Annual Research & Review in Biology

Volume 39, Issue 8, Page 87-98, 2024; Article no.ARRB.121148
ISSN: 2347-565X, NLM ID: 101632869
(Past name: Annual Review & Research in Biology, Past ISSN: 2231-4776)

Antioxidant, Anti-inflammatory and Cytotoxic Properties of *Cancerol S*, A Blend of Medicinal Plants, on Cancerous Cells in Culture

Marc Donald Wilfried Adico [a,b], Bagora Bayala [a,b,c,d*], Aminata Marie Simone Amsalet Traoré [a,b,c], Florencia Wendkuuni Djigma [a,b], Bapio Valerie Elvira Jean Telesphore Bazié [a,b], Paul Darga [b], Jean-Marc Lobaccaro [d] and Jacques Simporé [a,b]

[a] Laboratoire de Biologie Moléculaire et Génétique (LABIOGENE), Université Joseph KI-ZERBO, Burkina Faso.
[b] Centre de Recherche Biomoléculaire Pietro Annigoni (CERBA), Ouagadougou, Burkina Faso.
[c] Ecole Normale Supérieure, Koudougou, Burkina Faso.
[d] Université Clermont Auvergne, Institute Génétique, Reproduction, Développement, UMR CNRS 6293, INSERM U1103, 28, Place Henri Dunant, BP38, F63001, Clermont-Ferrand, France.

Authors' contributions

This work was carried out in collaboration among all authors. Authors MDWA, BB, PD, JML and JS designed the research work. Author MDWA performed the experiments. Authors MDWA, BB analyzed the data. Authors MDWA, BB and AMSAT wrote the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/arrb/2024/v39i82112

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/121148

Original Research Article

Received: 06/06/2024
Accepted: 08/08/2024
Published: 14/08/2024

**Corresponding author: E-mail: bayalabagora@gmail.com;*

Cite as: *Adico, Marc Donald Wilfried, Bagora Bayala, Florencia Wendkuuni Djigma, Bapio Valerie Elvira Jean Telesphore Bazié, Paul Darga, Jean-Marc Lobaccaro, and Jacques Simporé. 2024. "Antioxidant, Anti-Inflammatory and Cytotoxic Properties of Cancerol S, A Blend of Medicinal Plants, on Cancerous Cells in Culture". Annual Research & Review in Biology 39 (8):87-98. https://doi.org/10.9734/arrb/2024/v39i82112.*

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: High-performance thin-film chromatography (HPTLC) and the assay test were used for phytochemical screening of *Cancerol S*. The antioxidant power of *Cancerol s* was evaluated by free radical inhibition tests (DPPH and ABTS) and the Iron III Reduction Power (FRAP). The inhibition tests of 15-LOX (15-lipoxygenase) and COX1/COX2 (cyclooxygenases) were used to evaluate the anti-inflammatory capacity of *Cancerol S*.
Results: The total polyphenols and flavonoids content of the extract were respectively 115.6 ± 0.1 mg EAG/g and 43.4 ± 0.1 mg EQ/g dry extract. The extract showed excellent antioxidant activity, with inhibition of DPPH (EC_{50} = 8.3 ± 0.7 µg/mL) and ABTS (EC_{50} = 36.0 ± 1.1 µg/mL), and also reduced ferric ion Fe^{3+} (2.67 ± 0.1 mmol EAA/g extract). In addition, the extract moderately inhibited 15-LOX, COX-1 and COX-2. The HeLa cell line of cervical cancer was more sensitive to *Cancerol S* (EC_{50} = 84.6 ± 5.2 µg/mL) than the prostate cancer cell line DU 145 (EC_{50} = 140.7 ± 7.8 µg/mL).
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

The COVID-19 pandemic disrupts prevention methods and access to cancer treatment, affecting rates of incidence and mortality related to cancer. According to the latest report from the International Agency for Research on Cancer (IARC), there were 20 million new cases of cancer in 2022 and 9.7 million deaths worldwide. [1,2]. In Burkina Faso, the number of new cases in the same year was estimated at 14,538 and the number of deaths at 10,998, mainly due to breast, liver, cervical and prostate cancers [3]. The etiology of cancer is multifactorial and mainly due to genetic mutations and predispositions, chronic oxidative stress and inflammation [4,5]. Its treatment is mainly based on surgery, radiation therapy and chemotherapy [6,7]. 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. Thus, according to several scientists, traditional medicine could be a very promising approach in the treatment of cancer [8,9]. The use of medicinal plants and their molecules to treat cancer is therefore a 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 [10]. However, medicinal plants must first be studied to elucidate their toxicity and effectiveness. Four main activities are considered in the research of anticancer phytomedicines: antioxidant, anti-inflammatory, anti-angiogenic and cytotoxic [11]. Several recipes of anti-cancer medicinal plants developed by traditional health practitioners based on traditional knowledge, are marketed in Burkina Faso. This is one of the recipes called *Cancerol S*, made from the leaves of four plants from Burkina Faso (*Solanum incanum* (Nees) L., *Ocimum americanum* L., *Combretum micranthum* G. DON., *Combretum adenogonium* Steud ex A. Rich) which was the subject of our study. It is well known that these plants have excellent pharmacological properties, but no studies have scientifically tested the anti-cancer effect of these combined plants. Thus, the objective of this study was to verify the efficacy of the aqueous extract of *Cancerol S* in the treatment of cancer, based on antioxidant studies, The aim of this study is to develop a plant-based anti-inflammatory and cytotoxic drug on cancer lines.

2. MATERIALS AND METHODS

2.1 Phytochemical Study

2.1.1 Plant material

The plant material of the study is a recipe from four medicinal plants from Burkina Faso called *Cancerol S*. It was developed by the traditional health practitioner from dry leaves of these traditional plants with well-defined proportions,

and informally to avoid the popularization of the recipe (Table 1).

2.1.2 Extraction

The extraction of *Cancerol S* was carried out by aqueous decoction, respecting the indications of the traditional health practitioner. Thus, 15 grams of the mixture powder were added in 1.5 Liter of distilled water and boiled for 10 minutes. After cooling the mixture, filtering and centrifugation of the filtrate, the supernatant was collected and then lyophilized.

2.1.3 Determination of extraction yield and residual moisture content

Extraction yield: The extraction yield was determined by the formula for the ratio of the mass of the dry extract of *Cancerol S* to the mass of the dry vegetable powder.

Residual moisture content (THR): It was determined using the following formula:

THR (%) = (M-M')/M×100

M: Dry powder mass of the recipe
M': Powder mass of the recipe after drying

2.1.4 Phytochemical screening

The aqueous extract of *Cancerol S* was screened by high-performance thin-film chromatography (HPTLC) technique. This technique was used as described by Adico et al., [12] without modification.

Identification of flavonoids: Migration was performed using the solvent system composed of ethyl acetate-formic acid-acetic acid-water (50: 5.5: 5.5: 13, v/v/v/v).

Identification of cumarins: Ethyl Acetate-Methanol-Water-Chloroform (18:2.4:2.1:6, v/v/v/v) served as a migration solvent system.

Identification of tannins: The mixture Ethyl acetate-Methanol-Water-Chloroform (18: 2.4: 2.1: 6, v/v/v/v) was used for the migration of tannins [12].

2.1.5 Phytochemical determination

Determination of total polyphenols content (TPC): The total polyphenols (TPC) content of the extract was determined by the Folin-Ciocalteu method [13], as reported by Adico et al. [12], without modification. The calibration curve of the gallic acid equation: y = 16.698x + 0.086 (R^2=0.9979) was used for the calculation of this content. It was expressed in milligrams of gallic acid equivalent per gram of extract (mg EAG/g of extract).

Determination of total flavonoid content (TFC): The total flavonoid content (TFC) of the extract was determined by the aluminium trichloride method as described by Arvouet-Grand et al. [14], and taken up by Adico et al. [12], unchanged. It was expressed in milligrams (mg) of quercetin equivalent per gram (g) of dry extract (mg EQ/g of extract), with the equation of the quercetin curve corresponding to y = 16.038x + 0.0414 (R^2=0.9997).

2.2 Antioxidant Activity

2.2.1 DPPH radical scavenging activity

The ability of the extract to inhibit the radical $DPPH^{\blacksquare}$ was evaluated according to the method of Velázquez et al. [15] as reported by Adico et al., [12] in microplates. In practice, after half dilution of the extracts, 200µL DPPH (20 mg/L) was added to 100 µL of each concentration of the extract from the successive dilution in each well. The microplates were read by a spectrophotometer at 517 nm.

The semi-maximum effective concentration (EC_{50}) was determined from the inhibition percentage curves that were a function of the concentrations of the extracts. The formula used for expressing inhibition percentages was:

I (%) = (Abs (B)-Abs (E))/(Abs (B))×100

Abs (B): absorbances of the blank

Abs (E): absorbances of the aqueous extract of *Cancerol S*.

2.2.2 ABTS radical scavenging activity

The method of Re et al. [16] was used to evaluate the inhibitory capacity of $ABTS^{\blacksquare+}$ cations by aqueous extract of *Cancerol S.* The procedure used is that described by Adico et al. [12], without modification. Following half dilution of the extracts, 200 µL of ABTS solution was added to 20 µL of the different solutions for concentration of the extracts in the wells. After reading the microplates at 734 nm, the percentage of $ABTS^{\blacksquare+}$ radical inhibition was determined by the following formula:

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

I (%) = (Abs (C)-Abs (E)) / (Abs (C)) × 100
Abs (C): absorbances of the control
Abs (E): absorbances of the extract

The semi-maximum effective concentration (EC_{50}) was determined from the inhibition percentage curves that were a function of the concentrations of the extracts.

2.2.3 Iron reduction assay

The FRAP method, as described by Adico et al. [12] was used to evaluate the reducing power of Iron of aqueous extract of *Cancerol S*. In practice, 10µL of the concentration extract 1 mg/mL was added to 300µL of FRAP solution in a 96-well plate. The FRAP solution was composed of sodium acetate buffer, TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) and ferric chloride $FeCl_3$ (10:01:01, v/v/v). The absorbance was read at 593 nm.

2.3 Anti-inflammatory activity

2.3.1 15-lipoxygenase (15-LOX) Inhibition assay

The spectrophotometric method developed by Malterud and Rydland [17] was used with minor modifications to evaluate the inhibitory activity of *Cancerol S* aqueous extract on 15-lipoxygenase. The method used to inhibit this pro-inflammatory enzyme is described in the study by Adico et al. [12].

2.3.2 Cyclooxygenase (COX 1 and COX 2) inhibition assay

The commercially available colorimetric COX (Ovine/Human) inhibition kit (Cayman Chemical Company, USA, Number: 560131) was used to evaluate the inhibitory power of pro-enzymes inflammatory cyclooxygenase (COX-1 and COX-2) by *Cancerol S* extract. In this test, the activity of cyclooxygenases was measured using a colorimetric substrate, N, 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:

- **Enzymatic activity (COX 1 and COX 2)**: It was prepared by mixing 150 µL Buffer, 10 µL Hemin, 10µL Enzyme (COX 1/COX 2) and 10µL Extract dilution solvent (Methanol);
- **Activity of the extract**: It was made up of 150 µL of Tampon, 10µL of Hemin, 10µL of enzyme (COX 1/COX 2) and 10µL of the solution of the aqueous extract of *Cancerol S*;
- **The blank**: It was made up of 160 µL of Tampon, 10µL of Hemin and 10µL of the dilution solvent of the extract (Methanol);

20µL of the colorimetric substrate (TMPD) and 20µL of arachidonic acid were added to the three (3) reaction mixture. After incubation at 25ºC for 2 minutes, and absorbances were read at 590 nm. The following formula was used to determine the percentage of inhibition.

I (%) = (Absorbance (enzyme) - Absorbance (extract)) / (Absorbance (enzyme)) × 100

2.4 Cytotoxicity on Cultured Cancer Cells

2.4.1 Cultures of cells

The cytotoxic activity of *Cancerol S* aqueous extract was evaluated on prostate cancer cell lines DU 145 (ATCC HTB-81) and cervical HeLa

(ATCC CRM-CCL-2) *in vitro* culture. 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.

2.4.2 MTT test

The cytotoxicity test of *Cancerol S* was performed according to the MTT method, as described by Adico et al. [12]. In practice, 10,000 cells from each line were seeded into 96-well microplates and incubated. 24 hours mostly, the cells were brought into contact with different concentrations of *Cancerol S* extract and incubated again. After 72 hours, 10 µL of the MTT solution at a concentration of 5 mg/mL was added to each well. The revelation was made four hours mostly by adding 100 µL of isopropanol. The inhibition percentages were determined after plate readings at 570 nm.

I (%) = (Abs (control)-Abs (blank))/(Abs (extract)-Abs (blank))×100

2.5 Data Analysis

The tests of the study were performed in triplicate (n=3). The data obtained was presented as mean ± standard deviation. Statistical analyses, graphs and correlation determination between antioxidant/anti-inflammatory tests and polyphenolic compounds by the Pearson correlation coefficient performed using GraphPad Prism software version 8.0.2. The student test was used to compare two averages with a 5% significance threshold.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Phytochemical study

The residual moisture content of the *Cancerol S* powder was 4.2 ± 0.7%, with an extraction yield of 18.7 ± 0.3%. The aqueous extract of *Cancerol S* had a total phenolic content of 115.6 ± 0.1 mg GAE/g dry extract, and 43.4 ± 0.1 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	18.7 ± 0.3	4.2 ± 0.7	115.6 ± 0.1	43.41 ± 0.1

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

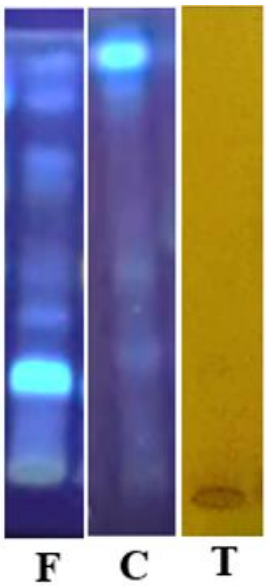

Fig. 1. Detection of flavonoids (F), coumarins (C) and tannins (T)

The migration profile of chemical groups after disclosure is shown in Fig. 1. This phytochemical targeting has made it possible to highlight flavonoids, coumarins and tannins.

3.1.2 Antioxidant activity

The results of antioxidant activity of *Cancerol S* extract are summarized in Table 3. They reveal good antioxidant activity of *Cancerol S*. Thus, $DPPH^{\blacksquare}$ and $ABTS^{\blacksquare+}$ radicals were inhibited with EC_{50} values of 8.3 ± 0.7 µg/mL and 36.0 ± 1.1 µg/mL. These values are lower than the references used in each case. The antioxidant power by iron reduction of *Cancerol S* was evaluated 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 (2.7 ± 0.1 mmol AAE/g extract), which is however lower than that of reference rutin.

3.1.3 Anti-inflammatory activity

Table 4 shows the result of inhibition of pro-inflammatory enzymes (15-LOX, and COX 1 and COX 2) by *Cancerol S* extract. The extract moderately inhibited 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 15.9 ± 1.1%; 37.2 ± 1.7% and 29.2 ± 1.3% respectively for 15-LOX, COX 1 and COX 2.

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 [18]. 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 3. Antioxidant activity the aqueous extract of *Cancerol S*

	DPPH EC_{50} (µg/mL)	**ABTS EC_{50} (µg/mL)**	**FRAP (mmol AAE/g extract)**
Cancerol S	8.3 ± 0.7***	36.0 ± 1.1****	2.7 ± 0.1****
Quercetin	4.4 ± 0.2	-	-
Trolox	-	2.5 ± 0.1	-
Rutin	-	-	3.9 ± 0.1

*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*

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	15.9 ± 1.1****	37.2 ± 1.7	29.2 ± 1.3
Indomethacin	91.5 ± 0.3	-	-

*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*

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

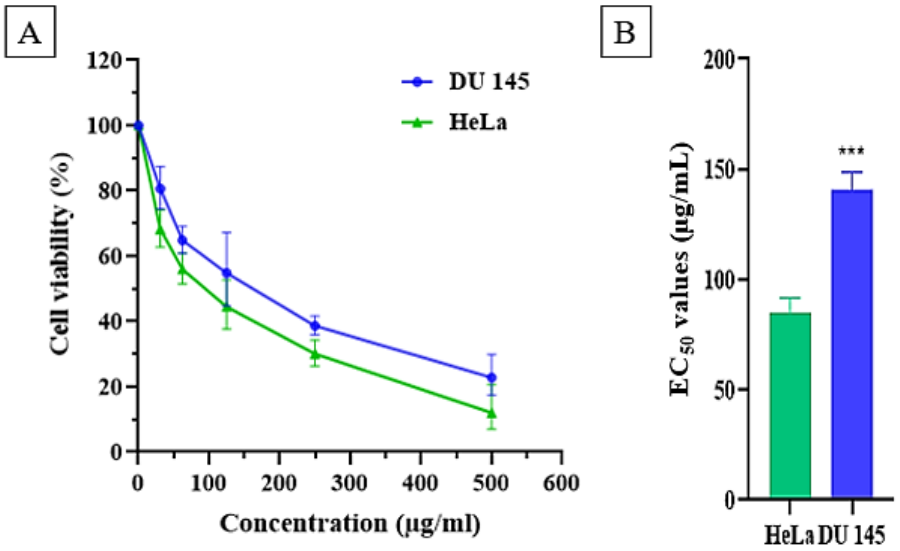

Fig. 2. Effect of *Cancerol S* aqueous extract on cancer cells

A: Dose-dependent antiproliferative activity of extract on prostate cancer DU 145 cell lines and cervical cancer HeLa. B: EC_{50} (µg/mL) of extract on prostate cancer DU 145 cell lines and cervical cancer HeLa

3.1.5 Cytotoxic activity of *Cancerol S* on cancer cells

Fig. 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 an effective half-maximum concentration (EC_{50}) of 140.7 ± 7.8 µg/mL on DU 145 cells line and 84.6 ± 5.2 µg/mL on HeLa cells line, with a statistically significant difference ($p = 0.0007$).

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 [19]. Combined therapy, defined as the combination of several compounds, is a new direction taken by scientists in the fight against cancer. It is one of the most effective and promising drug therapies in the fight against complex pathologies such as cancer [20]. 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 [21]. Thus, *Cancerol S*, a herbal recipe used in the treatment of people suffering from cancer has been studied with a view to scientifically prove 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 damage, resulting in genetic and epigenetic modifications not only in tumor suppressor genes and proto-oncogenes, but especially genes controlling cell survival, DNA repair and apoptosis processes. Damage could promote the initiation and growth of cancer [22].

The aqueous decoction method used in the study for the extraction of *Cancerol S* gave a good extraction yield. In addition, the dry vegetable powder from this recipe has a residual moisture content of less than 10%. This result indicates that *Cancerol S* has good conservation properties, Reducing all risks of contamination, deterioration of the microbiological quality of the recipe powder and alteration of bioactive molecules [23]. The phytochemical screening of the aqueous extract of *Cancerol S* revealed the presence of phytochemicals (flavonoids, coumarins and tannins) with excellent pharmacological properties. The polyphenols and flavonoid contents confirmed the presence of phenolic compounds and flavonoids in the aqueous extract of *Cancerol S*, which are well known for their antioxidant, anti-inflammatory and anticancer effects [24,25]. This is the case of a bioactive flavonoid, fisetin, formulated as nanocrystals has shown biopharmaceutical effects and excellent *In vitro* activity against solid tumors [26]. Our phytochemical results showed similar compositions to those obtained by other studies on the different plants constituting the recipe. 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 [27]. In addition, phytochemical tests of *Solanum incanum* aqueous leaf extract were positive for tannins and coumarins but not for flavonoids [28]. Zahoui et al., characterized sterols and polyterpens, polyphenols, flavonoids, tannins, quinones and alkaloids in the aqueous extract of the leaves of *Combretum micranthum* [29]. Finally, Nounagon et al., said that the leaves of *Combretum adenogonium* are rich in chemicals such as flavonoids, leucoanthocyans, anthocyanins, triterpenoids and tannins [30]. Thus, all these studies confirm that these plants are very rich in phytochemicals, and could explain the good phytochemical composition of *Cancerol S*.

The use of two or more methods in the evaluation of antioxidant activity gives a more reliable and understandable aspect on the antioxidant power of an extract. [31]. In our study, three antioxidant methods were used: the $DPPH^{\blacksquare}$ radical inhibition method, the $ABTS^{\blacksquare+}$ radical inhibition method and iron reducing power. The antioxidant effect of aqueous extract of *Cancerol S* was significant, with values of EC_{50} = 8.3 ± 0.7 µg/mL for DPPH (less than 10 µg/mL), of EC_{50}= 36.0 ± 1.1 µg/mL (less than 50 µg/mL) for ABTS and 2.67 mmol EAA/g of extract for FRAP. However, these values are statistically lower than the compounds used as a reference. The good antioxidant activity of this mixture would be due to the plants used for its preparation. For example, Touré et al. showed that the aqueous extract *Combretum micranthum* inhibited $DPPH^{\blacksquare}$ and $ABTS^{\blacksquare+}$ radicals with IC_{50} values of 9.1 ± 0.28 µg/mL and 11.8 ± 0.01 µg/mL respectively [32]. Other studies have reported that *Solanum incanum* and *Combretum adenogonium* had moderate antioxidant effects [33,34]. These results show that the phytochemicals in this mixture could act synergistically, thus increasing the antioxidant power of the recipe. 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, polyphenols are known to be powerful antioxidants. The mode of action of these phytochemicals is based on their ability to give electrons or hydrogen. In addition, they can block the production of free radicals by inhibiting the formation or disabling reactive species and free radical precursors. [35,36]. 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 [37–39]. Plant extracts with a half-maximum effective free radical scavenging

concentration (EC_{50}) of 10 µg/mL were shown to exhibit greater cytotoxicity [40].

Regarding the anti-inflammatory activity, the extract showed a moderate inhibitory effect on 15-LOX, COX-1 and COX-2 with an IC_{50} greater than 100 µg/mL. In addition, the positive correlation observed between TPC/TFC and inhibition of COX 1/COX 2 would be due to the effect of polyphenols including flavonoids, tannins and stilbenes. This inhibition can be achieved by blocking prostaglandins and thromboxanes via cyclooxygenases. Phytochemicals inhibiting pro-inflammatory enzymes are widely used in the fight against several diseases including cancer, because they also have a strong antioxidant activity [41,42]. 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 [43]. 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 involved in various pathological processes including cancer. The inhibition of these enzymes contributes to reduce the occurrence, proliferation, migration and cell survival of tumor cells [44].

The aqueous extract of *Cancerol S* inhibited the proliferation of both lines with a half-maximum effective concentration (EC_{50}) of 140.7 ± 7.8 µg/mL on DU 145 cells and 84,6 ± 5.2 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. Indeed, it is well known that inflammation and oxidative stress are two mechanisms promoting the onset and progression of cancer. Thus, the antioxidant and anti-inflammatory activities of *Cancerol S* are potentially related to its chemopreventive effectiveness. Furthermore, the inhibition of free radicals and pro-inflammatory enzymes by *Cancerol S* could also explain its cytotoxic effect on HeLa and DU 145 cancer cells, preventing the progression of cancer and the development of metastases. Numerous studies have shown that each of the medicinal plants in *Cancerol S* has the ability to inhibit the proliferation of several 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 [45]. Several studies have shown that *Solanum incanum* and its active compound, solamargine, can induce apoptosis in various cancer cells [46–48]. 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* [49]. 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 [50]. 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 [51]. Moreover, *Cancerol S* was more effective on the HeLa and DU 145 cancer lines compared to a recipe (*Acti-plus*) used by this same traditional health practitioner [12]. 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 [52]. 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*. *In vivo* studies would therefore be essential to conclude regarding the effects *Cancerol S*.

4. CONCLUSION

Cancerol S showed clear effects on both cancer lines in culture (DU 145 and HeLa). *Cancerol S* also reduced tumor growth 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ACKNOWLEDGEMENTS

The authors wish to express their deep gratitude to M. DARGA Paul, traditional health practitioner, author of the recipe *Cancerol S*, for having accepted to participate in the study and for his frank collaboration. DU 145 and HeLa cancer cells were graciously provided to CERBA/ LABIOGENE by the Institute of Genetics, Reproduction, Development, University of Clermont Auvergne, France.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ledford SG, Kessler F, Moss JL, Wang M, Lengerich EJ. The Impact of the COVID-19 Pandemic on Cancer Mortality in Pennsylvania: A Retrospective Study with Geospatial Analysis. Cancers. 2023;15(19): 4788.
2. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I and Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA A Cancer J Clinicians. 2024;74(3): 229–263.
3. GLOBOCAN. Estimated number of incident cases and deaths Burkina Faso, both sexes, all ages (excl. NMSC). 2020.
4. Turnbull C, Hodgson S. Genetic predisposition to cancer. Clin Med. 2005; 5(5):491–498.
5. 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.
6. Nave Op: Modification of Semi-Analytical Method Applied System of ODE. MAS. 2020;14(6):75.
7. 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.
8. 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.
9. 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.
10. Iqbal J, Abbasi BA, Mahmood T, Kanwal S, Ali B, Shah SA. Plant-derived anticancer agents: A green anticancer approach. Asian Pacific Journal of Tropical Biomedicine. 2017;7(12):1129–1150.
11. 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.
12. Adico MDW, Bayala B, Bazie VEJB, Traore L, Theophane A and Simpore J. Cytotoxic Properties on Prostate and Cervical Cancer Cells in Culture of «*Acti-plus*», a Recipe Based on Medicinal Plants from Burkina Faso. JDMP. 2023;9(4):148-155.
13. 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
14. 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.
15. 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.

16. 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.
17. Malterud KE, Rydland KM. Inhibitors of 15-Lipoxygenase from Orange Peel. J Agric Food Chem. 2000;48(11):5576–5580.
18. Ratner B. The correlation coefficient: Its values range between +1/−1, or do they? J Target Meas Anal Mark. 2009;17(2):139–142.
19. 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.
20. Alabi MA, Muthusamy A, Kabekkodu SP, Adebawo OO, Satyamoorthy K, Ajagun EJ. *In vitro* cytotoxicity of recipes derived from Nigerian medicinal plants (NMPs) on breast cancer cells. Int. j. biol. chem. sci. 2017; 1:90–97.
21. 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.
22. Trinchieri G. Cancer and Inflammation: An Old Intuition with Rapidly Evolving New Concepts. Annu Rev Immunol. 2012;30 (1):677–706.
23. 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.
24. 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.
25. Tuli HS, Garg VK, Bhushan S, Uttam V, Sharma U, Jain A. Natural flavonoids exhibit potent anticancer activity by targeting microRNAs in cancer: A signature step hinting towards clinical perfection. Translational Oncology. 2023; 27:101596.
26. Ma P, Seguin J, Ly NK, Henríquez LC, Plansart E, Hammad K, Gahoual R, et al. Designing fisetin nanocrystals for enhanced in cellulo anti-angiogenic and anticancer efficacy. International Journal of Pharmaceutics. 2022;4;100138.
27. 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.
28. 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.
29. 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.
30. 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.
31. Genskowsky E, Puente LA, Pérez-Álvarez JA, Fernández-López J, Muñoz LA and Viuda-Martos M. Determination of polyphenolic profile, antioxidant activity and antibacterial properties of maqui [*Aristotelia chilensis* (Molina) Stuntz] a Chilean blackberry: Antioxidant and antibacterial properties of maqui. J Sci Food Agric. 2016;96(12): 4235–4242.
32. Touré A, Xu X, Michel T and Bangoura M. In vitro antioxidant and radical scavenging of Guinean kinkeliba leaf (*Combretum micranthum* G. Don) extracts. Natural Product Research. 2011;25(11):1025–1036.
33. Fadl Almoulah N, Voynikov Y, Gevrenova R, Schohn H, Tzanova T, Yagi S, Thomas J, Mignard B, Ahmed AAA, El Siddig MA, Spina R and Laurain-Mattar D: Antibacterial, antiproliferative and antioxidant activity of leaf extracts of selected Solanaceae species. South African Journal of Botany. 2017;112:368–374.
34. Elnour MA. *Combretum adenogonium* Induce Anticancer and Antioxidant Effects in Prostate Cancer Cell Line. 2018;2(2): 138-140.
35. 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.
36. Zhang H, Li G, Han R, Zhang R, Ma X, Wang M. Antioxidant, Anti-Inflammatory, and Cytotoxic Properties and Chemical Compositions of *Filipendula palmata* (Pall.) Maxim. Evidence-Based Complementary and Alternative Medicine 2021;1–12.
37. 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.
38. 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.
39. 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.
40. 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.
41. Gonçalves S and Romano A: Inhibitory Properties of Phenolic Compounds Against Enzymes Linked with Human Diseases. In: Phenolic Compounds - Biological Activity. Soto-Hernndez M, Palma-Tenango M, Garcia-Mateos MDR (eds.). InTech; 2017.
42. Dey R, Dey S, Samadder A, Saxena AK and Nandi S: Natural Inhibitors against Potential Targets of Cyclooxygenase, Lipoxygenase and Leukotrienes. CCHTS. 2022;25(14): 2341–2357.
43. Rouzer CA, Marnett LJ. Cyclooxygenases: structural and functional insights. Journal of Lipid Research 2009;50:S29–S34.
44. Schneider C, Pozzi A. Cyclooxygenases and lipoxygenases in cancer. 2013.
45. 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.
46. Kuo K-W, Hsu S-H, Li Y-P, Lin W-L, Liu L-F, Chang L-C. Anticancer activity evaluation of the Solanum glycoalkaloid solamargine. Biochemical Pharmacology. 2000;60(12):1865–1873.
47. 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.
48. Wu C-H, Liang C-H, Shiu L-Y, Chang L-C, Lin T-S, Lan C-CE. *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.
49. 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.
50. 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.
51. 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.
52. Suffness M, Pezzuto JM. Assays related to cancer drug discovery. 6e édition, Academic Press. Hostettmann: Academic Press: London; 1991.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/121148