORE[1,2]

[1]*Laboratoire de Biologie Moléculaire et de Génétique (LABIOGENE). Département de Biochimie-Microbiologie. Université Joseph KI-ZERBO. 03 BP 7021. Ouagadougou 03. Burkina Faso.*
[2] *Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA). 01 BP 216 Ouagadougou 01. Burkina Faso.*
[3] *Ecole Normale Supérieure. BP 376 Koudougou. Burkina Faso.*
[4] *Laboratoire de Recherche-Développement de Phytomédicaments et Médicaments, Institut de Recherche en Sciences de la santé (IRSS), Centre National de la Recherche Scientifique et Technologique (CNRST), 03 BP 7047 Ouagadougou 03, Burkina Faso.*
[*] *Corresponding author; E-mail: b.bayala@labiogene.org ; bayalabagora@gmail.com; Tel: +226 76 69 61 66*

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ABSTRACT

The medicinal plants used in Burkina Faso are essential source of research for bioactive compounds against prostate and cervical cancer, which are major health problems in Burkina Faso and worldwide. This was done by hydro-acetic maceration of the powder of the leafy branches of *Combretum paniculatum* (*C. paniculatum*) and *Fadogia agrestis* (*F. agrestis*). These extracts were then fractionated using petroleum ether, dichloromethane, ethyl acetate, and n-butanol. A phytochemical screening of these extracts was performed using High-Performance Thin Layer Chromatography (HPTLC). Antioxidant activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl ($DPPH^{\blacksquare}$) and 2'-Azino-Bis-3-Ethylbenzothiazoline-6-Sulfonic Acid ($ABTS^{\blacksquare+}$). The extracts cytotoxicity was assessed by the MTT (Mitochondrial Tetrazolium Test) method on the LNCaP cell lines of prostate cancer and HeLa of cervical cancer. Phytochemical analysis of extracts and fractions revealed the presence of tannins, flavonoids, and steroids in these plants. For antioxidant activity, the ethyl acetate fraction of *F. agrestis* and the n-butanol fraction of *C. paniculatum* gave the best inhibition of $DPPH^{\blacksquare}$ with IC50 values of 0.246 ± 0.045 and 0.097 ± 0.008 µg extract/mL, respectively. Using the $ABTS^{\blacksquare+}$ method, the ethyl acetate fractions of *C. paniculatum* (1.1009 ± 0.0181 µM ET/g) and *F. agrestis* (0.0316 ± 0.0007 µM ET/g) showed the best activity. In addition, the fractions of dichloromethane (DCM) of *F. agrestis* (IC50 = 24.69 ± 9.51 µg/ml) and ethyl acetate (AE) of *F. agrestis* (IC50 = 129.9 ± 6.49 µg/ml) had better activity, respectively on the prostate and cervical cancer LNCaP lines. These properties could justify the use of these plants in traditional medicine.

Keywords: *Combretum paniculatum*, *Fadogia agrestis*, cancer, antioxidant and antiproliferative.

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INTRODUCTION

Cancer is a major public health problem, with approximately 17.5 million new cases and 8.7 million deaths worldwide in 2015 (Ogbole et al., 2017). Cancer deaths are projected to increase to 21 million by 2030 worldwide (Sylla and Wild, 2012; Iqbal et al., 2017). Africa, the continent least prepared to cope with this increase in cancer incidence and mortality, records nearly 70% of these deaths annually. Cervical cancer caused by the human papilloma virus (HPV) ranks fourth globally, with over 500,000 cases yearly (Sylla and Wild, 2012). It is the second most common cancer in Africa in women (Kagoné et al., 2022). In Burkina Faso, the prevalence rate is 12/100,000 women, with 838 yearly deaths (Zeba et al., 2019). Prostate cancer is generally more common in men over the age of 50. In Burkina Faso, there are approximately 4,305 new cases of prostate cancer due to increased screening (Kadanga et al., 2022). It is the second leading cause of cancer mortality in older men, with nearly 60% of deaths per year (Bayala et al., 2020a).

To limit the development of cancer, anti-cancer drugs such as camptothecin, podophyllotoxin, vinblastine, vincristine, and paclitaxel have been developed by pharmaceutical companies but remain inaccessible to African populations (Henkin et al., 2018; Bouhaous et al., 2022). Also, increased resistance to these anticancer drugs has forced researchers to turn to natural products of plant and marine origin (Sawadogo et al., 2012). In developing countries, people generally use traditional herbal therapies (Tine et al., 2019; Taïbi et al., 2020). Herbal medicine is, therefore, one of the greatest solutions to the health problems of populations in these developing countries. In West Africa, some plants have already been studied for their antiproliferative properties on cancer cells (Ayim et al., 2007; Adewole et al., 2020; Bayala et al., 2020b; Ambali et al., 2021; Kola et al., 2022; Coulibaly et al., 2023).

To contribute to the research of active substances for the management of prostate and cervical cancers, this study was initiated on *Combretum paniculatum* (*Combretaceae*) and *Fadogia agrestis* (*Rubiaceae*) plants used in traditional medicine in Burkina Faso, in order to participate in the search for a solution to this cancer scourge through the evaluation of antioxidant activity by the $DPPH^{\bullet}$ methods and $ABTS^{\bullet+}$ and cytotoxicity *in vitro* through the MTT test (Mitochondrial Tetrazolium Test) on cell lines of prostate and cervical cancer in culture of extracts of *Combretum paniculatum* and *Fadogia agrestis* from Burkina Faso.

MATERIALS AND METHODS

Plant material

The leaves of *C. paniculatum* and *F. agrestis* were harvested respectively at Louda in North-Central Burkina Faso (GPS coordinates 12°58'7.06'N / 01°5'16.98'W and in the Laba Forest in West-Central Burkina Faso (GPS coordinates 11°45'55.83'N / 02°41'7.94'W). The plants were identified by a botanist of the Joseph KI-ZERBO University InfoBio Laboratory and specimens were deposited under references 17900/6909 and 17901/6910. For this study, the freshly harvested plant material was dried at room temperature in the laboratory, away from light and sunlight.

Extraction and Fractionation

A 30 g mass of vegetable powder from each sample was macerated in 300 mL of a water-acetone solvent system (80:20, v/v) under mechanical agitation for 48 hours at room temperature. After filtration, the filtrate is evaporated under reduced pressure at 40°C. The aqueous extract is then used for fractionation. The filtrate is subjected to a liquid-liquid partition successively with petroleum ether, dichloromethane, ethyl acetate, and n-butanol.

Phytochemical screening

Phytochemical screening was performed by high-performance thin-layer chromatography (HPTLC). The solutions of the different plant fractions were screened on

glass HPTLC plates (20 cm x 10 cm) made of silica gel type 60 F_{254} (Merck, Darmstadt, Germany). Approximately 2 µL of each extract was deposited per strip (8mm) using a semi-automatic TLC Sampler (CAMAG, Linomat V, Switzerland).

Flavonoids

After deposition, the HPTLC plate was placed in a development vessel containing the solvent system: Ethyl Acetate-Formic Acid-Acetic Acid-Water (100:11:11:26, v/v/v/v). The plate was removed from the tank, dried, and heated to 105°C for 5 minutes after spraying with the Neu reagent. Flavonoids were revealed with a UV lamp at 366 nm (Wagner and Bladt, 1996).

Tannins

After deposition, the HPTLC plate was placed in a development vessel containing the solvent system: Ethyl Acetate-Formic Acid-Acetic Acid-Water (100:11:11:26, v/v/v/v). After elution, the plate was sprayed with $FeCl_3$ reagent 2% and observed in visible light (Wagner and Bladt, 1996).

Sterols and triterpenes

After deposition, the HPTLC plate was placed in a development vessel containing the solvent system: N-Hexane-Ethyl Acetate-Methanol-Water (3.5:11.9:1.41.6, v/v/v/v). The chromatographic plate was sprayed with sulfuric vanillin and heated to 105°C for 5 minutes. The observation was made in visible light (Wagner and Bladt, 1996).

Antioxydant activity

Antiradical activity by the DPPH method

The method used is that of Brand-Williams et al. (1995) with some modifications. The DPPH solution is prepared by solubilizing 30 mg of DPPH in 100 mL of methanol. Approximately 100 µL of the DPPH solution was mixed with 100 µL of extract. After incubation for 30 min at room temperature, the absorbance of the reaction mixture was read at 517 nm. Gallic acid and quercetin were used as standards, and the percentage of DPPH trapping activity of each extract was calculated as follows:

%Inhibition = [(As-(Ae-Ab)) /As] x 100

As: Absorbance of DPPH solution; **Ae**: Sample absorbance; **Ab**: Absorbance of white.

ABTS free radical scavenging activity

The trapping activity of the $ABTS^{\blacksquare+}$ radical was measured according to the protocol of Re et al. (1999). The concentration of compounds having a reductive effect on the ABTS radical ($ABTS^{\blacksquare+}$) (antioxidant) is calculated by the formula:

Concentration (µMET/g) **= (Cx D) / Ci**

C: concentration of the sample; **D**: dilution factor; **Ci**: concentration of the stock solution

Cytotoxicity evaluation

Study cell lines

LNCaP and HeLa cell lines were supplied to CERBA/LABIOGENE by the GReD Laboratory, UMR CNRS 6293-Clermont Université INSERM U1103, 24 avenue des Landais, BP80026, 24 avenue des Landais, F-63177, France. LNCaP cells have elongated star-shaped morphology and grow into islets with an estimated doubling time of about 24 hours, while HeLa cells have a rounded shape and grow into cell clusters with a doubling time estimated at 24 hours (Bayala et al., 2020a).

Cell culture

LNCaP and HeLa cells were grown in 75 cm^2 flasks in RPMI and DMEM media supplemented with 10% fetal calf serum (FCS, Bio-west, Nuaillé, France), 1% penicillin/streptomycin (Invitrogen, Oslo, Norway) and 1% L-glutamine. Cultures were maintained in the incubator at a humidified atmosphere containing 5% CO_2 at 37°C. These confluence cells were washed with PBS-1X, and harvested by trypsinization, and seeded in 96-well culture plates for MTT testing (Mitochondrial Tetrazolium Test).

Tests MTT

The MTT test was used to evaluate the effect of extracts on cell viability. To do this, the LNCaP and HeLa cell lines were seeded at a rate of 10,000 cells per well into plates of 96

flat-bottom wells (100 µL suspension per well) and incubated. After 24 hours, the cells were treated with variable concentrations (100 µL) of each extract or fraction and re-incubated for 72 hours. After 72 hours of incubation, the MTT solution was solubilized and brought into contact with 30 µL of MTT solution (5 mg/mL in PBS-1x) per well. The plates were then incubated for 2 hours and 45 minutes to produce formazan crystals. After that, the medium was removed by suction and 150 µL of MTT solvent (composed of 10% triton X100, 1N HCL, and isopropanol) was added to dissolve formazan crystals. The plates were then agitated for 20 minutes. The absorbance of the solution was measured at a 570 nm wavelength with a microplate reader (Biotek EL808 spectrophotometer). This calculated the inhibition percentage according to the formula:

%Inhibition = 100 - [(Ac - Ab) / (Ae - Ab)]

Ac: control absorbance; **Ab**: white absorbance; **Ae**: sample absorbance.

Statistical analysis

The results were expressed as a mean standard deviation. Cross-comparison is performed by the "Prism 5.0.3 One-Way ANOVA Test". The means are significantly different for $P < 0.05$.

RESULTS

Phytochemical screening

Phytochemical screening for the detection of sterols and terpenoids (Figure 1A), flavonoids (Figure 1B), and tannins (Figure 1C) was carried out on extracts obtained by successive exhaustion with four solvents with increasing polarity, thus twelve fractions (six of each plant). Apart from the petroleum and aqueous ether fractions (Figure 1B: Bands 4;9;11, and 12), flavonoids were present in most fractions, including DCM and total extracts (Figure 1B: Bands 1;2;6, and 7) and much more in ethyl acetate and n-butanol extracts (Figure 1B: Bands 3;5;8 and 10). The same ten-dance with tannins which constitute a subgroup of flavonoids was observed. Sterols and triterpenes were observed in dichloromethane and petroleum ether extracts.

Antioxidant activity by DPPH and ABTS test

The ability of total extracts and fractions of *C. paniculatum* and *F. agrestis* to inhibit radicals $DPPH^{\blacksquare}$ was assessed (Figure 2). The results of antioxidant Activity by inhibition of radicals $DPPH^{\blacksquare}$ *and* $ABTS^{\blacksquare+}$ of *C. paniculatum* and *F. agrestis* were presented in Table 1. Quercetin and gallic acid were used as reference compounds. Ethyl acetate (0.246 ± 0.045 µg extract/mL) and n-butanol fractions (0.537 ± 0.095 µg extract/mL) of *F. agrestis* had the best inhibition of $DPPH^{\blacksquare}$ radical respectively while the n-butanol fraction (0.097 ± 0.008 µg extract/mL) and the total extract (0.159 ± 0.053 µg extract/mL) of *C. paniculatum* were better. Concerning the inhibition of radical $ABTS^{\blacksquare+}$, the total extracts and fractions of *C. paniculatum* had the best inhibitions compared to those of *F. agrestis.*

Cytotoxic activity

The results of the extract and fractions of *F. agrestis* and *C. paniculatum* on the viability of the LNCaP lines of prostate cancer and HeLa of cervical cancer are shown in Figures 3 and 4, respectively. The different inhibition percentages of the extracts and fractions of *C. paniculatum* and *F. agrestis* from the MTT test determined the 50 inhibitory concentrations (IC50) reported in Table 2. At the maximum concentration of 500 µg/mL used for all the extracts of *C. paniculatum* and *F. agrestis*, it was observed that the fractions EP *C. paniculatum*, FA *C. paniculatum*, EP *F. agrestis*, FA *F. agrestis* had an IC50 at 500 µg/mL on LNCaP prostate cancer cells. However, on HeLa cervical cancer cells only FA *F. agrestis* had an IC50 greater than 500 µg/mL.

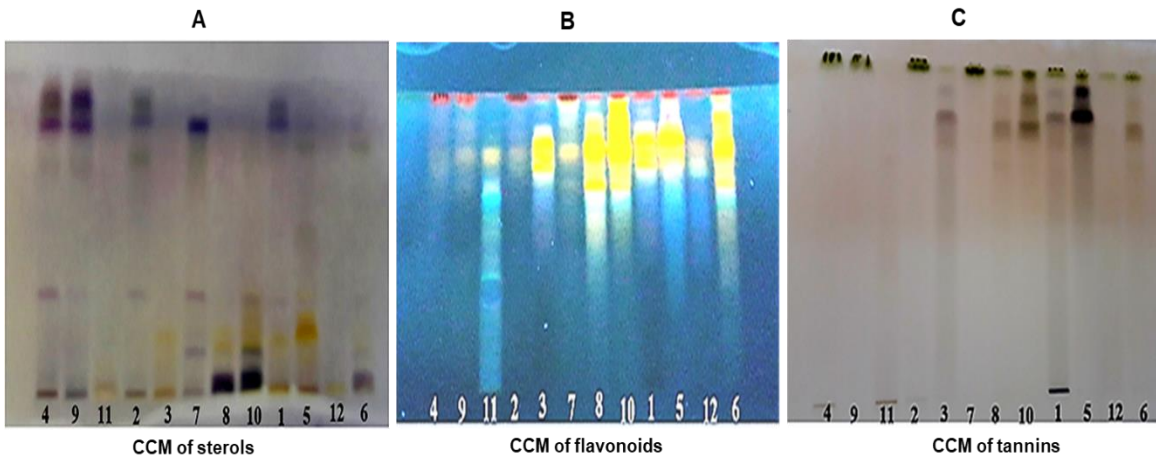

Figure 1: HPTLC chromatograms of sterols (sulphuric vanillin), flavonoids (Neu reagent), and tannins ($FeCl_3$ 2%).

Sterols are usually fluorescent to ultraviolet (366 nm) in blue, yellow, and green, while triterpenes are blue, yellow, green, and purple. As for polyterpenes, their colorations are blue and purple to the visible. This is what is observed on the HPTLC plates.

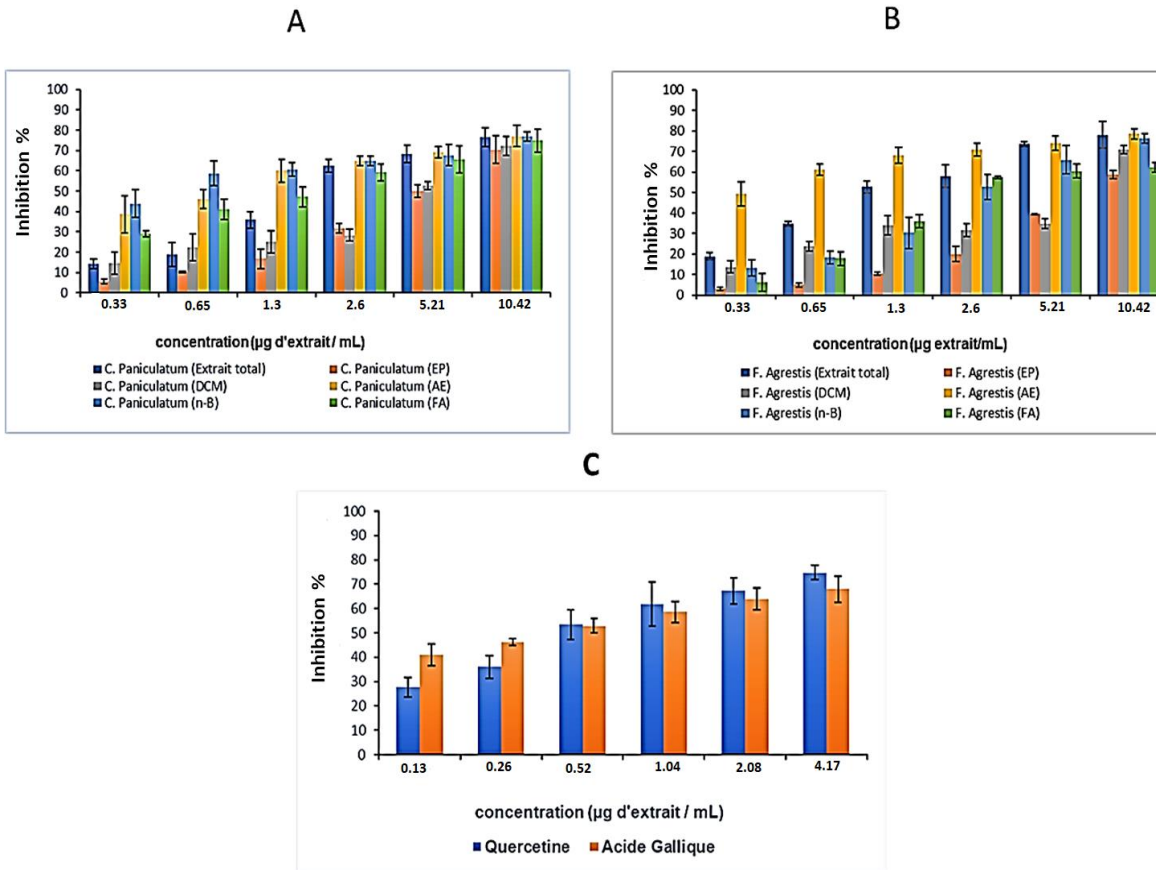

Figure 2: Histogram of antioxidant activities of extracts and reference compounds on $DPPH^{\blacksquare}$.

A. Anti-radical activity of *C. paniculatum* extracts; **B.** Anti-radical activity of *F. agrestis* extracts; **C.** Anti-radical activity of quercetin and gallic acid.

Table 1: Antioxidant Activity by inhibition of radicals $DPPH^{\blacksquare}$ *and* $ABTS^{\blacksquare+}$ of *C. paniculatum* and *F. agrestis* extracts.

	$DPPH^{\blacksquare}$ *test* : IC50 (µg extract/mL)		$ABTS^{\blacksquare+}$ *test* : Activity (µMET/g)	
	F. agrestis	***C. paniculatum***	***F. agrestis***	***C. paniculatum***
Total extract	1.110 ± 0.162^{e}	0.159 ± 0.053^{b}	0.0285 ± 0.0002^{g}	1.0165 ± 0.0178^{d}
Petroleum ether fraction	6.633 ± 0.072^{i}	4.283 ± 0.350^{h}	0.0178 ± 0.0012^{h}	0.1999 ± 0.0040^{e}
Dichloromethane fraction	1.967 ± 0.072^{g}	0.395 ± 0.043^{c}	0.0206 ± 0.0009^{h}	0.0953 ± 0.0029^{f}
Ethyl acetate fraction	0.246 ± 0.045^{b}	1.480 ± 0.259^{f}	0.0316 ± 0.0007^{g}	1.1009 ± 0.0181^{c}
n-butanol fraction	0.537 ± 0.095^{d}	0.097 ± 0.008^{a}	0.0245 ± 0.0004^{h}	0.9952 ± 0.0245^{b}
Aqueous fraction	2.272 ± 0.198^{g}	1.713 ± 0.212^{f}	0.0221 ± 0.0014^{h}	0.2135 ± 0.0077^{e}
Quercetin	0.400 ± 0.028^{c}		2.2386 ± 0.1540^{a}	
Gallic acid	0.091 ± 0.013^{a}		2.6577 ± 0.3073^{a}	

$DPPH^{\blacksquare}$, IC_{50} (µg extract/mL) and $ABTS^{\blacksquare+}$ in Trolox micromole equivalent/g extract (µM ET/g); quercetin and gallic acid were used as standards. Values are expressed as mean standard deviation values (n = 3 triplet experiments). Values with the same letter in each column can be considered identical ($P < 0.05$).

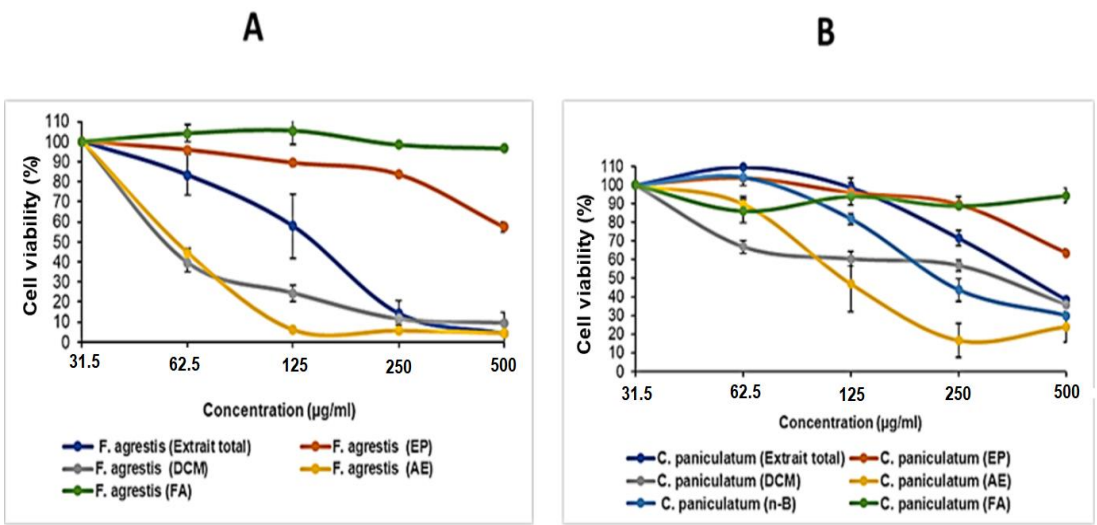

Figure 3 . Ouédraogo et al., 2023

Figure 3: Cytotoxicity of *Fadogia agrestis* and *Combretum paniculatum* to prostate cancer LNCaP cells.

A. *F. agrestis* activity on LNCaP cells; **B.** *C. paniculatum* activity on LNCaP cells.

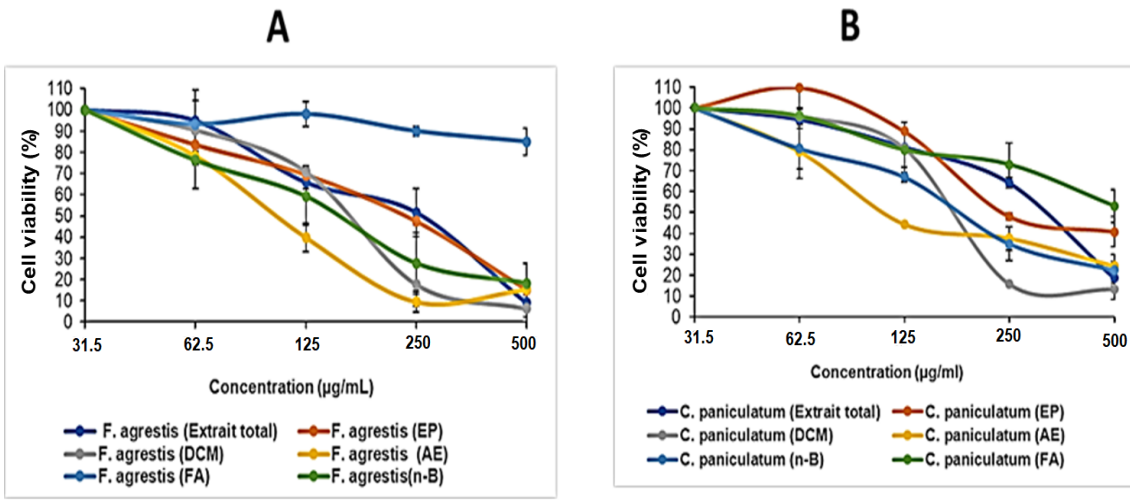

Figure 4 . Ouédraogo et al., 2023

Figure 4: Cytotoxicity of *Fadogia agrestis* and *Combretum paniculatum* on cervical cancer HeLa cells.
A. Activity of *F. agrestis* on HeLa cells; **B.** Activity of *C. paniculatum* on HeLa cells.

Table 2: IC50 of extracts and fractions of *Combretum paniculatum* and *Fadogia agrestis* on the cells HeLa of cervical cancer and LNCaP of prostate cancer.

Extract	IC_{50} (µg/mL)	
	LNCaP	HeLa
Total *C. paniculatum*	391.47 ± 12.14^{g}	233.34 ± 6.88^{e}
EP *C. paniculatum*	>500 (%$I_{500µg/mL}$ = 36.57 ± 2.02)	283.82 ± 32.44^{g}
DCM *C. paniculatum*	251.95 ± 6.82^{f}	237.21 ± 26.06^{f}
AE *C. paniculatum*	202.47 ± 9.74^{e}	151.46 ± 20.82^{b}
n-B *C. paniculatum*	260.90 ± 19.68^{f}	185.13 ± 16.34^{c}
FA *C. paniculatum*	>500 (%$I_{500µg/mL}$ = 5.67 ± 3.97)	484.68 ± 14.52^{h}
Total *F. agrestis*	143.94 ± 8.05^{c}	188.03 ± 11.42^{c}
EP *F. agrestis*	>500 (%$I_{500\ µg/mL}$ = 4.49 ± 2.55)	244.45 ± 30.02^{f}
DCM *F. agrestis*	24.69 ± 9.51^{a}	159.85 ± 5.18^{b}
AE *F. agrestis*	40.32 ± 7.15^{b}	129.9 ± 6.49^{a}
n-B *F. agrestis*	163.7 ± 5.54^{d}	207.04±21^{d}
FA *F. agrestis*	>500 (%$I_{500\ µg/mL}$ = 3.40 ± 0.49)	>500 (%$I_{500\ µg/mL}$ = 14.97 ± 6.37)

50% inhibitory concentration (IC50). Values are expressed as mean ± standard deviation values (n = 3 triplet experiments). IC50 values greater than 500 µg/mL are expressed as percent inhibition (%I500 µg/mL). Values with the same letter in each column can be considered identical (P < 0.05).

DISCUSSION

The phytochemical screening carried out on the fractions of *Combretum paniculatum* and *Fadogia agrestis* showed the presence of tannins, flavonoids, sterols, and terpenoids whose antioxidant properties are reported in the literature (Roger et al., 2011; Volobuff et al., 2019). However, flavonoids and tannins appeared in trace form in the petroleum ether extract and the aqueous fraction of the two plants. According to the literature, these compounds are essential in treating oxidative stress diseases (Traoré et al., 2019). They can interact with free radicals generated by the human body in response to the aggressions of our environment.

Plant extracts' relative anti-free radical activity reflects their ability to trap free radicals (Ahouagi et al., 2021; Wo\losiak et al., 2022). The study of the antioxidant properties of *C. paniculatum* and *F. agrestis* allowed the determination of the inhibition percentages of DPPH radicals ($DPPH^{\blacksquare}$) which are derived based on the concentrations of the extracts of both plants (Figure 2). The 50% inhibiting concentration (IC50) of $DPPH^{\blacksquare}$ free radicals determined graphically for each extract shows here that inhibition is concentration dependent. The extract with the smallest IC50 is the most antioxidant by the DPPH method (Bayala et al., 2020a). For example, the ethyl acetate fraction of *F. agrestis* and the n-butanol fraction of *C. paniculatum* exhibited the best activities with respective IC50 values of 0.246 0.045 µg extract/mL and 0.097 0.008 µg extract/mL. The IC50 of the gallic acid used as a standard (0.091 0.013 µg extract/mL) is similar to that of the n-butanol fraction of *C. paniculatum*, reflecting the potent inhibition of DPPH radicals ($DPPH^{\blacksquare}$) of this fraction of *C. paniculatum*.

In addition to the results of the antioxidant activity of the extracts and fraction of *F. agrestis* and *C. paniculatum* obtained by the anti-free radical test of $DPPH^{\blacksquare}$, a trap test of the $ABTS^{\blacksquare+}$ cationic radical proton was performed. The total extract (1.0165 0.0178 µM SD/g) and the ethyl acetate fraction (1.1009 0.0181µM SD/g) of *C. paniculatum* exhibited the greatest inhibitory effects of the cationic radicals $ABTS^{\blacksquare+}$. Thus, the antioxidant activity of these extracts goes through different mechanisms. Considering the two plants, the ethyl acetate fractions of *C. paniculatum* (1,1009 ± 0,0181 µM ET/g) and *F. agrestis* (0,0316 ± 0,0007 µM ET/g) rich in phenolic and flavonoid acids have the highest activities (Zongo et al., 2010; Floegel et al., 2011; Dieng et al., 2017; Kabran et al., 2021).

Medicinal plants are an alternative for preventing and treating cancer in many countries around the world (Haïdara et al., 2022). Current antitumor drugs have been isolated from natural plant products (Bailly et al., 2021). All extracts and fractions showed a cytotoxic effect on cervical cancer HeLa cells and prostate cancer LNCaP cells. However, the AE fraction of *F. agrestis* (IC_{50} = 129,9 ± 6,49 µg/ml) and the AE fraction of *C. paniculatum* (IC_{50} = 151,46 ± 20,82 µg/ml) had higher cytotoxic effects on HeLa cells. The DCM fraction of *F. agrestis* (IC_{50}= 24,69 ± 9,51 µg/ml) and the AE fraction of *C. paniculatum* (IC_{50}= 202,47 ± 9,74 µg/ml) were the best cytotoxic agents in the prostate cancer cell lines. According to the United States National Cancer Institute (NCI), an extract with an IC50 of less than 30 µg/mL may be considered a potential source of cytotoxic molecules (Boukhatem et al., 2022). The DCM fraction of *F. agrestis* (IC_{50}= 24,69 ± 9,51 µg/mL) had the best cytotoxic activity on the LNCaP cells of the prostate cancer in this study. Thus, this fraction could be a potential source of antitumor substances against prostate cancer.

Conclusion

Combretum paniculatum and *Fadogia agrestis* are plants rich in their chemical composition which reveals the presence of active substances such as flavonoids, tannins, sterols, and terpenes, as well as in their ability to inhibit radicals $DPPH^{\blacksquare}$ et $ABTS^{\blacksquare+}$. This

study showed that the ethyl acetate fractions of *F. agrestis* and *C. paniculatum* showed the best antiproliferative effects on cervical cancer cells, respectively. In contrast, the DCM fraction of *F. agrestis* and the ethyl acetate fraction of *C. paniculatum* on prostate cancer LNCaP cell lines exhibited the best antiproliferative effects. These activities could justify the various medicinal uses of *F. agrestis* and *C. paniculatum* in Burkina Faso.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

BO, BB, and JS designed the research; BO, BK and BB performed the experiments. BO, BB, and MK analyzed the data. BO, BB, MK, PFIZ, EO, LLC, MDWA, FWD, and JS wrote the manuscript. All authors read and approved the final manuscript.

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