Original Research Article

Antioxidant, anti-inflammatory and cytotoxic properties of *Cancerol S*, a blend of medicinal plants, on cancerous cells in culture

ABSTRACT

Aims: To evaluate *in vitro* the properties of a blend of medicinal plants called *Cancerol S* through its antioxidant, anti-inflammatory and cytotoxic potential on cancerous cells in culture.
Methodology: Phytochemical screening as well as total polyphenols and flavonoids assays were performed using characterization and assay tests, spectrometry and high-performance thin layer chromatography. Radical trapping tests (DPPH and ABTS) and iron III reducing power (FRAP) were used to measure the antioxidant capacity. Anti-inflammatory activity was studied using inhibition of 15-LOX (15-lipoxygenase) and COX1/COX2 (cyclooxygenases). Cytotoxic activity of *Cancerol S* was evaluated *in vitro* using MTT test.
Results: Total phenolic compounds (TPC) and total flavonoids (TFC) were found in abundance in the extract of *Cancerol S* with contents of 115.63 ± 0.08 mg GAE/g dry extract and 43.41 ± 0.02 mg QE/g dry extract respectively. The extract exhibited good scavenging capacity of DPPH (IC_{50} = 8.26 ± 0.73 µg/mL) and ABTS radicals (IC_{50} = 35.98 ± 1.12 µg/mL) and also reduced ferric ion Fe^{3+} (2.67 ± 0.02 mmol AAE/g extract). Study found that *Cancerol S* extract had relatively moderate inhibitory activity against 15-lipoxygenase, cyclooxygenase 1 and cyclooxygenase 2. Aqueous extract of *Cancerol S* inhibited the proliferation of both cell lines used in a dose-dependent manner with an IC_{50} value of 140.72 ± 7.76 µg/mL for prostate cancer cell line DU 145 and 84.59 ± 5.18 µg/mL for the HeLa cell line of cervical cancer.
Conclusion: These properties give a rational basis for the use of *Cancerol S* in traditional medicine in Burkina Faso.

Keywords: cytotoxic, antioxidant, anti-inflammatory, Cancerol S

1. INTRODUCTION

Cancer is one of the leading causes of death worldwide. According to the International Agency for Research on Cancer (IARC), there were 19.3 million new cases and 9.9 million cancer-related deaths in 185 countries worldwide in 2020 [1]. In Burkina Faso, the number of new cases in the same year was estimated at 12,000 and the number of deaths at 8,700, mainly due to breast, liver, cervical and prostate cancers [2]. The etiology of cancer is multifactorial and mainly due to the accumulation of mutations, genetic predisposition, chronic oxidative stress and inflammation of organs [3,4]. Conventional cancer treatments mainly include surgery, radiation and chemotherapy [5]. Despite progress in improving these treatments, serious side effects, multiple drug resistance, the unmanageable effects of metastatic tumors, the availability and accessibility of cancer drugs remain real challenges for the cancer scientific community. Faced with these difficulties, the development of alternative solutions is essential. Researchers have made efforts to find a new, more effective approach, and traditional medicine via these compounds has emerged as a very promising alternative for cancer treatment [6,7]. The use of medicinal plants and their molecules to treat cancer is therefore a

UNDER REVIEW

revolutionary field because they are simple, safe, environmentally friendly, inexpensive, fast and less toxic than conventional treatments. They are also functionally selective, acting specifically on tumor cells while sparing normal cells [8]. However, medicinal plants must first be studied to elucidate their toxicity, efficacy and sustainable availability for large-scale use. Four main activities are taken into account in the research of anticancer phytomedicines: antioxidant, anti-inflammatory, anti-angiogenic and cytotoxic [9]. Several anticancer medicinal plant recipes developed by traditional health practitioners based on traditional knowledge are marketed in Burkina Faso. One of the recipes, called *Cancerol S*, has been the subject of our study. *Cancerol S* is a blend of plants, provided by a tradipratician. It is composed of four plants from Burkina Faso (*Solanum incanum* (Nees) L., *Ocimum americanum* L, *Combretum micranthum* G. DON., *Combretum adenogonium* Steud ex A. Rich). These plants are commonly used, but the combination of these plants in the treatment of cancer has not yet been scientifically tested *in vitro* and/or *in vivo*. The objective of this study was therefore to scientifically verify *in vitro* the effectiveness of this recipe by evaluating its antioxidant, anti-inflammatory and antiproliferative properties of the aqueous extract of *Cancerol S* for a probable formulation of an anticancer drug with plant origin.

2. MATERIAL AND METHODS

2.1 Phytochemical study

2.1.1 Plant material

The plant drug called *Cancerol S* 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 tumors and cancers including breast, prostate and cervix tumors among others in Ouagadougou (Burkina Faso). It was developed mainly from the leaves of four traditional plants with well-defined proportions, and informally to avoid the popularization of the recipe (Table 1).

UNDER PEER REVIEW

Table 1. Medicinal plants of *Cancerol S*

Medicinal plants	Families	Part of plant used	Name in Mooré (Local language of Burkina Faso)
Solanum incanum (Nees) L.	Solanaceae	Leaves	Noraogo-Kuumbré
Ocimum americanum L.	Lamiaceae	Leaves	Yulin-gnu-raaga
Combretum micranthum G. DON. (Kinkeliba) Synonym : *Combretum Altum* Perr	Combretaceae	Leaves	Randga
Combretum adenogonium Steud ex A. Rich Synonym : *Combretum fragrans* F. Hoffm	Combretaceae	Leaves	Kwiguinga

2.1.2 Extraction

Cancerol S was extracted according to the instructions of the traditional health practitioner. Thus, the aqueous decoction method was used. This decoction was carried out by boiling 15 grammes of the recipe powder in 1.5 liters of distilled water for 10 minutes. The extract was

left at room temperature to cool before being filtered. The filtrate was then centrifuged at 2000 rpm for 10 minutes. The supernatant was recovered and freeze-dried.

2.1.3 Determination of extraction yield and residual moisture content

Extraction yield: The extraction yield was calculated using the following formula:

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

Where **M'** is the weight of the dry extract of *Cancerol S*, and **M** is the weight of the dry vegetable powder.

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:

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

2.1.4 Phytochemical screening

Phytochemical screening of the aqueous extract of *Cancerol S* 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 *Cancerol S* 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 five 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 became visible [10].

2.1.5 Phytochemical determination

Determination of total polyphenols content (TPC): The total phenolic content (TPC) of the aqueous extract of *Cancerol S* was quantified by spectrophotometry using the Folin-Ciocalteu method [11], with slight modifications. The reaction mixture was prepared with 1 mL of the aqueous extract solution of *Cancerol S* 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)

UNDER PEER REVIEW

of dry extract (mg GAE/g) was calculated from a gallic acid calibration curve. The measurements were made in triplicate.

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 *Cancerol S*, using quercetin as a reference [12]. The reaction mixture was prepared by mixing 1 mL of the aqueous extract solution of *Cancerol S* 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.

2.2 Antioxidant activity

2.2.1 DPPH Radical Scavenging Activity

The antiradical activity DPPH was evaluated at room temperature according to the method described by Velázquez et al. [13] with some modifications, and adapted for 96-well microplates. The aqueous extract of *Cancerol S* and quercetin were dissolved in methanol to obtain a concentration of 1 mg/mL, from which a series of successive dilutions (to 1/2) was carried out. In each well, 100 µL of each concentration of the aqueous extract of *Cancerol S* 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, the absorbances were read at 517 nm using the spectrophotometer (BioTek Instruments, Vinooski, Vermont, USA). Antioxidant activity was expressed as a percentage of inhibition of DDPH according to the formula:

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

where Abs (B) and Abs (E) represent, respectively, the absorbances of the blank and the aqueous extract of *Cancerol S*.

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 *Cancerol S*.

2.2.2 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 capacity of the aqueous extract of *Cancerol S* to trap $ABTS^{\bullet+}$ was determined according to the method of Re et al., adapted for 96-well microplates [14]. 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 is kept dark at room temperature for 12 hours. The mixture is then diluted in ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The diluted aqueous extract of *Cancerol S* and 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, Vinooski, Vermont, USA) at 734 nm after 30 minutes away from light, and were used to calculate the percentage of inhibition of $ABTS^{\bullet+}$ according to the following formula:

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

UNDER PEER REVIEW

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 *Cancerol S*.

2.2.3 Iron Reduction assay

The FRAP method (Ferric Reducing Antioxidant Power) is a colorimetric method based on the ability of an extract to reduce initially colorless ferric ions (Fe^{3+}) into ferrous ions (Fe^{2+}) of blue color. The ability of the aqueous extract of *Cancerol S* to reduce ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) was determined from a FRAP solution that was prepared by mixing sodium acetate buffer (300 mM) adjusted to a pH of 3.6; TPTZ (2,4,6-Tris(2-pyridyl) -s-triazine) solution (10 mM) and ferric chloride $FeCl_3$ in the proportions of 10: 01: 01 respectively. The aqueous extract of *Cancerol S* was dissolved in the extraction solvent to a concentration of 1 mg/mL. 10 µL of the extract was added to 300 µL of the FRAP solution in a 96-well plate. The absorbance was read after 30 minutes of incubation in the dark.

2.3 Anti-inflammatory activity

2.3.1 15-lipoxygenase (15-LOX) Inhibition assay

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 Malterud and Rydland [15] was used with minor modifications to evaluate the inhibitory activity of the aqueous extract of *Cancerol S* 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 *Cancerol S* 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 *Cancerol S*: It was prepared by mixing 146.25 µL of lipoxygenase, 3.75 µL of the solution of the aqueous extract of *Cancerol S* 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, Vinooski, Vermont, 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)

2.3.2 Cyclooxygenase (COX 1 and COX 2) inhibition assay

UNDER PEER REVIEW

The COX 1 and COX 2 inhibition test was performed using a commercially available colorimetric COX (Ovine/Human) inhibitor screening test kit (Cayman Chemical Company, Maine, USA, Number: 560131). In this test, the activity of cyclooxygenases was measured using a colorimetric substrate, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) as a co-substrate with arachidonic acid. For this test, three (3) types of reaction mixture were prepared:

- The enzymatic activity reaction mixture (COX 1 and COX 2): It was prepared by mixing 150 µL Buffer, 10 µL Hemin, 10 µL Enzyme (COX 1/COX 2) and 10 µL Extract dilution solvent (Methanol);

- The reaction mixture of the activity of the aqueous extract of *Cancerol S*: It was made up of 150 µL of Tampon, 10 µL of Hemin, 10 µL of enzyme (COX 1/COX 2) and 10 µL of the solution of the aqueous extract of *Cancerol S*;

- The blank reaction mixture: It was made up of 160 µL of Tampon, 10 µL of Hemin and 10 µL of the dilution solvent of the extract (Methanol);

20 µL of the colorimetric substrate (TMPD) and 20 µL of arachidonic acid were added to the three (3) reaction mixture. Each reaction mixture was prepared in triplicata in 96 well microplates. After incubation at 25ºC for 2 minutes, and absorbances were read at 590 nm. The percentage of inhibition was calculated using the following formula:

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

Abs (enzyme) = Absorbance (activity enzyme) – Absorbance (blank)

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

2.4 Cytotoxicity on cultured cancer cells

2.4.1 Cultures of cells

Prostate DU 145 (ATCC HTB-81) and cervical HeLa (ATCC CRM-CCL-2) cancer cell lines were used in this study. They were provided to CERBA/LABIOGENE by the iGReD Laboratory (CNRS-INSERM-Université Clermont Auvergne, France). The lines were grown in 75 cm^2 flasks in RPMI medium (for DU 145 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).

2.4.2 MTT test

The MTT test was used to assess the effect of the aqueous extract of *Cancerol S* on cell viability. To do this, cell lines DU145 and HeLa were seeded at a rate of 10,000 cells per well in 96 flat-bottom well plates (100 µL suspension per well) and incubated. After 24 hours, the cells were treated with varying concentrations (100 µL) of the extract and reincubed for 72 hours. After 72 hours of incubation, the MTT solution was solubilized and brought into contact with 10 µL MTT solution (5 mg/mL in PBS-1x) per well. After four (4) hours of incubation to allow the production of formazan crystals, 100 µL of isopropanol was added to dissolve formazan crystals. The plates were then stirred for 45 minutes. The absorbances were measured at a wavelength of 570 nm with a microplate reader (Biotek EL808

UNDER PEER REVIEW

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 *Cancerol S* – Absorbance (blank extract)

Abs (blank): Absorbance of blank

2.5 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, graphs and to determine the correlation of antioxidant/anti-inflammatory tests with polyphenolic compounds by the Pearson correlation coefficient. The student test was used to compare two means. The difference was considered statistically significant for a p value < 0.05.

UNDER PEER REVIEW

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical study

The dry plant powder of *Cancerol S* had a moisture content of 4.17 ± 0.67%. The aqueous solvent extraction efficiency of *Cancerol S* was 18.66 ± 0.32%. On the silica gel-coated HPTLC plates, the aqueous extract was screened to identify phytochemical groups such as flavonoids, coumarins, and tannins (Fig. 1).

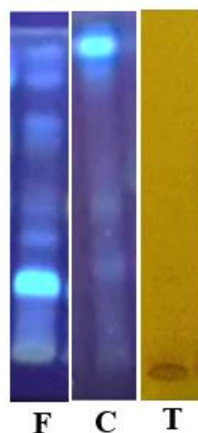

Fig. 1. Chromatogram of detection of flavonoids, coumarins and tannins in the aqueous extract of *Cancerol S*

F: Flavonoids, C: Coumarins, T: Tannins

The total polyphenol content of the aqueous extract of *Cancerol S* 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 = 16.038x + 0.0414 (R^2=0.9997). The results showed that the aqueous extract of *Cancerol S* had a total phenolic content of 115.63 ± 0.08 mg GAE/g dry extract, and 43.41 ± 0.02 mg QE/g dry extract as total flavonoids (Table 2).

Table 2. Phytochemical Analysis of the Aqueous Extract of *Cancerol S*

	extraction yield (%)	residual moisture content (%)	TPC (mg GAE/g dry extract)	TFC (mg QE/g dry extract)
Cancerol S	18.66 ± 0.32	4.17 ± 0.67	115.63 ± 0.08	43.41 ± 0.02

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

3.1.2 Antioxidant activity

The results obtained on the inhibition of DPPH and ABTS radicals (Table 3) by the aqueous extract of *Cancerol S* compared to quercetin and trolox respectively indicate that it has a lower inhibitory power. Nevertheless, the aqueous extract of *Cancerol S* exhibited excellent inhibition of the DPPH radical (IC_{50} = 8.26 ± 0.73 µg/mL). The reduction in ABTS radical production has been considered as significant (IC_{50} = 35.98 ± 1.12 µg/mL) since it is less than 50 µg/mL. The ferric reducing antioxidant content of the aqueous extract of *Cancerol S* was determined against a reference curve of ascorbic acid equation: y = 0.8337x + 0.0499 (R^2 = 0.9976). The result obtained indicates that the extract has a significant ferric ion reducing power (2.67 ± 0.02 mmol AAE/g extract), which is however lower than that of reference rutin.

Table 3. Antioxidant activity the aqueous extract of *Cancerol S*

	DPPH IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)	FRAP (mmol AAE/g extract)
Cancerol S	8.26 ± 0.73***	35.98 ± 1.12****	2.67 ± 0.02****
Quercetin	4.41 ± 0.20	-	-
Trolox	-	2.51 ± 0.09	-
Rutin	-	-	3.58 ± 0.06

*Values are presented as mean ± SD (n=3). In the same column, the extract value was compared to its corresponding reference (Quercetin/Trolox/Rutin) using the student test. **p<0.01, ***p<0.001 and ****p<0.0001 versus references.*

3.1.3 Anti-inflammatory activity

The results of the inhibitory activity of 15-LOX, and COX 1 and COX 2 by the aqueous extract of *Cancerol S* are presented in Table 4. The results show that the extract has a low inhibitory effect on lipoxygenase and cyclooxygenase, because at a concentration of 100 µg/mL, their inhibition percentages were less than 50% (IC_{50} > 100 µg/mL). The percentages of inhibition of the extract had 100 µg/mL were 15.85 ± 1.11%; 37.19 ± 1.65% and 29.22 ± 1.34% respectively for 15-LOX, COX 1 and COX 2.

UNDER PEER REVIEW

Table 4. Antioxidant activity the aqueous extract of *Cancerol S*

	Percent inhibition (%) at 100 µg/mL		
	15-LOX Inhibition (%)	COX 1 Inhibition (%)	COX 2 Inhibition (%)
Cancerol S	15.85 ± 1.11****	37.19 ± 1.65	29.22 ± 1.34
Indomethacin	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.*

3.1.4 Correlation between antioxidant, anti-inflammatory and polyphenolic compounds tests

The Pearson correlation coefficient (r) was used to identify a possible relationship between antioxidant/anti-inflammatory tests and polyphenolic compounds and antioxidant and anti-inflammatory tests. When this coefficient is between 0.7 and 1 or -1 and -0.7, the correlation is considered highly positive or negative, respectively. By account, if it is between 0.3 and 0.7 or - 0.7 and - 0.3, the correlation is said to be positive or moderate negative. A value of r between 0 and 0.3 or - 0.3 and 0, means a positive or negative poor correlation [16]. The results of the study showed that the elimination of DPPH radicals was moderately correlated with TFC (r = 0.467), while the FRAP test was strongly correlated with TFC (r = 0.891) and TPC (r = 0.982). These results suggest that flavonoids are probably the main compounds responsible for these antioxidant activities. The ABTS test was negatively correlated (r = -0.169) with flavonoids. In terms of anti-inflammatory activity, COX 1 and COX 2 inhibition were positively correlated with polyphenols (r = 0.5 and r = 1 respectively), with a significant difference between TPC and COX 2 inhibition. Finally, the study of the correlation between antioxidant potential and enzymatic inhibitors showed a very strong correlation (r = 0.982) between the FRAP-reducing power and the inhibition of COX 2 and a moderate correlation (r = 0.655) between FRAP reducing power and COX 1 inhibition (Table 5).

Table 5. Correlation matrix (Pearson correlation coefficients (r)) of the aqueous extract of *Cancerol S*

	DPPH	FRAP	ABTS	15-LOX	COX 1	COX 2	TPC	TFC
DPPH	1.000							
FRAP	0.015	1.000						
ABTS	-0.951	0.296	1.000					
15-LOX	0.894	-0.434	-0.989	1.000				
COX 1	-0.746	0.655	0.916	-0.965	1.000			
COX 2	0,202	0.982	0.112	-0.257	0.501	1.000		
TPC	0,203	0.982	0.111	-0.257	0.500	1.000	1.000	
TFC	0,467	0.881	-0.169	0.022	0.240	0.960	0.961	1.000

UNDER PEER REVIEW

3.1.4 Cytotoxic activity of *Cancerol S* on cancer cells

Figure 2 presents the results of the effect of the aqueous extract of *Cancerol S* on the viability of DU 145 cells, derived from metastatic prostate cancer, and HeLa cells, derived from cervical cancer. The extract at concentrations of 31.25 µg/mL to 500 µg/mL causes a dose-dependent inhibition of cell proliferation on both cell lines (Fig. 2). The extract had a half-maximum inhibitory concentration (IC_{50}) of 140.72 ± 7.76 µg/mL on DU 145 cells line and 84.59 ± 5.18 µg/mL on HeLa cells line, with a statistically significant difference ($p = 0.0007$).

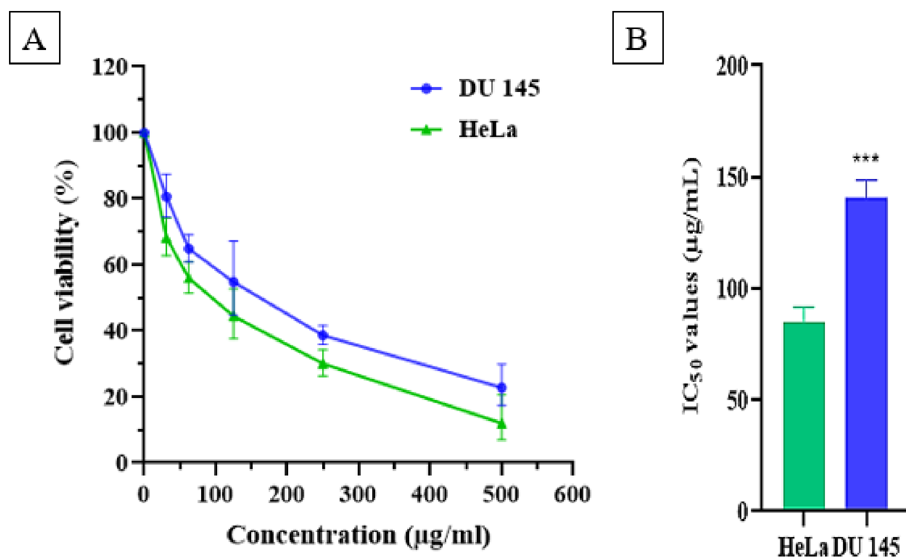

Fig. 2. Effect of *Cancerol S* aqueous extract on cancer cells

A: Dose-dependent antiproliferative activity of extract on prostate cancer DU 145 cell lines and cervical cancer HeLa. B: IC_{50} (µg/mL) of extract on prostate cancer DU 145 cell lines and cervical cancer HeLa. Values are presented as mean ± SD; n = 3 independent experiments

3.2 Discussion

Medicinal plants have long been used endogenously in the treatment of many pathologies. Research is needed to demonstrate their effectiveness and benefits to better use them in drug development projects. In recent years, medicinal plants have been at the center of the discovery of new powerful and effective anticancer compounds [17]. Combined therapy is a new approach in the fight against cancer. 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]. One of the fundamental advantages of combined therapy is the creation of a “synergy”, where the combined effect is greater than the sum of the individual effects [19]. Thus, the *Cancerol S*, a recipe developed by a traditional healer from four medicinal plants from Burkina Faso and used traditionally in the management of people suffering from cancer was studied in order to produce a scientific basis for its traditional use. This study aimed to verify the effectiveness of this formulation. Given the existing relationship between oxidative stress, inflammation and cancer, antioxidant and anti-inflammatory activities of *Cancerol S in vitro* was evaluated. In fact, during inflammation, inflammatory cells produce various reactive oxygen species that induce pathological damage and generate a pro-oxidant state. These reactive oxygen species can induce DNA strand breaks, DNA mutations and epigenetic changes in proto-oncogenes, tumor suppressor genes

and other genes encoding proteins that control apoptosis, survival, DNA repair and cell cycle. Damage could promote the initiation and growth of cancer [20].

The phytochemical study revealed that the extraction yield of the aqueous extract of *Cancerol S* was 18.66 ± 0.32% and the residual moisture content of 4.17 ± 0.67%, or less than 10%. This result shows that *Cancerol S* 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 [21]. Aqueous extraction is often used to recover very polar compounds such as phenolic compounds, flavonoids, tannins. Phytochemical analysis of the aqueous extract of *Cancerol S* has highlighted these chemical compounds. Quantitative tests yielded the total polyphenol content of 115.63 ± 0.08 mg GAE/g dry extract and total flavonoids of 43.41 ± 0.02 mg QE/g dry extract, confirming the effective presence of flavonoids that are known for their antioxidant effects, anti-inflammatory and anticancer [22,23]. Several studies have shown that the medicinal plants that make up this recipe follow this trend. Zengin et al., showed the presence of flavonoids, phenols and tannins in the aqueous, methanolic extracts of the leaves and flowers of *Ocimum americanum*, with the presence of compounds such as gallic acid, rutin, luteolin, quercetin and apigenin, all known for their antioxidant, anti-inflammatory and anticancer activities [24]. In addition, phytochemical tests of *Solanum incanum* aqueous leaf extract were positive for tannins and coumarins but not for flavonoids [25]. Zahoui et al., characterized sterols and polyterpens, polyphenols, flavonoids, tannins, quinones and alkaloids in the aqueous extract of the leaves of *Combretum micranthum* [26]. Finally, Nounagon et al., said that the leaves of *Combretum adenogonium* are rich in chemicals such as flavonoids, leucoanthocyans, anthocyanins, triterpenoids and tannins [27]. Thus, it appears clearly that these four medicinal plants of this recipe *Cancerol S* are rich in active phytochemical compounds both quantitatively and qualitatively, which could suggest the therapeutic effects of these plants and therefore of *Cancerol S*.

The antioxidant effect of the aqueous extract of *Cancerol S* studied using the anti-radical tests DPPH and ABTS, and the ferric ion reduction test (FRAP) was significant, with IC_{50} values = 8.26 ± 0.73 µg/mL for DPPH (less than 10 µg/mL), IC_{50} = 35.98 ± 1.12 µg/mL (less than 50 µg/mL) for ABTS and 2.67 ± 0.02 mmol AAE/g extract for FRAP. However, these values are statistically lower than the reference compounds. Moreover, correlations between TPC/TFC and DPPH and FRAP tests reflect that polyphenol, especially flavonoids, may be the compounds responsible for these antioxidant activities. Indeed, the antioxidant activity of polyphenols would be due to their redox properties that play an important role in the adsorption, capture, and neutralization of free radicals or in the decomposition of peroxides [28]. The lack of correlation between the ABTS test and TPC/TFC may be due to the fact that other compounds present in the aqueous extract of *Cancerol S* are responsible for the inhibition of ABTS radicals. In summary, the aqueous extract of *Cancerol S* is potentially antioxidant and could help prevent or reduce the pathogenicity of several diseases including cancer. Indeed, many plants have been shown to have a significant free radical trapping, and thus antioxidant, which is associated with cytotoxicity, and could therefore be used as therapeutic and preventive agents [29–31]. Plant extracts with a half-maximum effective free radical scavenging concentration (EC_{50}) of 10 µg/mL were shown to exhibit greater cytotoxicity [32].

Regarding the anti-inflammatory activity of the extract, the results showed an inhibitory effect of 15-LOX, COX-1 and COX-2 with IC_{50} greater than 100 µg/mL. In addition, the positive correlation observed between TPC and COX 1/COX 2 inhibition (r = 0.5 and r = 1 respectively) is due to the effect of polyphenols including flavonoids and tannins. The iron reduction capacity (FRAP) of the extract was positively correlated with the inhibition of COX 1 and COX 2 (r = 0.655 and r = 0.982 respectively). Indeed, COX are enzymes whose activity is controlled by the oxidation or reduction of iron [33]. Therefore, the inhibition of these enzymes by the aqueous extract of *Cancerol S* could be related to its ferric ion reducing capacity. COX and LOX and their eicosanoid products derived from arachidonic acid (prostanoids and HETE) are

UNDER PEER REVIEW

involved in various pathological processes including cancer. The inhibition of these enzymes contribute to reduce the occurrence, proliferation, migration and cell survival of tumor cells [34].

The aqueous extract of *Cancerol S* inhibited the proliferation of both lines with a half-maximum inhibitory concentration (IC_{50}) of 140.72 ± 7.76 µg/mL on DU 145 cells and 84,59 ± 5.18 µg/mL on HeLa cells. Thus, HeLa cells were more sensitive than DU 145 cells. In addition, cell proliferations were inhibited in a dose-dependent manner. Several studies have shown that each of the medicinal plants used in the development of *Cancerol S* has significant activities towards various cancer cell lines. *Solanum incanum* inhibited melanoma cells *in vitro* and reduced the growth of metastatic melanoma in vivo. It also induced apoptosis and caused cell cycle arrest in melanoma cells between the GO/G1 phases [35]. Several studies have shown that *Solanum incanum* and its active compound, solamargine, can induce apoptosis in various cancer cells [36–38]. Abdoul-Latif et al., evaluated the effects of *Ocimum americanum* leaf essential oil on thirteen (13) human cancer lines (K562, A549, HCT116, PC3, U87-MG, MIA-Paca2, HEK293, NCI-N87, RT4, U2OS, A2780, MRC -5 and JIMT-T1). The results showed that *Ocimum americanum* presented excellent and significant anticancer activity *in vitro* [39]. The anticancer activity of the roots, leaves and stems of *Combretum Adenogonium* was evaluated on the prostate cancer cell line (PC-3). *Combretum Adenogonium* root extract showed high activity against PC-3 cells with an IC_{50} of 24 µg/mL, while leaf and stem extracts had IC_{50} values > 100 µg/mL [40]. Gade et al., reported that *Combretum fragrans* (synonym for *Combretum Adenogonium*) strongly inhibited the growth of human U87 glioblastoma cells, C6 rat glioblastoma cells and PC-3 prostate cancer cells. Extract induces apoptosis by regulating ERK and Akt signaling pathways [41]. All these results show how sensitive cancer cells are to different therapies. Another determining factor concerns the chemical molecules of medicinal plants that depend on the geographical location.

According to the National Cancer Institute (NCI), a raw extract of anticancer medicinal plants is promising and intended for purification for the development of new anticancer drugs when its 50% inhibitory concentration (IC_{50}) *in vitro* is less than 30 µg/mL [42]. However, the use of *Cancerol S* as a functional food could be justified for the management of patients. Indeed, several mechanisms could increase its activity in vivo. Moreover, in some published studies, extracts whose results were insignificant in vitro gave better results *in vivo*. Some extracts that did not meet the criterion of the National Cancer Institute (NCI) produced significant results in *in vivo* studies [10,43,44]. *In vivo* studies would therefore be essential to conclude regarding the effects *Cancerol S*.

4. CONCLUSION

The *in vitro* cancer study of the aqueous extract of *Cancerol S* showed significant effects on both cancer lines (DU 145 and HeLa). *Cancerol S* also reduced tumor growth in vitro in a dose-dependent manner and was more cytotoxic on HeLa cells line than on DU 145 cells. In addition, it presented a fairly significant antioxidant activity, although its anti-inflammatory properties are moderate. Beyond, this study provides the first scientific rational showing that *Cancerol S* could be further developed as phytomedicine for cancer treatment. Toxicity studies as well as anticancer activity in vivo would however be necessary to identify the active ingredients as well as the molecular mechanisms involved.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA A Cancer J Clin. 2021;71(3):209–249.

UNDER PEER REVIEW

2. GLOBOCAN. Estimated number of incident cases and deaths Burkina Faso, both sexes, all ages (excl. NMSC). 2020.

3. Turnbull C, Hodgson S. Genetic predisposition to cancer. Clin Med. 2005;5(5):491–498.

4. Vaidya FU, Chhipa AS, Sagar N, Pathak C. Oxidative Stress and Inflammation Can Fuel Cancer. In: Maurya PK, Dua K, editors. Role of Oxidative Stress in Pathophysiology of Diseases. Springer Singapore: Singapore. 2020: 229–258.

5. Wang J-J, Lei K-F, Han F. Tumor microenvironment: recent advances in various cancer treatments. Eur Rev Med Pharmacol Sci. 2018;22(12):3855–3864.

6. Tariq A, Sadia S, Pan K, Ullah I, Mussarat S, Sun F, et al. A systematic review on ethnomedicines of anti-cancer plants: Anti-cancer plants. Phytother Res. 2017;31(2):202–264.

7. Adico MDW, Bayala B, Bunay J, Baron S, Simpore J, Lobaccaro J-MA. Contribution of Sub-Saharan African medicinal plants to cancer research: Scientific basis 2013–2023. Pharmacological Research. 2024;202:107138.

8. Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA, et al. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine. 2017;7(12):1129–1150.

9. Ngoua-Meye-Misso R-L, Sima-Obiang C, Ndong JDLC, Ndong-Atome G-R, Ondo JP, Ovono Abessolo F, et al. Medicinal plants used in management of cancer and other related diseases in Woleu-Ntem province, Gabon. European Journal of Integrative Medicine. 2019;29:100924.

10. Kola P, Manjula SN, Metowogo K, Madhunapantula SV, Eklu-Gadegbeku K. Four Togolese plant species exhibiting cytotoxicity and antitumor activities lightning polytherapy approach in cancer treatment. Heliyon. 2023;9(3):e13869.

11. Dolatyari A, Eyvazzadeh O, Nateghi L. Determination of Dunaliella Salina Phenolic Compounds in Laboratory Different Conditions. Chem Methodol. 2021;(Online First). doi:10.22034/chemm.2022.2.4.

12. Arvouet-Grand A, Vennat B, Pourrat A, Legret P. Standardisation d'un extrait de Propolis et identification des principaux constituants. Journal de Pharmacie de Belgique. 1994;49(6):462-468.

13. Velázquez E, Tournier HA, Mordujovich De Buschiazzo P, Saavedra G, Schinella GR. Antioxidant activity of Paraguayan plant extracts. Fitoterapia. 2003;74(1–2):91–97.

14. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine. 1999;26(9–10):1231–1237.

15. Malterud KE, Rydland KM. Inhibitors of 15-Lipoxygenase from Orange Peel. J Agric Food Chem. 2000;48(11):5576–5580.

16. Ratner B. The correlation coefficient: Its values range between +1/−1, or do they? J Target Meas Anal Mark. 2009;17(2):139–142.

UNDER PEER REVIEW

17. Alabi MA, Muthusamy A, Kabekkodu SP, Adebawo OO, Satyamoorthy K. Anticancer properties of recipes derived from nigeria and african medicinal plants on breast cancer cells *in vitro*. Scientific African. 2020;8:e00446.

18. Alabi MA, Muthusamy A, Kabekkodu SP, Adebawo OO, Satyamoorthy K, Ajagun EJ. I*n vitro* cytotoxicity of recipes derived from Nigerian medicinal plants (NMPs) on breast cancer cells. Int. j. biol. chem. sci. 2017;1:90–97.

19 Li S, Zhang B, Zhang N. Network target for screening synergistic drug combinations with application to traditional Chinese medicine. BMC Syst Biol. 2011;5(Suppl 1):S10.

20 Trinchieri G. Cancer and Inflammation: An Old Intuition with Rapidly Evolving New Concepts. Annu Rev Immunol. 2012;30(1):677–706.

21. Raja KS, Taip FS, Azmi MMZ, Shishir MRI. Effect of pre-treatment and different drying methods on the physicochemical properties of *Carica papaya* L. leaf powder. Journal of the Saudi Society of Agricultural Sciences. 2019;**18**(2):150–156.

22. Nile SH, Keum YS, Nile AS, Jalde SS, Patel RV. Antioxidant, anti-inflammatory, and enzyme inhibitory activity of natural plant flavonoids and their synthesized derivatives. J Biochem & Molecular Tox. 2018;32(1):e22002.

23 Tuli HS, Garg VK, Bhushan S, Uttam V, Sharma U, Jain A, et al. Natural flavonoids exhibit potent anticancer activity by targeting microRNAs in cancer: A signature step hinting towards clinical perfection. Translational Oncology. 2023;27:101596.

24. Zengin G, Ferrante C, Gnapi DE, Sinan KI, Orlando G, Recinella L, et al. Comprehensive approaches on the chemical constituents and pharmacological properties of flowers and leaves of American basil (*Ocimum americanum* L). Food Research International. 2019;125:108610.

25. Séré M. Study of the antibacterial capacity of extracts of *Solanum incanum* L., (Solanaceae) traditionally used for the management of avian cholera in rural areas of Burkina Faso. 2022.

26. Zahoui OS, Soro TY, Yao KM, Nene-Bi SA, Traoré F. Effet hypotenseur d'un extrait aqueux de *Combretum micranthum* G. Don (Combretaceae). *Phytothérapie* 2017;15(3):138–146.

27. Nounagon MS, Dah-Nouvlessounon D, N'tcha C, Akorede SM, Sina H, Noumavo PA, et al. Phytochemical Screening and Biological Activities of Combretum adenogonium Leaves Extracts. *ESJ* 2017;13(30):358.

28. Karou D, Dicko MH, Simpore J, Traore AS. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. African Journal of Biotechnology. 2005;4:823–828.

29. Chua M-T, Tung Y-T, Chang S-T. Antioxidant activities of ethanolic extracts from the twigs of *Cinnamomum osmophloeum*. Bioresource Technology. 2008;99(6):1918–1925.

UNDER PEER REVIEW

30. Hwang Y-J, Lee E-J, Kim H-R, Hwang K-A. In vitro antioxidant and anticancer effects of solvent fractions from *prunella vulgaris* var. lilacina. BMC Complement Altern Med. 2013;13(1):310.

31. Abu-Lafi S, Rayan B, Kadan S, Abu-Lafi M, Rayan A. Anticancer activity and phytochemical composition of wild *Gundelia tournefortii*. Oncol Lett. 2018. doi:10.3892/ol.2018.9602.

32. Sammar M, Abu-Farich B, Rayan I, Falah M, Rayan A. Correlation between cytotoxicity in cancer cells and free radical-scavenging activity: *In vitro* evaluation of 57 medicinal and edible plant extracts. Oncol Lett. 2019. doi:10.3892/ol.2019.11054.

33. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. *Journal of Lipid Research* 2009;50:S29–S34.

34. Schneider C, Pozzi A. Cyclooxygenases and lipoxygenases in cancer. 2013.

35. Yu S, Sheu H-M, Lee C-H. *Solanum incanum* extract (SR-T100) induces melanoma cell apoptosis and inhibits established lung metastasis. Oncotarget. 2017;8(61):103509–103517.

36. Kuo K-W, Hsu S-H, Li Y-P, Lin W-L, Liu L-F, Chang L-C, et al. Anticancer activity evaluation of the Solanum glycoalkaloid solamargine. Biochemical Pharmacology. 2000;60(12):1865–1873.

37. Liu L-F, Liang C-H, Shiu L-Y, Lin W-L, Lin C-C, Kuo K-W. Action of solamargine on human lung cancer cells - enhancement of the susceptibility of cancer cells to TNFs. FEBS Letters. 2004;577(1–2):67–74.

38. Wu C-H, Liang C-H, Shiu L-Y, Chang L-C, Lin T-S, Lan C-CE, et al. *Solanum incanum* extract (SR-T100) induces human cutaneous squamous cell carcinoma apoptosis through modulating tumor necrosis factor receptor signaling pathway. Journal of Dermatological Science. 2011;:S0923181111001216.

39. Mohamed Abdoul-Latif F, Elmi A, Merito A, Nour M, Risler A, Ainane A, et al. Essential Oils of *Ocimum basilicum* L. and *Ocimum americanum* L. from Djibouti: Chemical Composition, Antimicrobial and Cytotoxicity Evaluations. Processes. 2022;10(9):1785.

40. Elnour MA. *Combretum Adenogonium* Induce Anticancer and Antioxidant Effects in Prostate Cancer Cell Line. SBB. 2018;2(2). doi:10.31031/SBB.2018.02.000534.

41. Gade IS, Richard TS, Chadeneau C, Seite P, Vannier B, Atchade ADT, et al. Anticancer Activity of *Combretum fragrans* F. Hoffm on Glioblastoma and Prostate Cancer Cell Lines. Asian Pac J Cancer Prev. 2021;22(4):1087–1093.

42. Suffness M, Pezzuto JM. *Assays related to cancer drug discovery*. 6e édition, Academic Press. Hostettmann: Academic Press: London, 1991.

43. Manjula SN, Kenganora M, Parihar VK, Kumar S, Nayak PG, Kumar N, et al. Antitumor and antioxidant activity of *Polyalthia longifolia* stem bark ethanol extract. Pharmaceutical Biology. 2010;48(6):690–696.

UNDER PEER REVIEW

44. Ali M, Manjula S, Wani SUD, Parihar VK, Mruthunjaya K, Madhunapantula SV. Protective role of herbal formulation-divine noni against cisplatin-induced cytotoxicity in healthy cells by activating Nrf2 expression: An *in-vivo* and *in-vitro* approach. Phytomedicine Plus. 2021;1(1):100009.

UNDER PEER REVIEW