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Cytotoxic Properties on Prostate and Cervical Cancer Cells in Culture of «*Acti-plus*», a Recipe Based on Medicinal Plants from Burkina Faso

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Abstract: Medicinal plants are increasingly emerging as a real alternative that can contribute to the fight against cancer. The purpose of this study was to evaluate *in vitro* the antiradical, anti-lipoxygenase and cytotoxic properties on cancerous cells in culture of the medicinal recipe «*Acti-plus*». The extraction of «*Acti-plus*» was carried out according the instructions of the traditional health practitioner. Phytochemical screening, as well as the determination of polyphenols and total flavonoids were performed using characterization and assay tests by spectrometry as well as by high performance thin layer chromatography (HPTLC). Antiradical tests for trapping DPPH and ABTS radicals were used to measure the antioxidant potential. Anti-lipoxygenase activity was studied using 15-lipoxygenase (15-LOX) inhibition test. The cytotoxic activity of aqueous extract of «*Acti-plus*» was evaluated *in vitro* on human cancers cell using MTT test. The results of the study showed that aqueous extract of «*Acti-plus*» contains flavonoids, coumarins and tannins. Total phenolic compounds (TPC) and total flavonoids (TFC) were 61.95 ± 0.01 mg GAE/g dry extract and 43.01 ± 0.05 mg QE/g dry extract respectively. The extract exhibited excellent scavenging capacity of DPPH (IC_{50} = 9.33 ± 1.53 µg/mL) and ABTS (IC_{50} = 53.33 ± 0.89 µg/mL) radicals antioxidant potential. The study also found that the extract moderately inhibits 15-lipoxygenase at 100 µg/mL. Aqueous extract of «*Acti-plus*» inhibited in a concentration-dependent manner the proliferation of the three cell lines with best activity (IC_{50} = 0.317 ± 0.02 mg/mL) on prostate cancer PC-3 cells. These properties give a rational basis for the use of the recipe «*Acti-plus*» in traditional medicine in Burkina Faso.

Keywords: Cytotoxic, Antiradical, Anti-lipoxygenase, *Acti-plus*

1. Introduction

The use of medicinal plants in the treatment of pathologies become a very common practice especially in developing countries [1]. The World Health Organization (WHO) estimates that more than 80% of the African population uses traditional medicine for primary health care. This use is justified for reasons of traditional, cultural and economic knowledge [2, 3]. Medicinal plants remain an important source of discovery of new therapeutic molecules to improve the quality of population health. Today, it is estimated that in terms of research, only 6% of the world's plant biodiversity has been the subject of pharmacological investigations [4]. The world is confronted with several dreadful pathologies,

among which cancer occupies a prominent place. There is a real public health problem that has grown over the years in the world and particularly in Africa. Cancer statistics in Africa are staggering, with 702,827 deaths and 1,082,172 new cases in 2020 alone [5]. Unfortunately, by 2040, the number of new cancer cases per year in Africa is expected to reach 2,097,365 per 1,372,428 deaths, according to the Globocan database [6]. Conventional cancer treatments include surgery, radiation and chemotherapy. However, the many limitations associated with these therapies are still unresolved, such as serious side effects, multiple drug resistance and the high cost of these therapies [7, 8]. All these reasons have led African populations to turn to medicinal plants in the treatment of cancer. However, the contribution of scientists is crucial to highlight the beneficial, adverse or toxic effects of these plants for their safe use in cancer treatment. It is in this perspective that we are interested in a recipe of medicinal plants called «*Acti-plus*», developed by a tradipratician of health of Burkina Faso. This recipe is marketed and used as a dietary supplement for cancer prevention and as an adjuvant in the management of people suffering from cancer. The recipe «*Acti-plus*», provided by the tradipratician is composed of four plants from Burkina Faso including *Chrysanthelllum americanum* DC, *Petroselium crispum* (mill) fuss, *Apium graveolens* L. and *Guiera senegalensis* J. F. Gmel. These plants are well known by the population and commonly used endogenously in the treatment of inflammatory and oxidative pathologies, but the combination of these plants in the management of cancer has not yet been the subject of scientific tests *in vitro* and/or *in vivo*. The purpose of this study was therefore to scientifically verify (*in vitro*) the effectiveness of this recipe by evaluating its anti-radical, anti-lipoxygenase and cytotoxic properties of the aqueous extract of «*Acti-plus*» used in traditional medicine.

2. Materials and Methods

2.1. Plant Material

The plant drug called «*Acti-plus*» is a vegetable drug based on a mix of medicinal plants from Burkina Faso. This recipe was formulated by a traditional health practitioner, who uses it for the traditional management of people suffering from cancers including breast, prostate, liver and cervix in Ouagadougou (Burkina Faso). It was developed mainly from four traditional plants (Table 1).

Table 1. *Medicinal plants of «Acti-plus».*

Medicinal plants	Families	Part of plant used	Name in Moore (Local language of Burkina Faso)
Chrysanthelllum americanum DC Synonym: *Chrysanthelllum indicum* DC	Asteraceae	Leaves	Wall touko
Petroselium crispum (mill) fuss	Apiaceae	Leaves	Persil
Apium graveolens L.	Apiaceae	Leaves	Celeri
Guiera senegalensis J. F. Gmel	Combretaceae	Leaves	Wilin-wiiga

2.2. Extraction

The recipe «*Acti-plus*» was extracted according to the instructions of the traditional health practitioner. Thus, the aqueous decoction method was used. This decoction was made by boiling 8 grammes (one tablespoon) of the powder of the recipe in 1 liter of distilled water for 15 minutes. The extract was allowed to cool to room temperature before being filtered. The filtrate was then centrifuged at 2000 rpm for 10 minutes. The supernatant was recovered and freeze-dried.

2.3. Determination of Extraction Yield and Residual Moisture Content

2.3.1. Extraction Yield (%)

The extraction yield was calculated using the following formula:

$$\text{Extraction yield (\%)} = M'/M \times 100$$

M' is the weight of the dry extract of «*Actis-plus*», and M is the weight of the dry vegetable powder.

2.3.2. Residual Moisture Content (%)

The residual moisture content (%) was determined by taking a dry vegetable powder mass from the recipe (m) and placed in a Memmert brand oven for three hours at 105ºC. After cooling, this mass was re-weighed (m') and the residual moisture was calculated using the following formula:

$$\text{Residual moisture content (\%)} = (m-m')/m \times 100$$

2.4. Phytochemical Sceening

Phytochemical screening of aqueous extract of «*Acti-plus*» was carried out by high performance thin layer chromatography using HPTLC plates (20 cm × 10 cm) in glass covered with silica gel type 60 F254 (Merck, Darmstadt, Germany). Using a semi-automatic TLC Sampler V (CAMAG, Linomat V, Switzerland), 5 µL of the aqueous solution of «*Actis-plus*» was placed by strip on the silica gel of the HPTLC plates.

For flavonoids: After deposition, the HPTLC plate was placed in a double trough tank (CAMAG) containing the solvent system: Ethyl Acetate-Formic Acid-Acetic Acid-Water (50: 5.5: 5.5: 13, v/v/v/v) for migration. The plate was then removed from the tank, dried and heated to 105°C for 5 minutes. The revelation of flavonoids was performed by spraying with a solution containing 5 mL of a Natural Products reagent (1% of 2-aminoethyl-diphenylborinate in methanol) and 4 mL of the Macrogol reagent (5% of polyethylene glycol in 50 mL of ethanol). Flavonoids were revealed under UV at 366 nm.

For coumarin: The system of solvents for migration was constituted: ethyl acetate-methanol-water-chloroform (18: 2.4: 2.1: 6, v/v/v/v). The plate was sprayed with distilled water containing 5% KOH. Coumarins were identified at UV 366 nm.

For tannins: The solvent system for migration was formed: ethyl acetate-methanol-water-chloroform (18: 2.4: 2.1: 6, v/v/v/v). After drying the plate, it was sprayed with ethanol containing 2% of the iron trichloride III reagent. Under white light, tannins become visible [9].

2.5. Phytochemical Determination

2.5.1. Determination of Total Polyphenols Content (TPC)

The total phenolic content (TPC) of aqueous extract of «*Acti-plus*» was quantified by spectrophotometry using the Folin-Ciocalteu method [10], with slight modifications. The reaction mixture was prepared with 1 mL of the aqueous extract solution of «*Acti-plus*» and 1 mL of the Folin-Ciocalteu reagent (0.2 M). After eight minutes of incubation at room temperature, 2 mL of saturated sodium carbonate solution (7.5% in water) was added to the mixture. After thirty (30) minutes of incubation at room temperature, the absorbance was measured using a spectrophotometer (SHIMADZU UV-Vis, Japan) at 760 nm against a white sample (the extract was replaced by distilled water). The total polyphenol content expressed in milligrams (mg) of gallic acid equivalent per gramme (g) of dry extract (mg GAE/g) was calculated from a gallic acid calibration curve. The measurements were made in triplicate.

2.5.2. Determination of Total Flavonoid Content (TFC)

The aluminum trichloride method with some modifications was used to determine the total flavonoid (TFC) content of the aqueous extract of «*Acti-plus*», using quercetin as a reference [11]. The reaction mixture was prepared by mixing 1 mL of the aqueous extract solution of «*Acti-plus*» with 1 mL of a 2% aluminum trichloride ($AlCl_3$) methanolic solution. After thirty (30) minutes of incubation at room temperature, the absorbance was measured at 415 nm using a spectrophotometer (SHIMADZU UV-Vis, Japan). The total flavonoid content was estimated from a quercetin calibration curve. The result was expressed in milligrams (mg) of quercetin equivalent per gramme (g) of dry extract (mg QE/g). The measurements were made in triplicate.

2.6. Antiradical Activity

2.6.1. DPPH Radical Scavenging Activity

The DPPH antiradical activity was evaluated using the method described by Velázquez et *al*. [12] with some modifications and adapted in a 96-well microplate. The aqueous extract of «*Acti-plus*» and quercetin used as control were dissolved in methanol to obtain a concentration of 1 mg/mL, from which a series of successive dilutions (to 1/2) were carried out in the 96-well plate. In each well, 100 µL of each concentration of aqueous extract of «*Acti-plus*» was mixed with 200 µL of DPPH (20 mg/L). For each concentration, three repetitions were performed in the microplate. A blank consisting of 100 µL methanol and 200 µL DPPH was used. After 15 minutes of incubation at room temperature, the absorbances were read at 517 nm using the spectrophotometer (Biotek Instruments, USA). Antioxidant activity was expressed as a percentage of inhibition of DPPH according to the formula:

$$\text{Percentage of inhibition (\%)} = (\text{Abs (B)}-\text{Abs (E)}) / (\text{Abs (B)}) \times 100$$

where Abs (B) and Abs (E) represent, respectively, the absorbances of the blank and the aqueous extract of «*Acti-plus*».

Concentrations (µg/mL) trapping 50% of free radicals (IC_{50}) were determined using the percentages of antiradical activity curves based on the concentrations of the aqueous extract of «*Acti-plus*».

2.6.2. ABTS Radical Scavenging Activity

This method is based on the discoloration of the stable radical cation ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] into ABTS in the presence of antiradical compounds. The ability of the aqueous extract of «*Acti-plus*» to trap ABTS cations radicals was determined according to the method of Re *et al*., adapted for 96-well microplates [13]. The ABTS solution was prepared by dissolving 10 mg of ABTS in 2.6 mL of distilled water. A mass of 1.7212 mg of potassium persulfate was added and the mixture was kept dark at room temperature for 12 hours. The mixture was then diluted in ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The diluted aqueous extract of «*Acti-plus*» and the control solution of Trolox were prepared from a stock solution at 1 mg/mL. Then, 20 µL of the different solutions were mixed with 200 µL of the diluted ABTS solution in a 96-well microplate. The absorbances were read by spectrophotometer (Biotek Instruments, USA) at 734 nm after 30 minutes away from light, and were used to calculate the % inhibition of $ABTS^{\bullet+}$ according to the following formula:

$$\text{Percentage of inhibition (\%)} = (\text{Abs (C)}-\text{Abs (E/T)}) / (\text{Abs (C)}) \times 100$$

Where Abs (C) and Abs (E/T) are, respectively, the absorbances of the control (ABTS radical solution without extract or Trolox and Extract/Trolox (ABTS radical + Extract/Trolox).

Concentrations (µg/mL) trapping 50% of free radicals (IC_{50}) were determined using the percentages of antiradical activity curves based on the concentrations of the aqueous extract of «*Acti-plus*».

2.7. Anti-lipoxygenase Activity

The anti-lipoxygenase potential of the aqueous extract of «*Acti-plus*» was evaluated by the inhibition of 15-lipoxygenase which is a pro-inflammatory enzyme. The principle is to follow *in vitro* the production of leukotrienes and lipoxins by lipoxygenase in the presence of an inhibitory

substance. The spectrophotometric method developed by [14] was used to evaluate the inhibitory activity of the aqueous extract of «*Acti-plus*» on 15-lipoxygenase. In 96 well microplates, four types of reaction mixture were prepared:

The reaction mixture of the blank enzyme: It was prepared by mixing 146.25 µL lipoxygenase (400 U/ mL) with 153.75 µL borate buffer (0.2 M, pH 9).

The reaction mixture of enzymatic activity: It was composed of 3.75 µL borate buffer, 146.25 µL lipoxygenase and 150 µL linoleic acid (1.25 mmol).

The reaction mixture of the blank extracted: 146.25 µL lipoxygenase, 3.75 µL of the aqueous extract solution of «Acti-plus» and 150 µL of the borate buffer were mixed to constitute this reaction mixture,

The reaction mixture of the activity of the aqueous extract of «Acti-plus»: It was prepared by mixing 146.25 µL of lipoxygenase, 3.75 µL of the solution of the aqueous extract of «Acti-plus» and 150 µL of linoleic acid.

Each mixture was prepared in triplicata in 96-well microplates. The absorbance was read at the spectrophotometer (Biotek Instruments, USA) at 234 nm just after the addition of the substrate (linoleic acid). The percentage of inhibition was calculated using the following formula:

Percentage of inhibition (%) = (Abs (control)-Abs (extract)) / (Abs (control)) × 100

Abs (control): Absorbance (activity enzyme) – Absorbance (blank enzyme)

Abs (extract): Absorbance (extract activity) – Absorbance (blank extract)

2.8. Cytotoxic Activity on Cultured Cells

2.8.1. Cultures of Cells

Prostate lines PC-3 (ATCC CRL-1435) and DU 145 (ATCC HTB-81) and cervical HeLa (ATCC CRM-CCL-2) cancer cell lineages were used in this study. They were provided to CERBA/LABIOGENE by the iGReD Laboratory (CNRS-INSERM-Clermont Auvergne University, France). The cell lineages were grown in 75 cm^2 flasks in an RPMI medium (for DU 145 and PC-3 cells) and DMEM (for HeLa cells) supplemented with 10% fetal calf serum (FCS, Biowest, Nuaillé, France), 1% penicillin/streptomycin (Invitrogen, Oslo, Norway) and 1% L-glutamine. The cultures were kept in the incubator in a moistened atmosphere containing 5% CO2 at 37°C. These confluence cells were washed with PBS-1X and harvested by trypsinisation and then seeded in 96 well culture plates for MTT testing (Mitochondrial Tetrazolium Test).

2.8.2. MTT Test

The MTT test was used to assess the effect of aqueous extract of «*Acti-plus*» on cell viability. To do this, cell lines PC-3, DU145 and HeLa were seeded at a rate of 10,000 cells per well in 96 flat-bottom well plates (in 100 µL of complete culture medium per well) and incubated. After 24 hours, the culture medium was removed from the wells and replaced with 100 µL of culture medium containing varying concentrations of the aqueous extract of «*Acti-plus*» in each well and re-incubed for 72 hours. After 72 hours of incubation, the MTT solution was solubilized and put in contact with the cells at the rate of 10 µL of MTT solution (5 mg/mL in PBS-1x) per well. After four hours of incubation, 100 µL of isopropanol was added to dissolve the formed formazan crystals. The plates were then stirred for 45 minutes on a plate agitator [15]. The absorbances were measured at a wavelength of 570 nm with a microplate reader (Biotek EL808 spectrophotometer). This allowed the percentage of inhibition to be calculated using the formula:

Percentage of inhibition (%) = (Abs (control)-Abs (blank)) / (Abs (extract)-Abs (blank)) × 100

Abs (control): Absorbance of the control

Abs (extract): Absorbance of the aqueous extract of «*Acti-plus*» – Absorbance (blank extract)

Abs (blank): Absorbance of blank

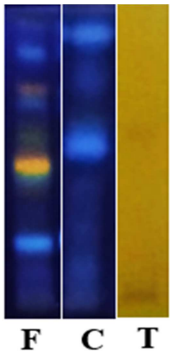
2.9. Statistical Analyses

The data were expressed as means ± standard deviation (SD) of three replicate determinations. GraphPad Prism software version 8.0.2 was used for statistical analysis and graph construction. A two-way analysis of variance (ANOVA) followed by Tukey multiple comparison and student tests were used to determine whether results had statistical significance. The difference was considered statistically significant for a p value < 0.05.

3. Results

3.1. Phytochemical Study

The dry vegetable powder of «Acti-plus» had a moisture content of 3.39 ± 0.02%. The aqueous solvent extraction efficiency of «*Acti-plus*» was 28.42 ± 0.32%. On the glass HPTLC plates covered with silica gel, the aqueous extract of «*Acti-plus*» was screened to identify phytochemical groups. Phytochemical targeting made it possible to highlight flavonoids, coumarins and tannins (Figure 1).



F: Flavonoids, C: Coumarins, T: Tannins

Figure 1. *Chromatogram of detection of flavonoids, coumarins and tannins in the aqueous extract of «Acti-plus».*

The total polyphenol content of the aqueous extract of «*Acti-plus*» was determined against a gallic acid reference curve of equation: y = 16.698x + 0.086 (R^2 = 0.9979), while the total flavonoids were determined from a quercetin reference curve equation: y = 19.038x + 0.0414 (R^2 = 0.9997). The results showed that the aqueous extract of «Acti-plus» has a total phenolic content of 61.95 ± 0.01 mg GAE/g dry extract and total flavonoids of 43.01 ± 0.05 mg QE/g dry extract (Table 2).

Table 2. *Phytochemical Analysis of the Aqueous Extract of «Acti-plus».*

	Aqueous extract of «*Acti-plus*»
Extraction yield (%)	28.42 ± 0.32
Residual moisture content (%)	3.39 ± 0.02
TPC (mg GAE/g dry extract)	61.95 ± 0.01
TFC (mg QE/g dry extract)	43.01 ± 0.05

TPC: Total polyphenol content, TFC: Total flavonoid content. Values are presented as mean ± SD (n=3).

3.2. Antiradical Activity

Thus, the results obtained on the inhibition of DPPH and ABTS radicals (Table 3) by the aqueous extract of «*Acti-plus*» compared to quercetin and trolox respectively indicate that it has a lower inhibitory power. Nevertheless, the aqueous extract of «*Acti-plus*» exhibited excellent inhibition of the DPPH radical (IC_{50} = 9.33 ± 1.53 µg/mL). The reduction in $ABTS^{\bullet+}$ production can be considered significant because the IC_{50} value was less than 150 µg/mL (IC_{50} = 53.33 ± 0.89 µg/mL).

Table 3. *Antiradical activity the aqueous extract of «Acti-plus»*

	Aqueous extract of «*Acti-plus*»	Quercetin	Trolox
DPPH IC_{50} (µg/mL)	9.33 ± 1.53**	4.41 ± 0.20	nd
ABTS IC_{50} (µg/mL)	53.33 ± 0.89****	Nd	2.51 ± 0.09

Values are presented as mean ± SD (n=3). In the same line, the extract value was compared to its corresponding reference (Quercetin/Trolox) using the student test. **p<0.01, ***p<0.001 and ****p<0.0001 versus references. nd: not determined

3.3. Anti-lipoxygenase Activity

The result of the inhibitory activity of 15-LOX, by the aqueous extract of «*Acti-plus*» is presented in Table 4. The result show that the extract moderately inhibits lipoxygenase compared to the reference (Indomethacin). In addition, the percentage of inhibition of 15-LOX by the extract at 100 µg/mL was 29.19 ± 1.38% (IC_{50} > 100 µg/mL).

Table 4. *Effect of «Acti-plus» aqueous extract on 15-lipoxygenase inhibition.*

	Aqueous extract of «*Acti-plus*»	Indomethacin
15-LOX Inhibition (%)	29.19 ± 1.38****	91.51 ± 0.34

Values are presented as mean ± SD (n=3). In the 15-LOX column, the extract value was compared to its corresponding reference (Indomethacin) using the student test. **p<0.01, ***p<0.001 and ****p<0.0001 versus the reference.

3.4. Cytotoxic Activity on Cancer Cells

Data on the cytotoxic activity of the aqueous extract of «*Acti-plus*» in cancer cells PC-3, DU 145 and HeLa are shown in Figure 2. They indicate a decrease in cell viability as a function of the concentration of the aqueous extract of «*Acti-plus*». The percentage of viability increases from 91.13% to 19.12% on PC-3 cells; from 92.06% to 30.08% on DU 145 cells and from 94.05% to 34.49% on HeLa cells when the concentration of the aqueous extract of «*Acti-plus*» varies from 0.03125 mg/mL to 1 mg/mL (Figure 2).

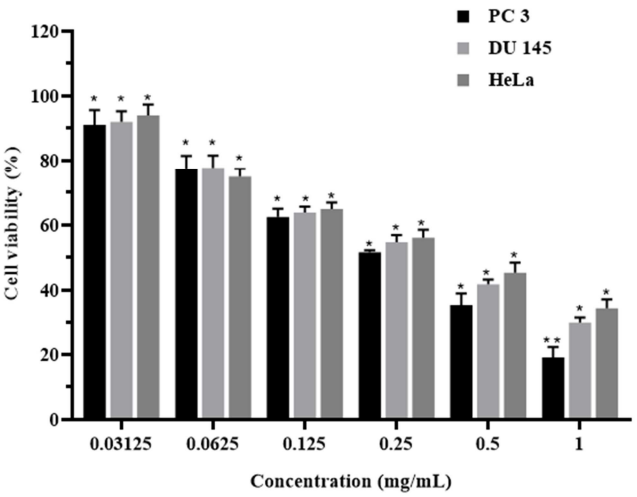


Figure 2. *Cytotoxic activity of « Acti-plus » aqueous extract on PC-3, DU 145 and HeLa cancer cells.*

For each concentration the same number of stars (*) with p<0.05, corresponds to percentages of cell viability without significant difference. While the number of different stars (*) corresponds to percentages of cell viability having a significant difference. Values are presented as mean ± SD; n = 3 independent experiments.

The extract had a half inhibitory concentration (IC_{50}) of 0.239 ± 0.01 mg/mL in the PC-3 cell line, 0.317 ± 0.02 mg/mL in the DU 145 cell line and 0.362 ± 0.02 mg/mL in the HeLa cell line (Table 5).

Table 5. *IC_{50} (mg/mL) of extract on cell lines (PC-3 and DU 145) of prostate cancer and HeLa of cervical cancer.*

Cell lines	IC_{50} of Aqueous extract of «*Acti-plus*» (mg/mL)
PC-3	0.239 ± 0.014
DU 145	0.317 ± 0.021***
HeLa	0.362 ± 0.027****

Values are presented as mean ± SD; n = 3 independent experiments. IC_{50} values were compared with each other using the two-way analysis of variance (ANOVA) followed by Tukey multiple tests, with a significant difference for p<0.05 (**p<0.01, **p<0.001 and ***p<0.0001)

4. Discussion

The plants have been explored for thousands of years and are traditionally used by various cultures. It is estimated that to date, about 70-95% of developing countries still use medicinal plants [16]. The practice of traditional medicine

has institutional and regulatory structures in most African countries, including Burkina Faso. Traditional healers are organized in association at regional and national levels. These actors are part of the health system and benefit from capacity building to equip them with the formulation of improved traditional medicines [17]. In Burkina Faso, several recipes of medicinal plants are developed by traditional health practitioners and used in several pathologies including cancer. The combination of medicinal plants is a new approach used in the treatment of diseases. It involves the interaction of two or more compounds with several simultaneous targets and is considered a more effective form of drug therapy against complex diseases such as cancer [18]. Thus, the recipe «*Acti-plus*» developed by a traditional practitioner from four medicinal plants from Burkina Faso and used in cancer prevention and management of cancer patients was the subject of this study in order to produce a scientific basis for its use traditional.

The extraction efficiency of the aqueous extract of «*Acti-plus*» was 28.42 ± 0.32% and the residual moisture content was 3.39 ± 0.02% (less than 10%). This result indicates that the recipe «*Acti-plus*» has good preservation properties according to the standards of the European Pharmacopoeia and could be kept with a low risk of contamination, deterioration of the microbiological quality of the recipe and/or alteration of the chemical principles [19]. Aqueous extraction is often used to recover very polar compounds such as polyphenols, flavonoids, tannins, saponins and coumarins.

Phytochemical analysis revealed these chemical groups in aqueous extract of «*Acti-plus*». Quantitative tests yielded a total polyphenol content of 61.95 ± 0.01 mg GAE/g dry extract and total flavonoids of 43.01 ± 0.05 mg QE/g dry extract, confirming the effective presence of flavonoids that are known for their anti-radical effects, anti-lipoxygenase and anticancer [20, 21].

The extract had excellent DPPH and ABTS radical free radical removal capacity, with IC_{50} values of 9.33 ± 1.53 µg/mL and 53.33 ± 0.89 µg/mL respectively. The study also found that the aqueous extract of «*Acti-plus*» moderately inhibits 15-lipoxygenase with an inhibition percentage of 29.19 ± 1.38% at the concentration of 100 µg/mL. Several studies have shown that the medicinal plants that make up this recipe follow these trends. Cao-Ngoc et *al*., showed that the aqueous extract of *Chrysanthelllum americanum* DC was mainly composed of flavonoids (flavanones, flavones and aurones) and phenolic acids (hydroxycinnamic acid) [22]. In a study conducted in Burkina Faso, Guenne et *al*., evaluated the antioxidant capacity and anti-lipoxygenase potential of *Chrysanthelllum americanum* DC. The results showed that the main components of *Chrysanthelllum americanum* DC are flavonoids, coumarins, tannins, polyphenols that inhibited free radicals DPPH and ABTS, with IC_{50} values of 10.10 ± 0.72 µg/mL and 65.30 ± 0.30 µg/mL respectively. It also moderately inhibited lipoxygenase with an inhibition percentage of 22.88 ± 2.38% at 100 µg/mL [23]. This plant is traditionally used in the treatment of fever, hepatitis, gastrointestinal pain, rheumatism and kidney disease. It also has healing and hepatoprotective properties [24, 25]. Aissani et al., reported that *Petroselium crispum* is mostly composed of flavonoids such as isorhamnetin, apigenin, quercetin, luteolin, and chrysoeriol [26]. These flavonoids could be responsible for the antiradical activity of *Petroselium crispum* because these compounds can easily yield hydrogen atoms to free radicals. The results of phytochemical analysis of research conducted by Al Aboody et *al*., showed that *Apium graveolens* contains tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, carbohydrates, proteins and anthraquinones [27]. Among the phytochemicals of *Apium graveolens*, he was able to identify several molecules namely caffeic acid, ferulic acid, apigenin, luteolin and kaempferol known for their powerful antioxidant properties [28]. Thus, the hydroethanolic extract of *A. graveolens* leaves produced excellent anti-radical activity with IC_{50} values of 11.25 ± 0.05 µg/mL for DPPH and 10.25 µg/mL for ABTS [27]. The antiradical activity of *Guiera senegalensis* leaves has been reported by Moriod et *al*., Sudan and Sombie et *al*., Burkina Faso [29, 30]. This activity would be due to the chemical compounds contained in the plant. Indeed, several studies have shown that extracts of *Guiera senegalensis* contain mainly flavonoids, tannins, saponins, coumarins, alkaloids [30, 31]. It appears clearly that these four medicinal plants of this recipe «*Acti-plus*» are rich in active phytochemical compounds both quantitatively and qualitatively, which justifies the therapeutic effects of these plants and thus of the recipe «*Acti-plus*».

The aqueous extract of the recipe «*Acti-plus*» inhibited the proliferation of the three cancer cell lineages with an IC_{50} of 0.239 ± 0.01 mg/mL on the PC-3 prostate cancer cell line, 0.317 ± 0.02 mg/mL on the DU 145 prostate cancer line and 0.362 ± 0.02 mg/mL on the HeLa cell line of cervical cancer. PC-3 cells were therefore more sensitive to the aqueous extract of «*Acti-plus*» than DU 145 and HeLa cells. Moreover, these three cell lines inhibited cell proliferation in a concentration-dependent manner. These results remain lower than those of Bayala et *al*., who found IC_{50} values of 21.1 µg/mL in line PC-3 and 146.17 µg/mL in line Hela with extracts of *Cymbopogon citratus* (DC) Stapf. and *Cymbopogon schoenanthus* (L.) Spreng. respectively [32, 33]. In addition, several studies have shown that the medicinal plants used to develop the recipe «*Acti-plus*» has anticancer activities towards various cancer cell lines. *Petroselium crispum* is known for its chemopreventive properties of cancer. Indeed, Tang et *al*., showed that *Petroselium crispum* a would protect normal 3T3-L1 cells from H_2O_2-induced DNA damage, suggesting its potential in cancer prevention. In addition, it blocked the migration of MCF-7 breast cancer cells, demonstrating its potential to prevent metastases [34]. This preventive effect of *Petroselium crispum* would be attributed to the activity of the flavonoids it contains, in particular apigenin and lutein which would have the ability to prevent motility and

cell invasion and would therefore act as chemopreventive agents of metastases [35, 36]. Köken et *al*., showed that *Apium graveolens* induces apoptosis of prostate cancer cells LNCaP by cleavating caspase 3 and poly-(ADP-ribose) polymerase (PARP) proteins [37]. Regarding the anticancer activity of the medicinal plant *Guiera senegalensis*, Kouame et *al*., evaluated the anticancer activity of aqueous extract of leaves of *Guiera senegalensis* against prostate cancer PC-3 cells, glioblastoma U373 and breast cancer MCF-7. This extract strongly inhibited MCF-7 cells (IC_{50}=2.1 ± 0.5 µg/mL) but moderately inhibited PC-3 cells (IC_{50}=219.29 µg/mL) and U373 cells (IC_{50}=258.18 ± 0.5 µg/mL) [38]. The cytotoxicity of guieranone A, one of the essential molecules of *Guiera senegalensis*, was performed on two cancer cell lines (HeLa and HCT-116) and fibroblasts of normal skin to study its anticancer potential. This component had considerable cytotoxic properties, with an IC_{50} of 9.2 and 6.9 M on HeLa and HCT-116 cell lines, respectively [39]. Although the in vitro cytotoxic activity of the aqueous extract of the recipe «*Acti-plus*» is moderate, an *in vivo* evaluation is necessary to draw precise conclusions on the preventive and anticancer effects of this recipe.

5. Conclusion

Our study focused on the recipe «*Acti-plus*», composed from four medicinal plants (*Chrysanthelllum americanum* DC, *Petroselium crispum* (mill) fuss, *Apium graveolens* L., *Guiera senegalensis* J. F. Gmel) from Burkina Faso. The aqueous extract of this recipe contains flavonoids, coumarins and tannins. It showed excellent antiradical potential against DPPH and ABTS radicals but moderately inhibited 15-lipoxygenase. *In vitro*, the aqueous extract of «*Acti-plus*» inhibited the viability of prostate and cervical cancer cells in culture. And this in a dependent concentration. This study provides scientific evidence of the traditional use of the recipe «*Acti-plus*». However, this study must be deepened by exploring the molecular mechanisms involved and consider *in vivo* studies on an animal model especially for prostate cancer androgeno-resistant to the image of the PC-3 cell.

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Conflicts of Interest

The authors declare no conflicts of interest.

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