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Antioxidant and antiproliferative activities on prostate and cervical cultured cancer cells of five medicinal plant extracts from Burkina Faso

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ABSTRACT

Medicinal plants are a potential source of drug discovery and development of cancer chemoprevention drugs. Thus, the aim of this work was to study the antioxidant and antiproliferative activities of hydromethanolic extracts of *Musa sapientum* L., *Cassia italica* (Mill.) Spreng., *Crateva adansonii* DC., *Euphorbia hirta* L. and *Ceratotheca sesamoides* Endl. from Burkina Faso. The antioxidative activity of hydromethanolic extracts of plant was assessed using DPPH radical scavenging assay and ABTS+ radical cation decolorisation assay. Antiproliferative activity was evaluated by MTT assay. Of these five plant extracts, hydromethanolic extract of *Euphorbia hirta* leaf twigs showed the best antioxidant activity both by DPPH (IC_{50} = 0.53 ± 0.04 µg extract / µg DPPH) and ABTS (C = 0.302 ± 0.003 µMET / g extract) methods. In addition, hydromethanolic extract of *Euphorbia hirta* leaf twigs showed the best antiproliferative activity on LNCaP cell lines of prostate cancer while the hydromethanolic extract of the *Ceratotheca sesamoides* leaf stems showed the best antiproliferative activity on the HeLa cell lines of cervical cancer. This work has shown not only the antioxidant and anticancer activities of these five local plants, but also the potential valorization of these species used in traditional medicine in Burkina Faso.
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Keywords: Cancer, antioxydant, antiproliferative, Medicinal plants, Burkina Faso.

INTRODUCTION

Traditional plants generally have many therapeutic properties (Sarr et al., 2015). Indeed, medicinal plants are a potential source of drug discovery and development of cancer chemoprevention drugs. Most African people still rely heavily on traditional medicine (Zank and Hanazaki, 2017). In fact, more than 80% of

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African population uses medicinal plants (Shewamene et al., 2020). Plants are reservoirs for novel chemical molecules and provide a promising line for research on cancer (Iqbal et al., 2017). Indeed, several extracts of African medicinal plants are known to have properties against cancer cells (Ambe et al., 2016; Bayala et al., 2019; Moore et al., 2016). *Musa sapientum*, *Cassia italica, Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides* are used in traditional medicine in Burkina Faso to treat inflammatory and oxidative diseases (Nacoulma, 1996). These plants are known in the literature for their many biological properties. Regarding *Musa sapientum*, ethanol extracts have anti-plasmodium and anti-toxoplasma activities *in vitro* (Leesombun et al., 2019) and an antidepressant activity possibly mediated by α1-adrenergic and dopaminergic D2 receptors and without anxiolytic effect (Salako et al., 2019). Stem extract of Musa sapientum showed anticonvulsant and antioxidant effects on both acute and chronic epilepsy experimental models (Reddy et al., 2018). Proteins extracted from flowers showed antibacterial effects against gram-positive and negative bacteria (Sitthiya et al., 2018). A significant antidepressant-like activity was found in *Musa sapientum* stem extract in experimental models in mice (Reddy et al., 2016). Methanol extract of stems has significant antihypercholesterolemic and antioxidant effects (Dikshit et al., 2016). At last, *Musa sapientum* decoction extract of fresh unripe peels exhibited strong antioxidant activity (Phuaklee et al., 2012).

Ethanolic extract of the whole parts of *Cassia italica* showed a dose-dependent inhibition of prostaglandin release effect using rat peritoneal leucocytes (Jain et al., 1997). Phytol, 1-hexyl-2-nitrohexane and 2-isopropyl-5-methylcyclohexyl 3- (1- (4-chlorophenyl) -3-oxobutyl) -coumarin-4-yl carbonate are three compounds identified in the extract *C. adansonii* that showed anti-inflammatory properties (Thirumalaisamy et al., 2018). Ahama-Esseh et al. (2017) pointed out that various *C. adansonii* leaf samples have anti-inflammatory activities. While dichloromethane / methanol extracts of stem bark exhibit anti-cancer activity on breast cancer cells MCF-7 and MDA-MB-231 (Zingue et al., 2016). The stem bark also possesses analgesic activity against peripheral and central mediated pain sensation and also antioxidant properties (Udeh & Onoja, 2015).

Euphorbia hirta has anthelmintic properties (Nsereko et al., 2019), as well anti-inflammatory and anxiolytic effects on neonatal asthmatic rats with inflammation (Xia et al., 2018). Isolated compounds caffeic acid and epicatechin 3-gallate showed antibacterial effect against *Pseudomonas aeruginosa* (Perumal et al., 2017). Aerial parts have *in vitro* antimicrobial activities against the bacterium *Aeromonas hydrophila* (Sheikhlar et al., 2017). Decoctions of leaves and bark are used for the treatment of dengue (de Guzman et al., 2016). *E. hirta* also inhibits the survival of MCF-7 cells with a half inhibitory concentration (IC50) value of 25.26 µg/mL at 24 h (Kwan et al., 2016). Chen et al. (2015) described the *in vitro* anti-inflammatory activity of fractionated *E. hirta* aqueous extract on rabbit synovial fibroblasts.

Toyin et al. (2012) showed antidiarrheal activity of aqueous leaf extract of *Ceratotheca sesamoides* in rats, while extract from leaves have antiviral activities (Obi et al., 2006).

According to these data, several other studies have been carried out on these five plants, but, to date, no study has yet been carried out on the anticancer activity of these five plants on prostate and cervical cancers cells. Based on that, we evaluated the antioxidant and antiproliferative activities of hydromethanolic extracts of *Musa sapientum*, *Cassia italica*, *Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides* from Burkina Faso on cells derived from prostate (LNCaP) and cervical (HeLa) cancers.

MATERIALS AND METHODS

Vegetal material and Extraction

The plant material of *Musa sapientum*, *Cassia italica, Crateva adansonii*, *Euphorbia hirta*, and *Ceratotheca sesamoides* were collected in August 2017 in Burkina Faso with respective GPS coordinates (Table 1). Taxonomic identities were confirmed by Dr. Abdoulaye SEREME, Plant Biology Researcher, Botanist of "Centre National de la Recherche Scientifique et Technologique (CNRST)". The different samples of harvested plants were dried in the laboratory away from sunlight and then reduced to powder. Each crude extract was obtained by hydromethanolic maceration (80:20) for 48 hours with frequent agitation. The mixture was filtered with Whatman No. 1 filter paper and the filtrate was concentrated by rotary evaporator with vacuum at 40 °C, poured in glass Petri dishes and brought to dryness at 40 °C oven.

Antioxidant activity

DPPH* (2,2-diphenyl-1-picryl-hydrazyl-hydrate) *radical scavenging assay

DPPH (Sigma-Aldrich, L'Ile d'Abeau, France) radical scavenging activity was measured as described by Velasquez (Velázquez et al., 2003) with modifications. Briefly, plant extract at 0.625mg / mL was diluted at different concentrations in a 96-well plate. Then, 100 µL of each extract concentration was mixed with 100 µL of DPPH (30 mg / L in methanol). After 30 min of incubation in the dark, the absorbance was read at 517 nm using a UV / Visible spectrophotometer for Radical scavenging capacity. Gallic acid was used as a control. The radical scavenging activity was expressed as a percentage inhibition according to the formula:

$$\text{RSC (\%)} = \frac{\text{Absorbance Blank} - \text{Absorbance Sample}}{\text{Absorbance Blank}} X\ 100$$

RSC: Radical scavenging capacity.

Concentrations were expressed in µg of extracts / µg of DPPH by formula:

$$\text{Concentration} = \frac{\text{Mass of extract}}{\text{Mass of DPPH}}$$

That is to say:

$$\text{C} = \frac{\text{Concentration of extract x Volume of extract}}{\text{Concentration of DPPH x Volume of DPPH}}$$

C: Concentration.

The concentration necessary for scavenging 50% of the DPPH radicals was then determined graphically according to the formula: IC50 = f (log C) with being the concentration of extract and the equation of the regression line y = ax + b is used to determine IC_{50}.

$ABTS^{+\cdot}$ radical cation decolorization assay

The spectrophotometric analysis of $ABTS^{+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) scavenging activity was determined according Re et al. (1999). Briefly, preparation of $ABTS^{+}$ solution was done by dissolving 10 mg of ABTS in 2.6 mL of distilled water. Then, 1.7212 mg of potassium persulfate was added and the mixture kept in the dark at room temperature for 12 hours. The mixture was then diluted with ethanol in order to obtain an absorbance of 0.70 ± 0.02 to 734 nm. In 96-well plates, 50 µL of ethanolic extract solution at an initial concentration of 0.625 mg / mL (*Musa sapientum*, *Cassia italica*, *Crateva adansonii* and *Ceratotheca sesamoides*) and of 0.125 mg/mL (*Euphorbia hirta*) were added to 200 µL of freshly prepared $ABTS^{+}$ solution. The same process was carried out for gallic acid at an initial concentration of 0.0125 mg/mL used as standard. The mixture made in the 96-well plates was then incubated in the dark at room temperature (25 ºC) for 15 min and the absorbance was read at 734 nm against a standard curve of 5,7,8-tetramethyl-2-carboxylic acid 6-hydroxy-2 (Trolox, Sigma-Aldrich) using a spectrophotometer. The plant extract activity on the radical cation $ABTS^{+\cdot}$

was expressed in micromoles Trolox equivalent per gram of extract (µmol TE / g) using the following formula: C = (cx D) / Ci, C being the concentration of plant extract in µmol TE / g; c, the concentration of the sample read; D, the dilution factor and Ci, concentration of the stock solution.

Cancer cell lines and culture conditions

LNCaP (Lymph Node Cancer of the Prostate) cells are an androgen responsive prostate cancer cell line with a low metastatic potential derived from a lymph node metastasis (Horoszewicz et al., 1983). HeLa (Henrietta Lacks) cells derived from tumor of the cervix (C, 1974). All these cells are available through the GReD (Génétique, Reproduction & Développement) Laboratory (University Clermont-Auvergne, France) and others manipulations were carried out in GReD and CERBA/LABIOGEME Laboratories. They are cultured and maintained at 37 °C in a chamber moistened with 5% CO_2 in 75 cm^2 flasks of tissue culture, in medium supplemented with 10% fetal calf serum (FCS, Biowest, Nuaillé, France), 1% penicillin and 1% streptomycin (Invitrogen, Oslo, Norway). Cells were maintained in RPMI-1640 (Roswell Park Memorial Institute) medium (Invitrogen).

Antiproliferative activity

3[4,5-dimethylthiazol-2-yl]-diphenyltetrazolium bromide (Sigma-Aldrich) assay (MTT) was used to measure the cell survival. Briefly, 50,000 cells/mL were seeded for 24 h in 96-well plates. After 24 h, extracts were added. And after 72 h incubation, the number of living cells was measured as described (Bayala et al., 2014, 2018) using a microplate reader type Bio-Rad 11885 at 490 nm. Experiments were performed in sextuplicate with three independent experiments on each cell line.

Statistical analysis

All data are presented as mean ± standard deviation. The data were analyzed by analysis of variance followed by the Turkey multiple comparison test. The analyses were performed using XLSTAT 7.1 software. $P < 0.05$ was used as a criterion for statistical significance.

Table 1: GPS geographic location of plants.

Plant	GPS coordinates	
	X	Y
Musa sapientum	662753	1369094
Cassia italica	678088	1374329
Crateva adansonii	679992	1373734
Euphorbia hirta	662723	1369080
Ceratotheca sesamoides	678084	1374327

RESULTS

The antioxidant activities of the hydromethanolic extracts of *Musa sapientum*, *Cassia italica*, *Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides* are presented in Table 1. From these results, it appears that the extracts of the leafy twigs of *Euphorbia hirta* presented the highest inhibition of DPPH with IC50 of 0.53 ± 0.04 µg extract / µg DPPH ($p < 0.05$) and *Crateva adansonii* bark the lowest inhibition (IC50 of 15.73 ± 2.04 µg extract / µg DPPH) (Table 1). In ABTS radical cations inhibition, leafy twigs extract of *Euphorbia hirta* also showed the highest activity (0.302 ± 0.003 µMET / g of extract) ($p < 0.05$) while those of *Crateva adansonii* bark hydromethanolic extracts has the lowest activity (0.024 ± 0.002 µMET / g of extract) (Table 2). The gallic acid used as a standard exhibited a good activity with an IC50 of inhibition of the DPPH radicals of 0.11 ± 0.04 µg extract / µg DPPH and an inhibition concentration of the ABTS cation radicals of 2.665 ± 0.314. µMET / g extract (Table 2).

The results of the tests of the antiproliferative activity are recorded in Table 2. This table presents the different IC_{50} of *Musa sapientum*, *Cassia italica*, *Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides* hydrometanolic extracts. Extract of the leafy twigs of *Euphorbia hirta* has antiproliferative activity on LNCaP cell lines of prostate cancer are 251.15 ± 6.5 µg / mL. In HeLa cell lines of cervical cancer, leaf stems of *Ceratotheca sesamoides* had an activity of 723.25 ± 3.82 µg / mL. Figure 1 shows the viability of LNCaP cells according to the concentrations of each extract used. As for Figure 2, it also demonstrates the viability of HeLa cells of cervical cancer according to the concentrations of each extract. Cisplatin was used as standard on LNCaP cells of prostate cancer and HeLa cells of cervical cancer (Figures 1 and 2).

Table 2: Antioxydant activity of hydromethanolic extracts of *Musa sapientum*, *Cassia italica*, *Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides.*

Sample	**Antioxydant activity**	
	DPPH, IC_{50} (µg extract / µg DPPH)	**ABTS, C (µMET/g extrait)**
Musa sapientum (F.).	5.7 ± 0.91^{d}	0.032 ± 0.005^{d}
Cassia italica (Rx F.)	5.13 ± 1.06^{d}	0.029 ± 0.004^{a}
Crateva adansonii (F.)	3.65 ± 0.88^{c}	0.041 ± 0.004^{c}
Euphorbia hirta (Rx F.)	0.53 ± 0.04^{b}	0.302 ± 0.003^{b}
Ceratotheca sesamoides (Tg F.)	2.02 ± 0.94^{c}	0.027 ± 0.004^{d}
Crateva adansonii (E.)	15.73 ± 2.04^{e}	0.024 ± 0.002^{d}
Gallique acid	0.11 ± 0.04^{a}	2.665 ± 0.314^{a}

IC_{50}, Inhybitory concentration 50; C, Concentration; DPPH, (2,2-diphenyl-1-picrylhydrazyl); ABTS (2,20-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]); Values are expressed as mean values ± SD. n = 3 independent experiments in triplicate for the measurement of antioxidant activity; DPPH activities is expressed as IC_{50} (µg Extract /µg DPPH) and ABTS activities are given in µmol Throlox equivalent/g of Extract. a, b, c, d, e, from the largest to the smallest activity, the same letters are used for statistically identical activities and different letters when they are statistically different in each column ($p < 0.05$). F, Leaf; Rx F, leafy twigs; Tg F, Leaf Stem; E, Bark. Galic acid was used as standard.

Table 3: IC_{50} of hydromethanolic extracts tested on LNCaP human prostate cancer cell lines and HeLa human cervical cancer cell lines.

Sample	IC_{50} (µg/mL)	
	LNCaP cell lines	**HeLa cell lines**
Musa sapientum (F.)	>1000	>1000
Cassia italica (Rx F.)	>1000	>1000
Crateva adansonii (F.)	>1000	>1000
Euphorbia hirta (Rx F.)	251.15 ± 6.50***	>1000
Ceratotheca sesamoides (Tg F.)	599.85 ± 4.76*	723.25 ± 3.82$^{\$}$
Crateva adansonii (E.)	585.35 ± 3.19**	>1000
Cisplatin	3.4 ± 0.5****	5.12 ± 1.1$^{\$\$}$

IC_{50}, Inhybitory concentration 50; Values are expressed as mean values ± standard deviation. n = 3 independent experiments in sextuplicate; *, **, *** and $^{\$, \$\$}$ ($p < 0.05$) from lowest to highest activity and significantly different compared respectively. F, Leaf; Rx F, leafy twigs; Tg F, Leaf Stem; E, Bark. Cisplatin was used as standard.

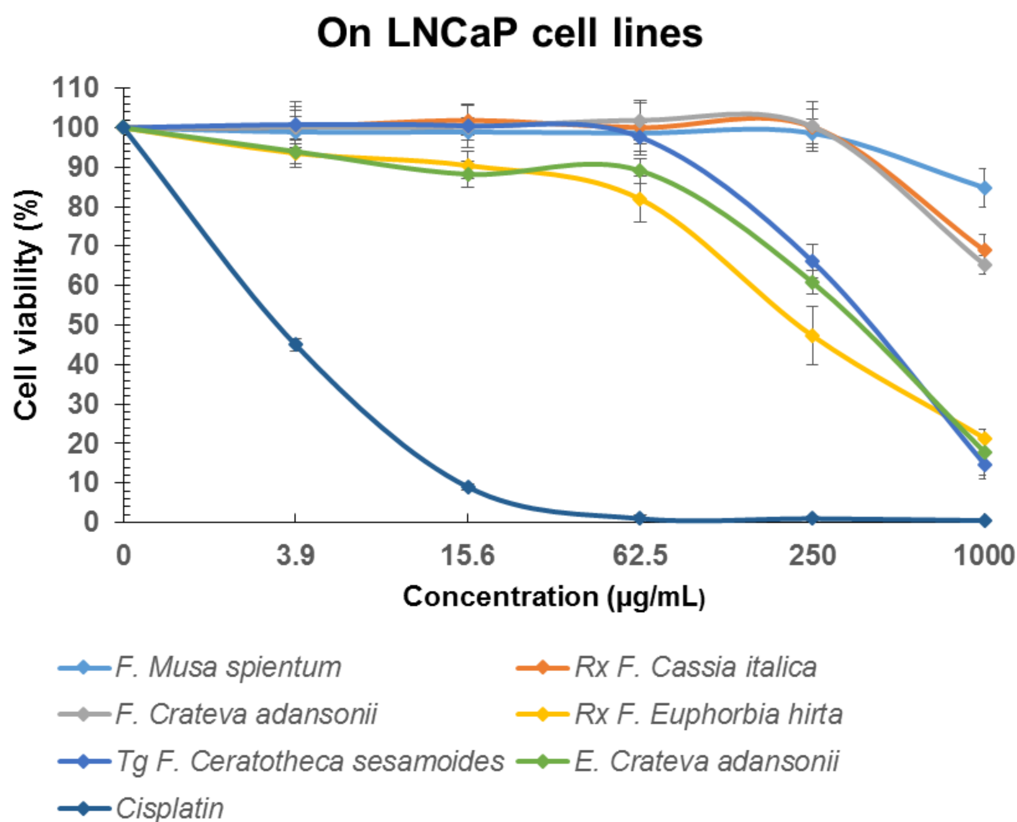

Figure 1: Dose-dependent anti-proliferative activity of hydromethanolic extracts of plant on human LNCaP cell lines of Prostate cancer.

Cell lines were treated for 72 h. Experiments were performed 3 times in sextuplicates. F, Leaf; Rx F, leafy twigs; Tg F, Leaf Stem; E, Bark. Cisplatin was used as standard.

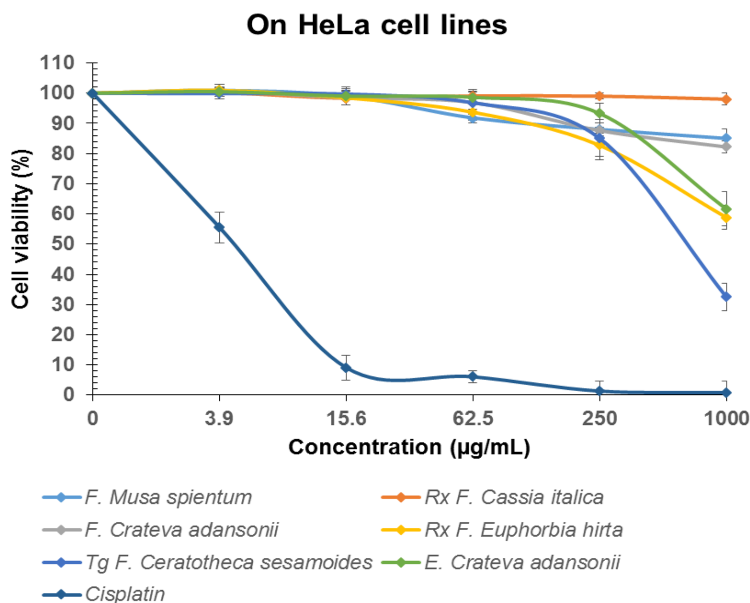

Figure 2: Dose-dependent anti-proliferative activity of hydromethanolic extracts on human HeLa cells lines of cervical cancer.

Cell lines were treated for 72 h. Experiments were performed 3 times in sextuplicates. F, Leaf; Rx F, leafy twigs; Tg F, Leaf Stem; E, Bark. Cisplatin was used as standard.

DISCUSSION

Oxygen reactive species (ORS) are involved in physiological processes at low levels. However, excess production of ORS can become toxic to major cell components, lipids, proteins and nucleic acids (Ouattara et al., 2020) causing oxidation in the body. The DPPH and ABTS method are used to determine the antioxidant activity in vitro. The difference between DPPH anti-radical and anti-radical ABTS activities at the origin of the antioxidant activity is mainly at the level of their mechanism of action brought into play. Indeed, the DPPH involves free radicals while the ABTS involves radical cations. So, the hydromethanolic extracts of *Musa sapientum*, *Cassia italica*, *Crateva adansonii*, *Euphorbia hirta* and *Ceratotheca sesamoides* all exhibited antioxidant activities depending on the inhibition of radicals DPPH and cation radicals ABTS. Hydromethanolic extracts of the leafy twigs of *Euphorbia hirta* presented the higher inhibition of DPPH as well of ABTS radical cations. This antioxidant activity could be justified by a high content of antioxidant compounds contained in *Euphorbia hirta* (Basma et al., 2011). Indeed, extract of the aerial parts of *Euphorbia hirta* contains many acidic compounds (Yang et al., 2020) that could justify this antioxidant activity. High total phenolic and flavonoid contents, suggested that *E. hirta* methanolic extract is a potential antioxidant agent for the development of local natural products for disease treatment (Ismail et al., 2019). Moreover, concerning *Crateva adansonii*, it should also be noted that hydromethanolic extract of its leaves are more active than those of its bark. Thus, for the same plant, the activity may vary according to the parts used. The standard gallic acid exhibits stronger antioxidant activity compared to the natural hydromethanolic extracts from plants (Table 2). Indeed, gallic acid is a pure compound compared to extracts which are composed of a complex mixture of several

compounds which could have antagonistic effects.

Hydromethanolic extracts from *Musa sapientum* leaves, *Cassia italica* twigs and leaves, *Crateva adansonii* leaves do not present strong inhibitory effects on both LNCaP and HeLa cell lines with IC_{50} > 1000 µg/mL (Figures 1 & 2). These three extracts would therefore be active in high concentrations. Furthermore, these extracts are a mixture of several compounds whose antagonistic actions between them could also explain their low overall activity. Conversely, hydromethanolic extracts from the leafy twigs of *Euphorbia hirta* has a significant antiproliferative activity on the LNCaP cell lines from prostate cancer with IC50 of 251.15 ± 6.5 µg / mL ($P < 0.05$) (Table 3). This activity is also higher than that of leaf stems of *Ceratotheca sesamoides* on HeLa cell lines of cervical cancer whose IC50 is 723.25 ± 3.82 µg / mL ($P < 0.05$) (Table 3). The presence of phenolic compounds in *Euphorbia hirta* could explain this activity (Basma et al., 2011). Moreover, the phytochemical screening and chromatography revealed the presence of saponin, sterol, terpene, alkaloids, polyphenols, tannins and flavonoids on *Euphorbia hirta* extract (Yvette Fofie et al., 2015). Indeed, terpene (Gill et al., 2016), polyphenols (Costea et al., 2019; Miyata et al., 2019) and sterol (Blanco-Vaca et al., 2019) are known to have anticancer activities. Previous studies have shown that *Euphorbia hirta* exhibited significant inhibition of the survival of breast cancer MCF-7 cells with an IC_{50} of 25 µg/mL at 24 h (Kwan et al., 2016). This antiproliferative activity exerted by the hydromethanolic extract of *Euphorbia hirta* is concentration dependent but remains low compared to cisplatin used as standard. Comparable effects could be observed with *Ceratotheca sesamoides* leaf Stem and *Crateva adansonii* bark hydromethanolic extract. Leaf stems of *Ceratotheca sesamoides* as cisplatin are also concentration dependent on HeLa cells of cervical cancer.

Conclusion

This work evaluated for the first time the antioxidant and antiproliferative activities of the hydromethanolic extracts of plants from Burkina Faso on cultured cancer cells. Even though the active compounds are yet to be identified and need to be investigated further, this work constitutes a scientific basis and also allow the valorization of these local medicinal plants of Burkina Faso.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

BB, JS and JML designed the research; BB and TMZ performed the experiments and analyzed the data. BB, TMZ, FWD, CN, SB, JML and JS wrote the manuscript. All authors read and approved the final manuscript.

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