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Cytotoxic and Anti-inflammatory Properties of Two Medicinal Plant Recipes Decoctions, *acphyx* and *chryfa*, Used in Traditional Medicine of Burkina Faso

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Authors' contributions

This work was carried out in collaboration among all authors. Author BAGL designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors KS and BB managed the analyses of the study. Author BBM managed the literature searches. Authors SJ and GIP supervised the study, reviewed and edited of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Cancer is a major public health problem worldwide and in Burkina Faso. This study aimed to evaluate the cytotoxic and anti-inflammatory properties of ACPHYX and CHRYFA, two medicinal plant recipes used against inflammatory diseases in traditional medicine in Burkina Faso.
Study Design: The anti-inflammatory and anti-proliferative activity on prostate and cervical cancer cells of a recipe orients on its possible anti-cancer properties. Then would help to realize ethnopharmacological evidence study for its validation or not as a phytomedicine against prostate or cervical cancer.
Place and Duration of Study: The studies were carried out in the laboratories of the Pietro Anignoni Biomolecular Research Center (CERBA) and the Health Sciences Research Institute (IRSS/CNRST) from December 2021 to March 2023 in Burkina Faso.
Methodology: An aqueous decoction of *ACPHYX* recipe and *CHRYFA* recipe, were made. The phytochemical groups of interest and the phenolic compound content of the extracts were determined. The antioxidant test was performed by the DPPH and FRAP methods. The cytotoxic activity on cancer cells was evaluated by the MTT test. Anti-inflammatory activity was assessed by pro-inflammatory enzyme inhibition tests (15-lipoxygenase, cyclooxygenases), anti-edema and antihistamine tests.
Results: *ACPHYX* decoction showed the best antioxidant activity by DPPH method (IC50 of 12.80 ± 0.5µg/mL). The chelation capacity evaluated by FRAP method was also better with *ACPHYX* decoction (1.94 ± 0.296 mmol EAA/g). ACPHYX decoction also showed the best cytotoxic activity on prostate cancer DU145 cells (175.5 ± 3.79 µg/mL) and cervical cancer HeLa cells (302.5 ± 64.36 µg/mL). In vitro, the extracts inhibited 60% edema at 400 mg/kg dose and 79.32% scratching inhibition at 600 mg/kg dose.
Conclusion: The strongest anti-inflammatory activity in vitro and *in vivo* was observed with ACPHYX extract. ACPHYX recipe compared to CHRYFA showed better cytotoxic and anti-inflammatory properties. Its use could help to treat tumor and inflammatory conditions through further studies.

Keywords: Acphyx; Chryfa; cytotoxicity; anti-inflammatory; Burkina Faso.

1. INTRODUCTION

According to the studies realized in 2020, about 19.3 million new cases of cancer were recorded worldwide with a 10 million deaths. It now appears as a major public health problem in the world (Sung et al. 2021).

In addition, many studies indicate an exponential increase of the rate of cancer-related mortality in Africa for the next 20 years. it could be exceeding the global average by [1].

In Burkina Faso, the cancer situation is become a major concern, kind egard the number of deaths recorded in 2020 [1]. The majority of sub-Saharan African countries, such as Burkina Faso, are facing to the diagnostic challenge and the cancer treating. Today, cervical cancer is the second most common cancer among women. It the leading cause of cancer deaths in sub-Saharan Africa [2] Prostate cancer is also the second most common malignant tumor among men [3]. Effective cancer treatment is only

possible when the disease was early diagnosis [4]. Unfortunately, the majority of cancers are diagnosed late. Often at the metastasis stage which lead to death [5]. Chemotherapy causes some consequences often high toxicity effects. The research for new ways of therapy is necessary. And medicinal plants are remaining an important source to discovery the new biomolecules active against cancer. The present study was to evaluate the anti-inflammatory and cytotoxic efficacy of two plants recipes named *ACPHYX* and *CHRYFA* used in traditional medicine in Burkina Faso for the treatment of inflammation diseases.

2. MATERIALS AND METHODS

2.1 Biological Materials

The plant material was composed by two plants recipes, *ACPHYX* and *CHRYFA* which gotten with the healer. The *ACPHYX* recipe was made with the plants of *Acacia nilotica* (L.) Willd. ex Delile, *Phyllanthus amarus* (Schumach. & Thonn), *Xylopia aethiopica* (Dunal) A.Rich, *Acanthospermum hispidum* (DC) and *Terminalia macroptera* (Guill. & Perr). *CHRYFA* recipe was constituted by the plants of *Guiera senegalensis* J.F. Gmel, *Chrysanthemum indicum* Lin, *Moringa olifera* Lam and *Faidherbia albida* (Del).

The animal material was DU145 cells from prostate cancer and HeLa cells from cervical cancer. These are immortal cancer cells used in medical research. Female albino mouse of NMRI strain, weighing between 22 and 25g, provided by the IRSS animal facility, were also used for *in vivo* tests of anti-inflammatory activity.

2.2 Substances and Reagents Related to Pharmaceuticals

The following products from Sigma-Aldrich and Cayman were required for the various tests: MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium), Trypan blue, L-glutamine, Fetal Calf Serum (SVF), Acetylsalicylic acid, Trolox, linoleic acid, lipoxygenase, cyclooxygenases 1&2, DTNB, Buffer, DiheptanothiolPC, DPPH (2,2-diphenyl-picrylhydrazine), ABTS [2,2'-azinobis (3-ethyl benzoin-6-sulphonate)], Trolox, quercetin, tannic acid, FCR 2N (Folin Ciocalteu reagent).

2.3 Phytochemistry

2.3.1 Extraction

It's a decoction that was made according to the healer indication. So, 75 g of powder from each recipe, *ACPHYX* and *CHRYFA,* were mixed in 1L of distilled water. The mixture was let boiling during 30 min. The extracts obtained was filtered and the filtrate was centrifuged at 2000 rpm/min for 10 minutes. The supernatant was collected in a crystallizer and lyophilized. The yield (R) of the extraction was determined (European Pharmacopoeia 10th edition).

$$R\ (\%) = (M/Pe) \times 100$$

R, represents the yield (%), M represents the dry extract's mass (g), and Pe represents the plant matter sample.

2.3.2 Phytochemical screening

Phytochemical screening of the extracts was performed by HPTLC on silica gel 60 F254 plates [6].

- Terpenoids, proanthocyanidins, saponosides and tannins were eluted by the chloroform/ethyl acetate/methanol/distilled water (6:18:2,4:2,1, v/v/v/v) system. Terpenoids were revealed by Liebermann Bürchard reagent, proanthocyanes by sulfuric anisaldehyde, saponosides by sulphuric anisaldehyde reagent (after heating) and tannins by FeCl3 (2%).
- Flavonoids were eluted by éthyl acetate / formic acid / acetic acid / water (100 :11 :11: 26, v/v/v) system. It revealed by reagent of NEU.

2.3.3 Determination of the total phenolic content

Total phenolic compounds were determined by Singleton et al, method [7]. The reaction mixture was constituted by 0.5 mL of extract, 0.5 mL of FCR 2N and 1.5 mL of 20% sodium carbonate solution. It was incubated at room temperature during 40 min and the absorbance read at 760 nm. The white control was distilled water. A standard curve was plotted with tanic acid (1-5 µg/mL). The total phenolic concentration of the extract was determined by the formula:

$$T_{PT} = \frac{C_{Tube}}{C_i} \times D$$

TPT is the total phenolic content of the extract expressed as tannic acid equivalent (EAT)/g, CTube is the concentration in µg EAT/mL in the test tube, D the dilution factor and Ci the concentration in mg/ml in the stock solution.

2.3.4 Determination of total flavonoid content

The flavonoids content determination was performed according to the Abdel-Hameed et *al*, method [8]. Two (2) mL of extract at 1 mg/mL concentration in methanol were mixed with 2 mL of 2% aluminum trichloride in methanol. After 40 min incubation and centrifugation at 2000 revolutions/min for 10 min, the absorbance was measured at 415 nm. The quercetin absorbance (0…10 mg/mL) was used as reference compound. The tests were realized in triplicate.

The flavonoids content extract was determined in quercetin equivalent (EQ) according to the formula:

$$T_{Flav} = \frac{A \cdot m_0}{A_0 \cdot m}$$

TFlav the flavonoid content of the extract expressed in µg EQ/mL, A the absorbance of the extract, A0 the absorbance of quercetin, m is the mass of the extract in mg and m0 is the mass of quercetin in mg.

2.4 Cytotoxic Activity of Extracts on Prostate Cancer DU145 Cells and Cervical Cancer Hela Cells

2.4.1 The culture process of DU145 and HeLa cells

Cell lines were grown in a RPMI or DMEM medium supplemented with 10% fetal calf serum, 1% penicillin-streptomycin and 1% L-glutamine. Prostate cancer cell lines were maintained in the RPMI-1640 environment and cervical cancer cell lines were maintained in the DMEM environment. Cells were grown in single-layer culture flasks held in a wet incubator at 37°C with 5% CO2. After 24 hours of incubation, the cells adhere and begin to multiply. Culture medium were renewed every 48 hours.

2.4.2 Viability testing by the MTT method

The principle has based on the reduction of soluble yellow 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) in Formazan purple insoluble by mitochondrial reductases, non-mitochondrial and plasma from living cells [9].

Before to start test, cells were counted using 100 µL of suspension and 100 µL of trypan blue which is a vital dye that only colors dead cells. Malassez cell was used for counting living cells under a reverse phase microscope. Cell lines were seeded in 96-well plates at 10,000 cells into 100µL of culture medium and incubated for 24 h to ensure adequate adhesion of cells. After 24 hours of incubation, the extracts at different concentrations (1; 0.5; 0.25; 0.125; 0.0625 mg/mL) were placed in contact with the cells. The cell and extract mixture were again let in incubation for 72h. The next step was to reveal at MTT after 72 hours of incubation. So, a 10 µL MTT (5 mg/mL) was distributed in each well of the plate and incubated for 4h. After this, in each well of the plate, a 100 µL of isopropanol was added. The reaction mixture was stirred using a plate shaker for 45 minutes. The number of living cells is directly proportional to the intensity of the violet color. After stirring, the plate was read by the spectrophotometer at 570 nm. The percentage of growth inhibition was calculated according to the formula below.

$$\text{Inhibition}(\%) = 100 - \frac{(\text{Sample absorbance} - \text{White absorbance})}{(\text{Absorbance control} - \text{White absorbance})} * 100$$

2.5 Anti-inflammatory Activity

2.5.1 Inhibition of 15-lipoxygenase (LOX)

Lipoxygenase inhibition was performed according to Malterud and Rydland method, with some modifications (Malterud and Rydland 2000). So, in a 96-well microplate, the following reaction mixtures were made. An enzyme blank constituted by 153.75µL borate buffer and 146.25µL LOX solution at a concentration of 820.51 U/mL. The enzyme activity was a mixture of 3.75µL borate buffer, 146.25µL of LOX solution and 150 µL of linoleic acid solution at concentration 1,25 mM. The extract blank was constituted by 146.25 µL of the concentration LOX solution 820.51 U/mL, 3.75 µL of the extract and 150 µL borate buffer. The extract activity was a mixture of 146.25 µL of the concentration LOX solution 820.51 U/mL, 3.75 µL of extract and 150 µL of linoleic acid solution (substrate) concentration 1,25 mM. Each reaction mixture is made in triple and at 100 µg. mL concentration of the extract.

The reaction mixture absorbance variation was measured at 234 nm, immediately after the addition of linoleic acid.

The percentage of lipoxygenase inhibition calculated follows the formula:

$$\mathrm{I}\,(\%) = 100 \times [(\mathrm{DO.enzyme} - \mathrm{DO.echantillon})/\mathrm{DO.enzyme}]$$

DOenzyme= DOEn.actif – DOEn.blanc ;
DOechantillon= DOactif Ech – DOblanc Ech

2.5.2 Inhibition of cyclooxygenases (COX-1 et COX-2)

The cyclooxygenase inhibition by extracts was assessed using the Cayman's manufacturer procedure (Item No. 760111, 2018) Cayman (Chemical Company, 2018). A reaction mixture constituted by 10µL of extract, 10µL of prepared enzyme, 10µL of hemin and 150µL of diluted buffer. The same mixture is made without the extract in another well. A blank constituted by 160µL diluted buffer, 10µL of hemin and 10µL of the solvent dilution of the extract. The plat was stirred and let to incubate for 5 min. Then 20µL of arachidonic acid (substrate) and a colorimetric substrate were added into all wells. The plate was let in incubation for 2 min before the reading at 590 nm. The reaction mixtures were made in triple with salicylic acetic acid as reference. The following formula has been used to determine the percentage of cyclooxygenase inhibition by the extract.

$$\mathrm{I}\,(\%) = 100 \times [(\mathrm{AEA} - \mathrm{AIA})/\mathrm{AEA}]$$

AEA: Activity enzyme test absorbance; Enzyme test activity: (Enz Test abs – blank abs);

AIA: Activity inhibition test absorbance; Activity Inhibition Test: (Abs Inh Test - blank abs)

2.5.3 DPPH free radical test

The anti-free radical activity of extracts was assessed by Kim et al, method Kim et al. [10]. So, in a 96-well microplate, 20 µL of extract at different concentrations was added to 200 µL of DPPH solution (0.077 mmol/L in methanol). The mixture was incubated in the dark during 30 min. The absorbance was measured at 517 nm. The Trolox was used as a control. The blank was composed of 200 µL DPPH and 20 µL ethanol. The percentage inhibition calculated according to the formula.

$$\mathrm{Inhibition}(\%) = \frac{\mathrm{A0}-\mathrm{A1}}{\mathrm{A0}} \times 100$$

A percentage inhibition curve of the DPPH was plotted against the sample concentration. On the curve, the concentration required to degrade 50% DPPH (IC_{50}) was determined. IC_{50}: 50% inhibitory concentration expressed as µg/mL of extract.

2.5.4 Iron reduction test by FRAP

The extract ability to reduce ferric ion was determined according to Widowati et *al*. technique Widowati et al. [11]. The FRAP solution was prepared by the mixing of 300Mm sodium acetate buffer adjusted at 3.6 pH, 10 Mm of TPTZ (2,4,6-tris (2-pyridyl) solution -s-triazine) and 20 Mm of ferric chloride $FeCl_3$ (10:01:01, v: v). The 100 µL extract solutions was added to 03 mL FRAP solution. After 30 min incubation, the mixture absorbance was read at 593 nm against a standard curve of ascorbic acid (0.025-0.5). All preparations and analyses were done in triple. Quercetin was used as reference substances.

2.5.5 Anti-edema test *in vivo* with carrageenan

The test was performed according to Winter et *al*, method [12]. Groups of six mouse were formed. Mouse were fasted for 16 hours before the test. The aqueous extract of *ACPHYX* at the doses of 200, 400 and 600 mg/kg bw, were administered orally to the individual mouse batches, one hour before carrageenan injection at 0.05 mL under the plantar aponeurosis of the posterior leg. Also, the control with acetylsalicylic acid (AAS) at a dose of 100 mg/kg bw and blank (water) were assessed. The volume of the treated leg was measured 1 h before carrageenan injection and again 1, 3 and 5 hours after carrageenan injection. The volume variation of the treated leg allowed to evaluate the anti-inflammatory power of the substance. The anti-inflammatory activity was evaluated by reduction percentage of edema of treated rats compared to white controls using the following formula:

$$\mathrm{Inhibition}(\%) = \frac{(\mathrm{A} - \mathrm{B})}{(\mathrm{A})} * 100$$

A : is the difference in mean volume increase of mouse paw from the white control; B : is the mean difference in mouse leg volume increase from treated lots.

2.5.6 Antipruritic test *In vivo*

The test was performed according to Chandrashekhar et al. [13]. Groups of six mouse were formed. Before the test, the mouse were unfed for 24 hours. Aqueous extract of ACPHYX at 200 mg/kg, 400 mg/kg and 600 mg/kg dose were administered orally 1h before of the 48/80 compound injection. Then 3 mg/kg dose of 48/80 compound was injected subcutaneously on the back of the mouse to cause scratching behavior. Sodium cromoglycate at 10 mg/kg dose was reference substance which used. The control batch received only the 48/80 compound. The scratching number of the whole body were counted for 20 minutes. The antipruritic effect was evaluated using the following formula.

$$\text{Inhibition}(\%) = \frac{(\text{A} - \text{B})}{(\text{A})} * 100$$

A: Is the average number of scratches in white mouse that have not been treated; B: is the average number of mouse scratches in the batches processed.

2.6 Results Analysis

Statistical analysis of the results was performed on the basis of statistical processing using the software Graph Pad Prism version 5. The statistical test Oneway ANOVA test was used to compare the extract with the references. Differences considered significant if P (p-value) is less than 0.05 compared to the control or reference. The IC50 were plotted by linear or logarithmic regression with Excel software.

3. RESULTS

3.1 Phytochemistry

3.1.1 Yield and phytochemical identified

The extraction yields can be found in the Table 1.

Table 1. Extraction yields

Extracts	Yield (%)
Acphyx	24.36 ± 0.43
Chryfa	20.09 ± 0.09

Values expressed Mean ± S.E.M

Chemical screening revealed the presence of phytochemical groups of interest in extracts such as flavonoids, anthocyanins, tannins and triterpene sterols. These chemical groups are shown in the Table 2.

3.1.2 Total flavonoid and phenolic contents

The assay determined the total flavonoid and phenolic content of both extracts listed in Table 3.

3.2 Cytotoxic Activity of Extracts on Cancer Cells

3.2.1 Cytotoxic activity on prostate cancer DU 145 line

The inhibitory effect of *ACPHYX* and *CHRYFA* decoctions on prostate cancer line cells DU145 at different concentrations was presented in the Fig. 1. The results analysis showed for *ACPHYX* an inhibitory concentration IC_{50} = 175.5 ± 3.79 µg/mL against a IC_{50} = 423.0 ± 45.60µg/mL for *CHRYFA.*

Table 2. Phytochemicals group identified

Chemicals group	*Acphyx*	*Chryfa*
Polyphénols/tannins	+	+
Flavonoïds	+	+/-
Stéroïds/terpénoïds	+	+
Caroténoïds	+	+
Chlorophylles	+	+
Saponosides	+	+
Anthracénosides	(-)	+/-
Anthocyanins	+	+
Alkaloids salts	(-)	(-)
Cardénolides	(-)	(-)
Cumarines and derivatives	(-)	(-)
Reducing compounds/sugars	+	+

+ = Presence ;(-) =undetected; +/- = Signs

Table 3. Total flavonoid and phenolic content

Extracts	Phenolic content (µg/mL)	flavonoid content (µg/mL)
Acphyx	233.73 ± 4.22 ***	25,17 ± 1.43 ns
Chryfa	84.40 ± 0.11	28.14 ± 0.14

*Values expressed Mean ± S.E.M, *P = .05; ** P = .01 is considered significant compared to other extracts;; *** P = .001 compared to other extracts; ns= is considered non-significant compared to other extracts,*

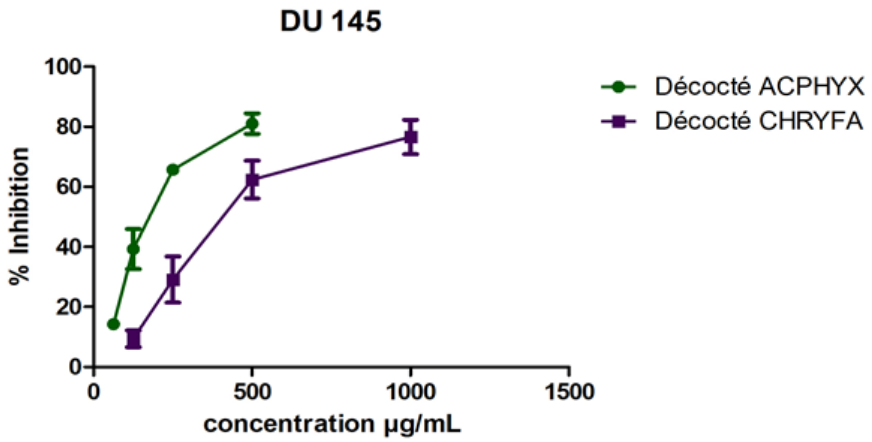

Fig. 1. DU145 cells line inhibition at different concentrations of *ACPHYX* and *CHRYFA* extracts during 72h

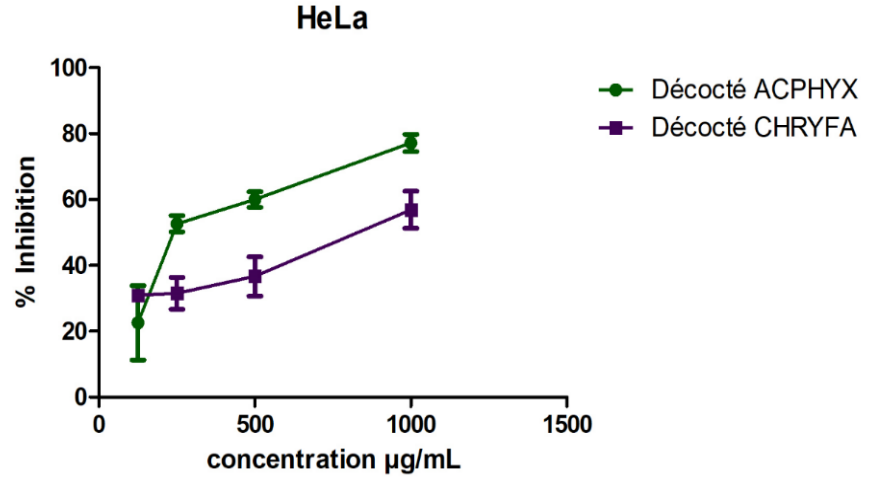

Fig. 2 Hela cell line inhibition at different concentration of *ACPHYX* and *CHRYFA* extracts during 72h

3.2.2 Cytotoxic activity on the HeLa line of cervical cancer

The effect of *ACPHYX* and *CHRYFA* extracts on the HeLa cells line of cervical cancer was presented in the Fig 2. The results analysis showed an IC_{50} = 302.5 ±64.36 µg/mL for *ACPHYX* extract and IC_{50} = 864.3 ± 70.29 µg/mL for *CHRYFA* extract.

3.3 Anti-inflammatory Activity

3.3.1 Inhibition of 15-lipoxygenase (Lox) and cyclooxygenases (COX1 & COX2)

Enzyme inhibition percentage by both extracts at 100g/mL concentration is reported in Table 4.

Table 4. Percentage (%) of inhibition (Inh) of enzymes

Extract (n=3)	%Inh COX-2	%Inh COX-1	%Inh 15-LOX
ACPHYX (100 µg/mL)	48.77± 1.61 **	47.52± 1.75**	39.09±6.81 **
CHRYFA (100 µg/mL)	20.5 ± 2.41**	29.75± 0.01 **	16.81±0.240**
AAS (10µg/mL)	16.58±1.45	-	-

*Values expressed Mean ± S.E.M; **P = 0.01 is considered significant compared to other extracts;***P =.001 compared to other extracts; ns is considered insignificant compared to other extracts, AAS: Acetyl Salicylic acid*

Table 5. 50% Inhibitory concentration (IC_{50}) of extracts

Extract (n=3)	IC_{50} (µg/mL)
ACPHYX	12.80 ± 0.5***
CHRYFA	51.95 ± 7.49***
Trolox	6.19 ± 0.04

Values expressed Mean ± S.E.M; **P = .01 is considered significant to control (Trolox); ***P = .001 versus reference (Trolox); ns= is considered non-significant to Trolox.

3.3.2 Anti-radical effect on DPPH

The results contained in the Table 5 of the DPPH anti-radical test of the extracts showed very appreciable 50% inhibitory concentration.

3.3.3 Iron reduction by FRAP

The activity of reduction of the ferric ion (Fe^{3+}) into ferrous ion (Fe^{2+}) of the two extracts in the Fig. 3.

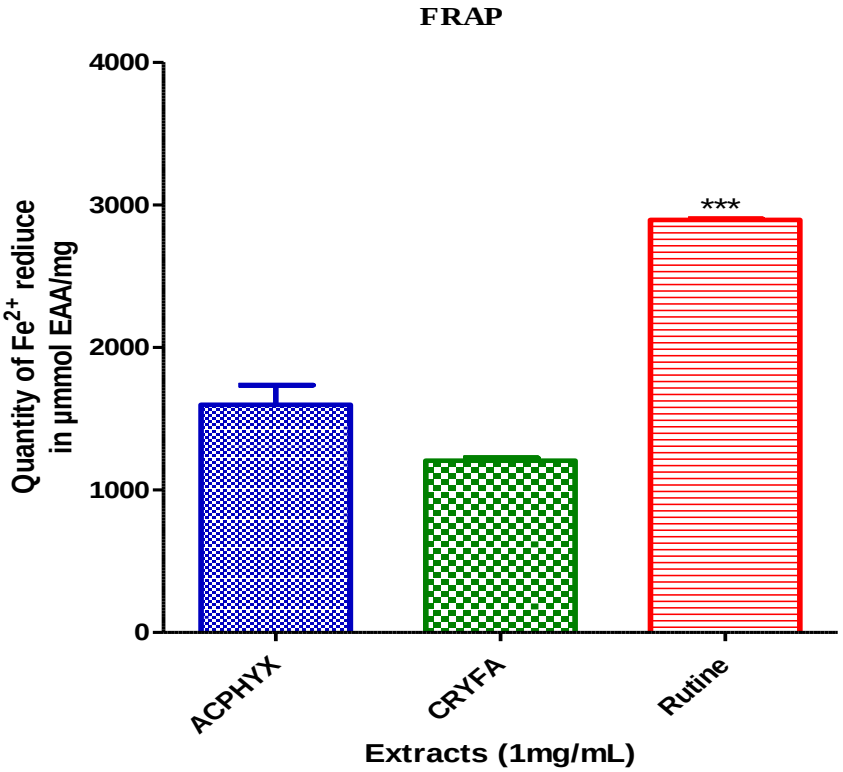

Fig. 3. Antioxidant effect of extracts evaluated by the FRAP method

*Results are expressed in µmol ascorbic acid equivalent/mg (µmol EAA/mg) and represent the Mean ± ESM from three duplicate experiments **P = .01 is considered significant in relation to the control (Rutin); ***P =.001 versus the reference (Rutin); ns= is considered not significant in relation to rutin.*

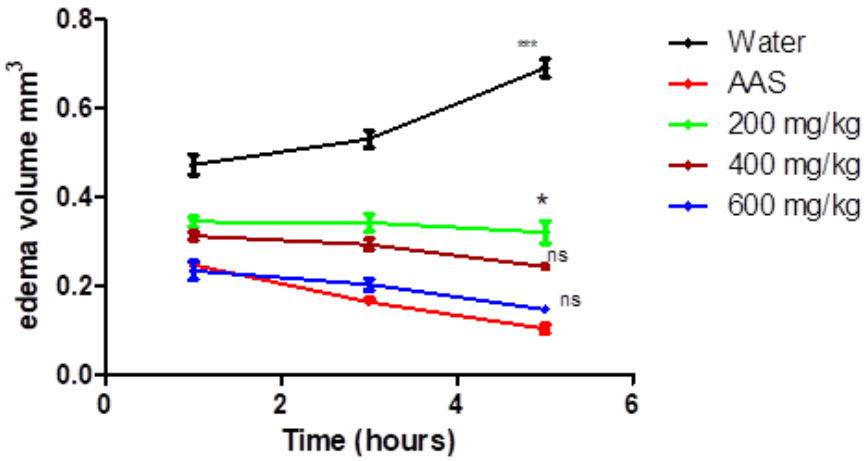

Fig. 4. The effect of *ACPHYX* DA on mouse leg edema caused by carrageenan compared to AAS (Acetyl Salicylic Acid)

****P =.001 is considered significant in relation to the control; ns= is considered not significant in relation to the control. N=6*

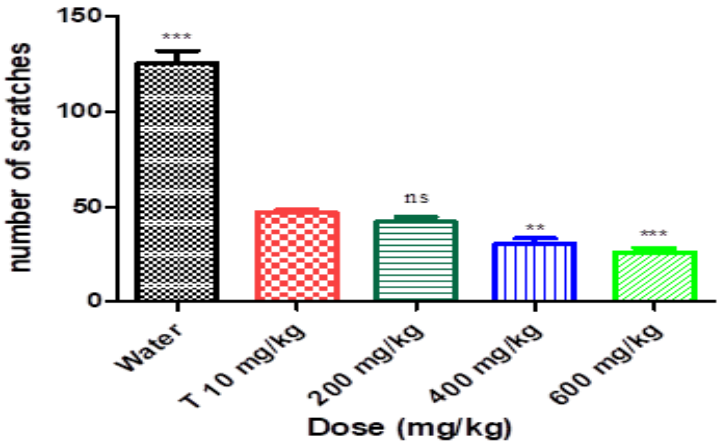

Fig. 5. Scratching inhibition effect of *ACPHYX* and T compound (sodium cromoglycate).

***P =.01, ***P =.001 considered significant Vs sodium cromoglycate. ns= is considered not significant Vs sodium cromoglycate. N=6*

3.3.4 Anti-edema effect

The anti-inflammatory activity evaluated by the carrageenan edema inhibition test by DA *ACPHYX* gave the results shown in the Fig. 5. Volume inhibition of edema was observed as dose-dependent. However, it is still lower than that of AAS.

3.3.5 Anti-allergic effect

The *ACPHYX* extract showed a dose-dependent inhibition of scratching. The results are shown in the Fig. 5.

4. DISCUSSION

The phytochemical screening showed the presence of an interesting chemical group such as flavonoids, sterols, triterpenes, carotenoids, saponosides and anthocyanins in both the extract from the *ACPHYX* and *CHRYFA* recipes. The same phytochemical groups were found in extracts of *Acacia nilotica* [14] *Acanthospermum hispidum* [15] *Guiera senegalensis* [16] which are component plants of these recipes. The phenolic compound content in *ACPHYX* extract was significantly higher than *CHRYFA* extract. This could be justified by the presence of the *Acacia*

nilotica plant in the *ACPHYX* recipe, which is knowing that a rich phenolic compound plant [17].

The antiradical activity by DPPH method showed a strong antioxidant activity of the extracts. A*CPHYX* extract showed better anti-radical activity compared to the *CHRYFA* extract. *ACPHYX* extract was also presente better activity than Phyllanthus amarus (40 µg/mL) and *Xylopia aethiopica* (2278.89±104 µg/mL) according to Chandan et al. [18] and Ataba et al. [19] which are the component plants of *ACPHYX*. This strong anti-radical activity of *ACPHYX* and *CHRYFA* extract reflects their capacity to protect the body against excess free radicals causing inflammatory diseases.

Antioxidant activity by FRAP method revealed the capacity of ACPHYX and *CHRYFA* extracts to reduce ferric ion. *ACPHYX* extract showed a better reducing power than *CHRYFA*. It is also better than *Terminalia macroptera* (81.17± 0.5 µM ET/mL), the component plant of *ACPHYX* recipe according to Sombié et al. [20]. But it less powerful than reference compound rutin. This iron-reducing capacity of the extracts could be explained by their richness in polyphenols and flavonoids [21].

The pro-inflammatory enzymes inhibition such as lipoxygenase and cyclooxygenases by extracts was very appreciable. *ACPHYX* extract showed a better inhibition of pro-inflammatory enzymes than *CHRYFA* extract almost 50%. *ACPHYX* inhibition activity on lipoxygenase is close that to *Acanthospermum hispidum,* which showed 49.02 ± 1.7 % inhibition [22]. But higher than *Acacia nilotica* (36.05% ± 2.89 and 56.48 ± 0.29) on cyclooxygenases [17] which are the component plant of *ACPHYX* recipe. This strong inhibitory activity of pro-inflammatory enzymes by *ACPHYX* mean a potential anti-inflammatory property. The anti-inflammatory property could be explained by its high content of phenolic compounds, which are cyclooxygenases and lipoxygenases inhibitors [23]. The *CHRYFA* extract showed a low inhibitory power 30% on the pro-inflammatory enzymes.

The IC_{50} results show that the extracts get cytotoxic activity on HeLa cells of cervical cancer and DU 145 cells of prostate cancer. The highest activity was obtained with *ACPHYX* extract on all of cells lines tested at 500 µg/mL. But *CHRYFA* extract showed low activity on all of cells lines cancer. So, for the two lines studied, DU 145 cells were the most sensitive (175.5 ± 3.79 µg/mL for decoction *ACPHYX* and 423.0 ± 45.60 µg/mL for decoction *CHRYFA*), compared to HeLa cells. This cytotoxic activity of the extracts could be explained by the presence of phenol compounds which get antitumor properties [24]. For each extract, the viability of DU 145 and HeLa cells decreased as the concentration of the extracts increased.

The results of *in vivo* anti-edema test of *ACPHYX* extract showed an effective anti-edema property. The extract inhibited edema development at 50% for 400 mg/kg (59.64%) dose. The extract effect is more significant at the second phase of inflammation. This means that the extract would act by the leukotrienes or prostaglandins production blocking by lipoxygenase or cyclooxygenase inhibition. The values of lipoxygenase and cyclooxygenase inhibition percentage *in vitro* of *ACPHYX* extract could corroborate this mechanism action *in vivo*. The effect of Acetyl Salicylic Acid (ASA) are more important than *ACPHYX* edema inhibition (85.05%). The phenolic compounds of *ACPHYX* extract may be responsible for this anti-edema property [25]. The anti-edema effect of *ACPHYX* recipe appears better than *Acacia nilotica*, which inhibition is 68.73 ± 0.01% at 600 mg/kg dose [17] and *Terminalia macroptera* (56.53% inhibition) [26].

The antipruritic activity of *ACPHYX* extract was better by his scratching inhibition at 79.32% for 600 mg/kg dose. Flavonoids, tannins inhibit the release of histamine induced by the compound 48/80 [13]. Flavonoids and tannins presence in the extract could justify this antipruritic efficacy of *ACPHYX* recipe [27].

5. CONCLUSION

This study showed that *ACPHYX* and *CHRYPHA* extracts get cytotoxic, anti-inflammatory, and antipruritic (antihistaminique) properties. The strongest anti-inflammatory activity in vitro and *in vivo* was observed with ACPHYX extract. It also presented the best cytotoxic activity on prostate cancer DU 145 cells and on cervical cancer HeLa cells. These results would be justifying the use of *ACPHYX* in inflammatory and tumour-related diseases.

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 the writing or editing of this manuscript.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the Institutional Ethics Committee for Health Sciences Research of the Research Institute for Health Sciences (IRSS) (Ethics N/Ref: A015-2022/CEIRES).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sharma R, Fronterre C, Ssentongo AE, Yenney K, Amponsah-Manu F, Ssentongo P. Mapping cancer in Africa: A comprehensive and comparable characterization of 34 cancer types using estimates from GLOBOCAN 2020. Front Public Health. 2022;10:83983. Available:https://doi.org/10.3389/fpubh.2022.83983
2. Jedy-Agba E, Joko WY, Liu B, Buziba NG, Borok M, Korir A, Masamba L, et al. Trends in cervical cancer incidence in Sub-Saharan Africa. Br J Cancer. 2020;123(1):148–154. Available:https://doi.org/10.1038/s41416-020-0831-9
3. Kadanga ES, Zouré AA, Zohoncon TM, Traoré L, Ky BD, Yonli AT, Traoré DDA, et al. Carriage of mutations R462Q (RS 486907) and D541E (RS 627928) of the RNASEL gene and risk factors in patients with prostate cancer in Burkina Faso. BMC Med Genomics. 2022;15(1):123. Available:https://doi.org/10.1186/s12920-022-01279-9
4. Baratedi WM, Tshiamo WB, Mogobe KD, Mcfarland DM. Barriers to prostate cancer screening by men in Sub-Saharan Africa: An integrated review. J Nurs Scholarsh. 2020;52(1):85–94. Available:https://doi.org/10.1111/jnu.12529
5. Robert J. Biologie de la métastase. Bull Cancer. 2013;100(4):333–342. Available:https://doi.org/10.1684/bdc.2013.1724
6. Wagner H, Bladt S. Plant Drug Analysis: A Thin Layer Chromatography Atlas. Berlin, Heidelberg: Springer Berlin Heidelberg; 1996. Available:https://doi.org/10.1007/978-3-642-00574-9
7. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Methods in Enzymology. Elsevier; 1999. p. 152–178. Available:https://doi.org/10.1016/S0076-6879(99)99017-1
8. Abdel-Hameed E-S S. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. Food Chem. 2009;114(4):1271–1277. Available:https://doi.org/10.1016/j.foodchem.2008.10.046
9. Nga NTH, Ngoc TTB, Trinh NTM, et al. Optimization and application of MTT assay in determining density of suspension cells. Anal Biochem. 2020;610:113937. Available:https://doi.org/10.1016/j.ab.2020.113937
10. Kim KS, Lee S, Lee YS, Jung SH, Park Y, Shin KH, Kim B-K. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. J Ethnopharmacol. 2003;85(1):69–72.
11. Widowati W, Rani AP, Hamzah RA, Arumwardana S, Afifah E, Kusuma HSW, Rihibiha DD, Nufus H, Amalia A. Antioxidant and antiaging assays of *Hibiscus sabdariffa* extract and its compounds. Nat Prod Sci. 2017;23(3):192.
12. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544–547.
13. Chandrashekhar VM, Halagali KS, Nidavani RB, Shalavadi MH, Biradar BS, Biswas D, Muchchandi IS. Anti-allergic activity of German chamomile (*Matricaria*

recutita L.) in mast cell-mediated allergy model. J Ethnopharmacol. 2011;137(1): 336–340.
14. Rather LJ, Shahid-Ul-Islam, Mohammad F. *Acacia nilotica* (L.): A review of its traditional uses, phytochemistry, and pharmacology. Sustainable Chem Pharm. 2015;2:12–30.
15. Dos Santos ECF, Machado JCB, Ferreira MRA, Soares LAL. *Acanthospermum hispidum* DC: An updated review on phytochemistry and biological activities. Mini Rev Med Chem. 2022;22(5):684–700.
16. Kouame J, Gnoula C, Pale E, Bassole H, Guissou IP, Simpore J, Nikiema J-B. Étude des propriétés cytotoxiques et anti-radicalaires d'extraits de feuilles et de galles de *Guiera senegalensis* J. F. Gmel (Combretaceae). Sci Technol, Sci de la Santé. 2009;32(1 & 2):9–23.
17. Boly A, Gilchrist L, Ouedraogo N, Traore A, Traore KT, Belemlilga M, Belemnaba L, Ouedraogo M, Lompo M, Ouedraogo S, Guissou IP. HPLC analysis and anti-inflammatory properties studies of trunk barks of *Acacia nilotica* var. *adansonii* (Guill and Perr) O. Ktze (Mimosaceae). Int J Pharm Pharm Sci. 2021;13(1):40–46.
18. Chandan S, Umesha S, Balamurugan V. Antileptospiral, antioxidant and DNA damaging properties of *Eclipta alba* and *Phyllanthus amarus*. Sci Rep. 2012;10(1):231.
DOI: 10.4172/scientificreports.231
19. Ataba E, Essoham G, Gnatoulma K, Toudji GA, Ritter M, Ameyapoh AH, Edlom Tchadié P, Batawila K, et al. Toxicity, chemical composition, anti-inflammatory and antioxidant activities of plants used for the treatment of helminth infections in the Kara and Central region of Togo. J Appl Biosci. 2020;156(1):16114–1631.
20. Sombie EN, Tibiri A, N'do JY-P, Traore TK, Ouedraogo N, Hilou A, Guissou PI, Nacoulma OG. Ethnobotanical study and antioxidant activity of anti-hepatitis plant extracts of the Comoé province, Burkina Faso. Int J Biol Chem Sci. 2018;12(3):1308–1319.
21. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010;2(12): 1231-1246.
22. Bayala B, Hilou A, Nacoulma O, Simpore J. Ethnomedicinal and pharmacognostical investigation on three medicinal Asteraceae species from Burkina Faso: *Bidens engleri* O. E. Schulz, *Acanthospermum hispidum* DC., and *Ageratum conyzoides* L. Int J Pharm Biol Sci. 2021;9(4):329-342.
23. Chandel P, Rawal RK, Kaur R. Natural products and their derivatives as cyclooxygenase-2 inhibitors. Future Med Chem. 2018;10(20):2471-2492.
24. Bamba B, Bourahima K, Comoé KDB, Ouattara A, Doukourou DN, Kamou Kamou R, Ouattara K. Teneurs en phenols totaux, activites antioxydantes des maceres et decocte des feuilles de *Uvaria chamae* P. Beauv. (Annonaceae). Int J Biol Chem Sci. 2021;15(1):54-67.
Available:https://doi.org/10.4314/ijbcs.v15i1.6
25. John KK, Ouattara-Soro FS, Abizi G, Zougrou NE, Kouakou KR, Begbin KE, Kple TK, Kablan KJ-J, Koffi S. Activite anti-inflammatoire et études phytochimiques de l'extrait aqueux des écorces *Distemonanthus benthamianus* Baill. (Caesalpiniaceae: Leguminosae - Caesalpinioideae). Eur Sci J. 2021;17(7).
Available:https://doi.org/10.19044/esj.2021.v17n7p74
26. Ballo MT, Conlon P, Lavy-Shahaf G, Kinzel A, Vymazal J, Rulseh AM. Association of tumor treating fields (TTFields) therapy with survival in newly diagnosed glioblastoma: A systematic review and meta-analysis. J Neuro-Oncol. 2023;164 (1):1-9.
27. Cayman Chemical Company. Use brochure cox (ovine) colorimetric inhibitor screening assay kit (Item No. 7600111). Available from: www.caymanchem.com

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