https://www.scirp.org/journal/ns Natural Science, 2024, Vol. 16, (No. 9), pp: 131-145

Antibacterial, Anti-Inflammatory and Antioxidant Activities of an ACAZY Herbal Formula Used in Traditional Medicine to Treat Respiratory Infections

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Keywords: ACAZY, Anti-Inflammatory, Antioxidant, Antibacterial, *In Vitro*

Received: July 26, 2024 **Accepted:** September 7, 2024 **Published:** September 10, 2024

ABSTRACT

ACAZY is a plant formula used in traditional medicine in Burkina Faso to treat respiratory infections. After phytochemical analysis, this study evaluated extracts' anti-inflammatory, antioxidant and antibacterial properties from the ACAZY recipe. Three extractions, an aqueous macerate (AM), an aqueous decoction (AD) and an hydroethanolic macerate (HEM) of the ACAZY recipe powder were carried out. Phytochemical screening of the extracts was carried out using high-performance thin-layer chromatography (HPTLC) and the determination of phenolic compounds. The anti-inflammatory potential was assessed *in vitro* using pro-inflammatory enzyme inhibition tests. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric-reducing antioxidant power (FRAP) antioxidant properties were also determined. The antibacterial activity was

evaluated on *Staphylococcus aureus* and *Streptococcus pneumoniae* strains. Phytochemical analysis revealed the presence of flavonoids, saponins, tannins, anthracenosids, sterols and triterpenes in the extracts. The extracts inhibited pro-inflammatory enzymes by more than 40% at only 100 µg/mL. The extracts also showed potent antibacterial activity with a minimum inhibitory concentration 1 mg/mL on *Staphylococcus aureus* and 2 mg/mL on *Streptococcus pneumoniae*. The extracts in the ACAZY formula have shown anti-inflammatory and antioxidant properties *in vitro*. The AD also showed an antibacterial activity. This justifies its use in traditional medicine to treat acute respiratory infections.

1. INTRODUCTION

According to the World Health Organization (WHO) (2014), acute respiratory infections cause about 4 million deaths worldwide each year. This mortality rate is particularly high in low- and middle-income countries and affects more infants, children and the elderly [1]. Acute respiratory infections (ARIs) are one of the leading causes of death and illness in children under the age of 5 [2-5]. Influenza and the common cold are the most common ARIs, affecting both the upper and lower respiratory tracts diseases [4]. Respiratory infections account for 6% of the global burden of disease, higher than the burden of cancer, HIV infection, ischaemic heart disease, diarrhoeal diseases and malaria [5, 6]. Worldwide, an estimated 6.6 million children under the age of 5 die each year; 95% of these deaths occur in low-income countries, and ARI accounts for a third of all deaths [5, 6]. In 2007, according to the Global Burden of Disease (GBD) report, lower respiratory tract infection caused 704,000 deaths in children under the age of 5 [7]. Acute respiratory infections are a global public health problem and one of the main reasons why young children are admitted to hospitals [8]. More than 12 million children under the age of 5 are admitted to hospital each year because of ARI [6]. In Burkina Faso, acute respiratory infections are among the reasons for the highest number of outpatient consultations in health establishments, with 6,193,114 cases according to the Ministry of Health 2023 [9]. The germs responsible for these acute respiratory infections are generally viruses and bacteria that cause a strong inflammatory response. *Streptococcus pneumoniae* is the most common cause of bacterial pneumonia in many countries [10]. In 2020, the number of deaths from COVID-19 alone was around 2 million worldwide. This would place COVID-19 among the top 10 causes of death worldwide, with other diseases such as ischaemic heart disease, stroke, chronic obstructive pulmonary disease (COPD), lower respiratory tract infections and neonatal conditions ranking highest [11]. In low- and middle-income countries, amoxicillin remains the antibiotic of choice for first-line treatment [10]. However, health professionals are currently facing the emergence of antibiotic-resistant bacterial strains. Antimicrobial resistance affects humans, animals, and the environment and constitutes a public health problem [12-15]. However, health professionals are currently facing the emergence of antibiotic-resistant bacterial strains. The management of these acute respiratory infections, particularly those of bacterial origin, is still largely based on antibiotics. Research into new antimicrobial agents is underway to overcome this resistance and improve tolerability [16]. Natural resources in general, and medicinal plants, are among the most important sources of new effective antimicrobial molecules [17]. In Burkina Faso and throughout Africa, several medicinal plant species are used to treat respiratory infections [18]. As part of the effort to develop these medicinal plants, a scientific study of their antibacterial, antiviral, antioxydant and even anti-inflammatory properties could reassure people that they are effective in treating acute respiratory infections. It is in this context that the present study is being carried out to evaluate the anti-inflammatory, antioxidant and antibacterial efficacy of a formula called ACAZY, composed of four (04) plants: *Acacia nilotica (A. nilotica)*, *Faidherbia albida (F. albida)*, *Zanthoxylum zanthoxyloides (Z. zanthoxyloides)* and *Zingiber officinale (Z. officinale)*, plants used in traditional medicine in Burkina Faso to treat respiratory infections [19-23]. This study evaluated the anti-inflammatory and antibacterial properties of extracts from the ACAZY medicinal plant formula using enzymatic and antibacterial *in vitro* tests.

2. MATERIALS AND METHODS

2.1. Biological Material

The plant material was a recipe named ACAZY, a mixture of powder of four (04) medicinal plants collected in Burkina Faso which are *Acacia nilotica* (L.) Willd. ex Delile, *Faidherbia albida* (Delile). A.Chev, *Zanthoxylum zanthoxyloides* (Lam.) and *Zingiber officinale*. The description and other taxonomic data was conformed to World Flora Online with id: wfo-0000205536 for *A. nilotica*, wfo-0000188059 for *F. albida*, wfo-0000685053 for *Z. zanthoxyloides* and wfo-0000339326 for *Z. officinale*. The bacterial strains used were *Streptococcus pneumoniae* 49619-ATCC, *Staphylococcus aureus* MRSA-43300-ATCC and *Staphylococcus aureus* MSSA-6538-ATCC.

2.2. Pharmacological Substances and Reagents

The following reference products were required for the various tests Acetylsalicylic acid, chloramphenicol, trolox, linoleic acid, lipoxygenase, cyclooxygenases 1&2, DTNB, buffer, diheptanoythiol-PC, TSA (Trypticase Soy Agar), Müller Hinton (MH), DPPH (2,2-diphenyl-picrylhydrazine), ABTS [2,2'-azinobis (3-ethylbenzoin-6-sulfonate)], quercetin, tannic acid, FCR 2N (Folin Ciocalteu Reagent). These products were supplied by Sigma-Aldrich and Cayman.

2.3. Phytochemical Studies

2.3.1. Extraction

Extractions were performed according to ethno-pharmacological indications. Three extractions were performed. An aqueous maceration and an alcoholic maceration (90% v:v) using 150 g of powder in 500 mL of solvent for 24 hours, then a decoction using 75 g of powder in one litre (1 L) of water boiled for 30 minutes. The three extracts obtained were filtered and centrifuged. The macerate and aqueous decoction supernatants were collected in crystallisers, lyophilised and stored in Falcon tubes (50 mL) at −20˚C. The supernatant of the alcoholic macerate was concentrated by means of a rotavator and then dried in an oven. The freeze-dried and dried extracts of the aqueous macerate, decoction and alcoholic macerate obtained were stored in the refrigerator for further testing.

2.3.2. Phytochemical Screening by HPTLC

Phytochemical screening of the extracts was performed on HPTLC plates (10 cm × 5 cm) silica gel 60 F254 (Merck, Darmstadt, Germany) [24]. 10 µL of each extract (C = 40 mg/mL) was applied in 0.8 cm strips using a microlitre Hamilton syringe along the baseline 1 cm from the bottom edge of the plate. The distance between the spots was 0.8 cm. The distance between the plate's first spot and left edge and between the plate's last spot and right edge is 20 mm. Upward linear development with 10 mL mobile phase was carried out in a CAMAG double trough glass chamber lined with filter paper and previously saturated with mobile phase vapour for 30 minutes. The development distance was approximately 70 mm. After development, the plates were dried in an oven at 37˚C. In the double trough chamber, the mobile phase consisted of [25]:

- Terpenoids, proanthocyanins, saponins and tannins were eluted with chloroform/ethyl acetate/methanol/distilled water system 6:18:2.4:2.1, v/v/v/v. Terpenoids were eluted with Liebermann-Bürchard reagent, proanthocyanins with sulfuric anisaldehyde, saponins with sulfuric anisaldehyde reagent (after heating) and tannins with $FeCl_3$ (2%).
- The flavonoids were eluted with the ethyl acetate/formic acid/acetic acid/water system 100:11:11:26, v/v/v and eluted with the NEU reagent.

2.3.3. Total Phenolic Content

Total phenolic compounds were determined by the method of Singleton *et al.* [26]. These compounds react with Folin Ciocalteu Reagent (FCR) in an alkaline medium. The loss of a phenolic proton in the alkaline medium results in a phenolate anion capable of reducing the FCR in which the molybdate is reduced,

forming a blue coloured molybdenum oxide complex with an absorption maximum at 760 nm. The intensity of the blue colour is proportional to the amount of total phenolics present in the test sample. The reaction mixture consisted of 0.5 mL extract, 0.5 mL 2N FCR and 1.5 mL 20% sodium carbonate solution. It was allowed to stand at room temperature for 40 min and then the absorbance was measured at 760 nm using a spectrophotometer (Agilent 8453). In the white control tube, the extract was replaced by distilled water. A standard curve was plotted with tannic acid (1 - 5 µg/mL). Tests were performed in triplicate. The total phenolic concentration of the extract was calculated using the formula:

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

T_{PC} T is the total phenolic content of the extract expressed as tannic acid equivalent (TEA)/g, C_{Tube} is the concentration in mg TEA/mL in the assay tube, D the dilution factor and C_i the concentration in mg/mL in the stock solution.

2.3.4. Total Flavonoid Contents

Flavonoids were determined by the method described by Abdel *et al.* [27]. Two (2) mL of extract at a concentration of 1 mg/mL in methanol was mixed with 2 mL of 2% aluminium trichloride in methanol. After 40 min, the absorbance was measured at 415 nm using a spectrophotometer (Agilent 8453). The white control tube consisted of 2 mL of methanol. The absorbance of quercetin (0.10 mg/ml), used as a reference compound, was measured under the same conditions. The tests were performed in triplicate. The amount of flavonoids in the plant extract in quercetin equivalent (QE) was determined according to the regression equation from the calibration curve:

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

where T_{Flav} is the flavonoid content of the extract expressed in mg EQ/mg, A is the absorbance of the extract, A_0 is the absorbance of quercetin, m is the mass of the extract in mg and m_0 is the mass of quercetin in mg.

2.4. *In Vitro* Antiinflammatory and Antioxidant Activity

2.4.1. 15-Lipoxygenase (15-LOX) Inhibition

The 15-LOX inhibition assay was performed according to the method described by Malterud and Rydland with some modifications [28]. The following reaction mixtures were prepared in a 96-well microtitre plate. An enzyme blank consisting of 153.75 µL borate buffer and 146.25 µL LOX solution at 820.51 U/mL. Enzyme activity, a mixture of 3.75 µL borate buffer, 146.25 µL LOX solution at 820.51 U/mL and 150 µL linoleic acid solution at 1.25 mM. The extract blank consisted of 146.25 µL of 820.51 U/mL LOX solution, 3.75 µL extract and 150 µL borate buffer. The activity of the extract consisted of 146.25 µL LOX solution at 820.51 U/mL, 3.75 µL extract and 150 µL linoleic acid solution (substrate) at 1.25 mM. Each reaction mixture was run in triplicate with an extract concentration of 100 µg/mL in the microplate wells. Changes in the reaction mixture were monitored by photospectrometry at 234 nm immediately after the addition of linoleic acid.

2.4.2. *In Vitro* Inhibition of Cyclooxygenases (COX-1 and COX-2)

The inhibitory potency of the cyclooxygenase extract was assessed according to the procedure of the Cayman manufacturer (Item No. 760111, 2023).

A reaction mixture of 10 µL of extract with 10 µL of prepared enzyme, 10 µL of haemin and 150 µL of diluted buffer was made in one well of a multi-well plate. The same mixture is made without the extract in another well, but supplemented with 10 µL of the extract dilution solvent. A blank consisting of 160 µL diluted buffer, 10 µL haemin and 10 µL extract dilution solvent was also prepared. The whole mixture was vortexed and incubated for 5 min. Then 20 µL of prepared arachidonic acid (substrate) and a colorimetric substrate were added to all wells. The plate was vortexed a second time and incubated for 2 min before

reading with a photospectrometer at 590 nm. The reaction mixtures were in triplicate with salicylic acetic acid as the reference.

2.4.3. DPPH Free Radical Scavenging Assay

The ability of the extracts to reduce DPPH free radicals was determined by the method of Kim *et al.* [29]. A series of concentrations were determined from the starting concentration (1 mg/ml) of the samples. On a 96-well microplate, each well for each concentration was filled and placed on a plate with 200 µL DPPH solution (0.04 mg/mL) and 20 µL of the diluted extract or reference. After incubation for 30 minutes, the absorbance was read at 490 nm using a spectrophotometer. The blank without sample was prepared under the same conditions and consisted of 200 µL DPPH and 20 µL ethanol. A curve of percentage DPPH inhibition was plotted as a function of sample concentration. The concentration required to inhibit 50% of DPPH (IC_{50}) was determined from the curve.

2.4.4. Ferric-Reducing Antioxidant Power (FRAP) Assay

The reducing power of the extracts was assessed using the technique of Silue *et al.* [30]. The FRAP solution was prepared by mixing 300 mM sodium acetate buffer adjusted to pH 3.6, 10 mm TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) solution and 20 mm ferric chloride $FeCl_3$ in the ratio 10:01:01. 300 µL FRAP solution was added to 10 µL extract solution. The absorbance of the mixture was measured at 593 nm after 30 min of reaction. The increase in absorbance in the reaction medium was proportional to the increase in iron reduction. A calibration curve (0.025 - 0.5) was first established by preparing a series of ascorbic acid solutions. All preparations and analyses were performed in triplicate.

2.5. *In Vitro* Antibacterial Activity

2.5.1. Bacterial Strains

Three respiratory pathogenic bacterial strains were used. These were *Streptococcus pneumoniae* (49619-ATCC), *Staphylococcus aureus* MRSA (3300-ATCC) and *Staphylococcus aureus* MSSA (6538-ATCC).

The bacterial inoculum for the test was prepared from the stock solution of the fresh bacterial strain. The optical density (OD) of the inoculum was adjusted to between 2 and 3 at 600 nm. After adjustment, the final inoculum contained 10^6 - 10^7 CFU/mL.

2.5.2. Bacterial Sensitivity Test

The sensitivity of the bacterial strains to the extract was tested using the agar diffusion technique [31]. 0.5 mL of bacterial inoculum was inoculated into Petri dishes by flooding with solid TSA (trypticase soy agar) medium. Approximately 6 mm diameter wells were made in the solid TSA bacterial medium using a sterile cone. 100 µL of a 10 mg/mL concentration of the extract was added to each well. After allowing the extract to diffuse into the TSA medium for 30 minutes at room temperature in a fume hood, the dishes were incubated in an oven at 37˚C for 24 hours. At the end of 24 h, the presence or absence of an inhibition zone was observed under a magnifying glass and the diameter of the inhibition zone was measured. Chloramphenicol at 0.5% was used as a control. The results were interpreted according to Ponce *et al.*, (2003) [32].

2.5.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC is the lowest concentration of extract at which no growth is visible to the naked eye after an incubation period of 18 to 24 hours. It was determined by the test tube dilution technique [33]. A cascade dilution in test tubes of the extract dissolved in the MH liquid medium was used to obtain a series of concentrations of 8, 4, 2, 1, 0.5 and 0 mg/mL (bacterial control). At each concentration, 50 µL of bacterial inoculum (1% of the volume of the extract solution) was added. The tubes were then incubated in an oven at 37˚C for 18 and 24 hours. At the end of the incubation period, the turbidity of each tube caused by bacterial growth was observed in the negative control tube (extract solution without bacteria). The tube with the

lowest concentration for which no opacity was observed with the naked eye was considered the MIC. The MBC is the minimum bactericidal concentration of the extract at which no more than 0.01% of the bacteria survive. For this purpose, 5 µL of the contents of the tubes in which no turbidity was observed, starting with the MIC tube, were inoculated onto TSA medium in Petri dishes. After 24 hours of incubation at 37˚C in an oven, the concentration of the dish in which no bacterial growth was observed.

2.6. Statistical Analysis

The analysis of the results of the *in vitro* tests was carried out based on statistical processing using Graph Pad Prism software version 10.0.2. Data were given as mean ± error standard of the mean (SEM) obtained from three independent experiments and analysed with Student's t-test for paired data. The differences in data were considered to be statistically significant ($p < 0.05$) compared to control.

3. RESULTS

3.1. Extraction Yields

The extraction yields are shown in the Table 1.

Table 1. Yields for the different extractions of the ACAZY recipe.

Extracts	Extraction yields (%)
Aqueous extracts	15.68 ± 0.25
Hydroethanolic macerate	26.71 ± 0.91
Aqueous decoction	25.94 ± 0.31

Values are expressed as mean ± SEM.

3.2. HPTLC Screening

The results of phytochemical screening by TLC revealed the presence of phytochemical groups shown in Figure 1.

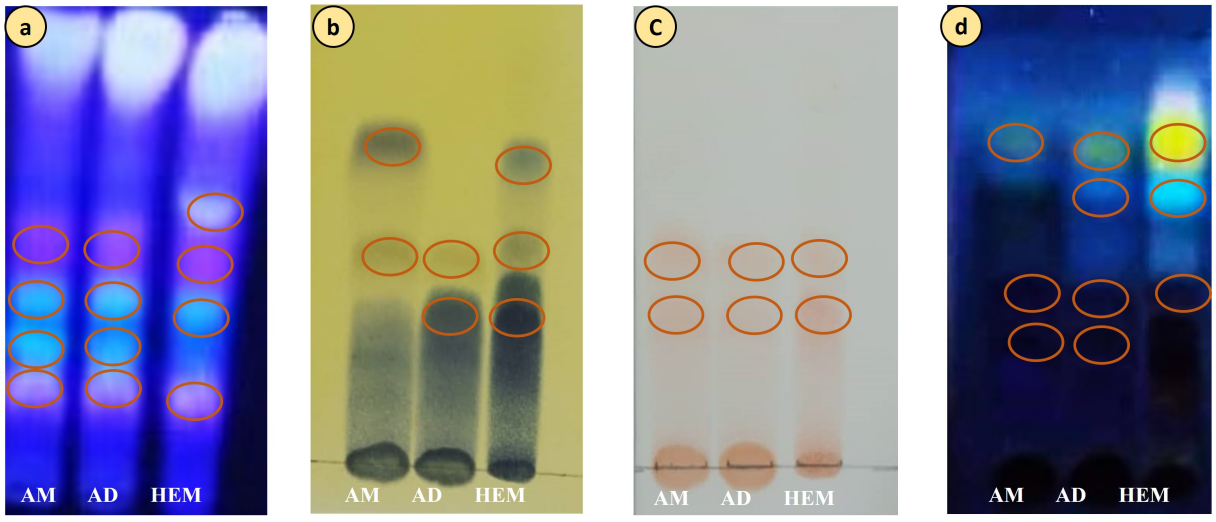


Figure 1. Chromatographic profile of aqueous macerate, aqueous decoction and ethanolic macerate. (a) Flavonoids; (b) Tannins; (c) Proanthocyanins, (d) Sterols and triterpenes.

3.3. Total Flavonoid and Phenolic Total Contents

The assay was used to determine the total flavonoid and phenolic content of the different extracts from the ACAZY recipe, as shown in Table 2.

Table 2. Total phenolics, and flavonoids contents in different extracts of the ACAZY recipe.

Extracts	Total phenolic content (mg TAE/g extract)	Flavonoid content (mg QE/g extract)
Hydroethanolic macerate	422.41 ± 6.29 ns	4.03 ± 0.16 ns
Aqueous macerate	271.12 ± 1.62***	4.57 ± 0.32 ns
Aqueous decoction	414.08 ± 3.53 ns	8.54 ± 0.40**

Values are expressed as mean ± SEM, TAE: tannic acid equivalent; QE: quercetin equivalent. **$p < 0.01$ is considered significant compared to aqueous decoction; ***$p < 0.001$ compared to aqueous decoction; ns is considered not significant compared to other extracts. (one-way ANOVA analysis followed by Dunnett's test).

3.4. Inhibition of 15-Lipoxygenase (15-Lox) and Cyclooxygenases (COX1 & COX2)

The enzyme inhibition percentage of anti-inflammatory properties against 15-LOX and COX1 & COX2 of the three extracts was determined in Table 3.

Table 3. Enzyme inhibition percentage.

Extracts (n = 3)	%Inh COX-2	%Inh COX-1	%Inh LOX
AM (100 µg/mL)	33.78 ± 1.34ns	26.03 ± 0.58**	59.72 ± 3.61**
AD (100 µg/mL)	37.76 ± 1.07ns	42.15 ± 2.34ns	42.22 ± 2.90***
HEM (100 µg/mL)	42.39 ± 0.81*	45.66 ± 0.88ns	87.44 ± 0.5
ASA (10 µg/mL)	16.58 ± 1.45	-	-
Indomethacin (100 µg/mL)	-	-	91.47 ± 0.8

Values are expressed as mean ± SD; **$p < 0.01$ is considered significant compared to other extracts; ***$p < 0.001$ compared to other extracts; ns is considered not significant compared to other extracts; AM: aqueous macerate; AD: aqueous decoction; HEM: ethanolic macerate.

3.5. DPPH Anti-Free Radical Effect

The results of the DPPH anti-free radical test of the extracts, shown in Figure 2. showed very significant 50% inhibition concentrations (IC_{50}).

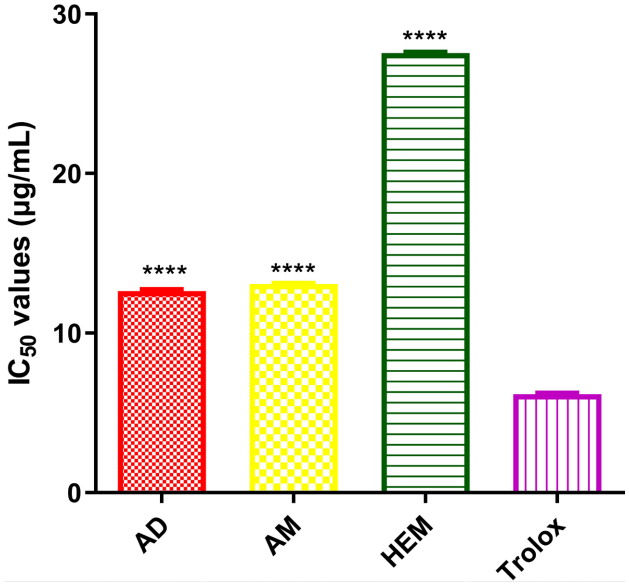
3.6. Ferric-Reducing Antioxidant Power (FRAP)

The activity of ACAZY extracts in reducing ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) is illustrated in Figure 3.

3.7. Bacterial Sensitivity

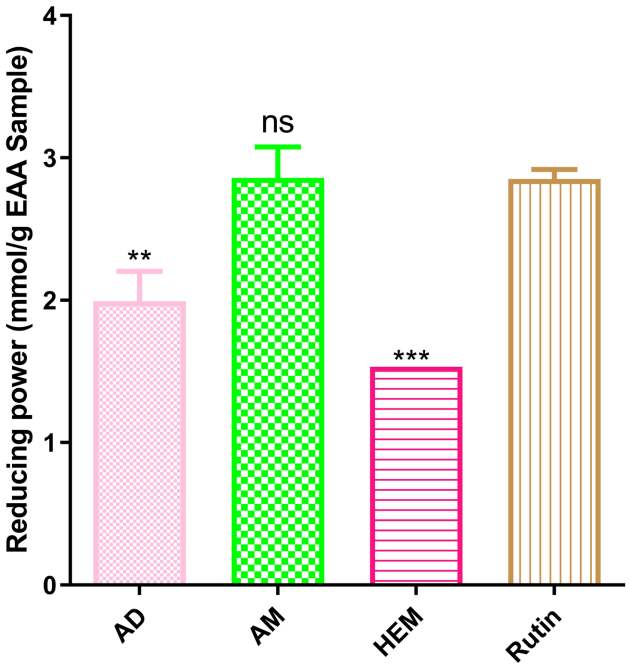
All 3 bacterial strains showed sensitivity to 10 mg/mL aqueous decoction, with inhibition diameters

greater than 10 mm. The results are shown in the tables (Table 4 and Table 5) below.



Values are expressed as mean ± SD; **p < 0.01 is considered significant compared to control (Trolox); ***p < 0.001 compared to the reference (Trolox); ns is considered non-significant compared to Trolox; AM: aqueous macerate; AD: aqueous decoction; HEM: ethanolic macerate.

Figure 2. Anti-free radical effect of the extracts in the recipe by DPPH method.



Values are expressed as mean ± SD; **p < 0.01 is considered significant compared to control (Trolox); ***p < 0.001, **p < 0.01 compared to the reference (Rutin); ns is considered non-significant compared to Rutin; AM: aqueous macerate; AD: aqueous decoction; HEM: ethanolic macerate.

Figure 3. Anti-free radical effect of the extracts in the recipe by FRAP method.

Table 4. Inhibition diameter of aqueous decoction on bacterial strains.

S. No.	*Streptococcus pneumoniae* 49619-ATCC	*Staphylococcus aureus* MRSA-43300-ATCC	*Staphylococcus aureus* MSSA-6538-ATCC
Aqueous decoction Inhibition diameter (mm)	13.25 ± 0.35***	14.25 ± 0.35***	14.5 ± 0.70***
Chloramphénicol Inhibition diameter (mm)	27.5 ± 0.7	23.05 ± 0.1	22.5 ± 0.7

Values are expressed as mean ± SD, *p < 0.05; **p < 0.01; ***p < 0.001 is considered significant compared to the positive control (chloramphenicol).

Table 5. MIC and MBC parameters for aqueous decoction.

S. No.	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI
Streptococcus pneumoniae 49619-ATCC	2 ± 0.0ns	4 ± 0.0ns	2
Staphylococcus aureus MRSA-43300-ATCC	1 ± 0.0ns	2 ± 0.0ns	2
Staphylococcus aureus MSSA-6538-ATCC	1 ± 0.0ns	4 ± 0.0ns	4

Values are expressed as mean ± SD; **p < 0.01 is considered significant compared to other extracts; ***p < 0.001 compared to other extracts; ns is considered not significant compared to other extracts, S. No: Strain number.

4. DISCUSSION

Phytochemical analysis revealed the presence of chemical groups of interest such as tannins, flavonoids, sterols, triterpenes, saponins and proanthocyanins in the three extracts. The test showed that the aqueous decoction and the alcoholic macerate were richer in phenolic compounds than the aqueous macerate. These results on the three plants that make up the ACAZY recipe *Zanthoxylum zanthoxyloides*, *Acacia nilotica* var adansonii, *Zingiber officinale*, are consistent with those reported by some authors [34-36]. In order to assess the anti-inflammatory potential of the extracts, tests to inhibit the pro-inflammatory enzymes lipoxygenase and cyclooxygenase were used. The alcoholic extract (HEM) showed a better lipoxygenase inhibitory activity (over 80%) than the aqueous extracts (AD and AM). On the other hand, for cyclooxygenases (COX1 and COX2), the alcoholic extract of HEM and the aqueous extracts of AM and AD showed a good inhibitory activity, relatively close to over 40%. These results are close to those obtained by Boly *et al.* with the aqueous extract of *Acacia nilotica*, which was 36% for COX1 [37]. Atèhèzi *et al.* also reported good cyclooxygenase inhibitory activity of Zanthoxylum zanthoxyloides extracts [38]. This confirms the enzyme inhibitory activity of extracts from the ACAZY formulation of *Zanthoxylum zanthoxyloides* and *Acacia nilotica* plants. The chemical groups flavonoids and tannins may be responsible for the enzyme inhibitory activity of ACAZY recipe extracts [39]. The extracts also showed strong antioxidant activity, similar to the reference compound Trolox. In fact, the anti-free radical activity of the extracts from the ACAZY recipe was better than that of its constituents, *Acacia nilotica*, *Zingiber officinale* and *Zanthoxylum zanthoxyloides* [40-43]. The iron-reducing capacity of the extracts was found to be very effective, with an activity of more than 1000 µmol·mL ascorbic acid equivalent. The AM extract showed the highest iron reducing power (2858.76 ± 306 µmol·mL AA equivalent) compared to the AD and HEM extracts. All extracts showed high iron-reducing power compared with the constituent plants *Gingiber officinale* and *Acacia nilotica* [40, 44-46]. These results strongly suggest the potential of the formulation as a natural antioxidant. The iron reduction

potential of the recipe is higher than that of Acacia nilotica, which is 152.79 ± 7.43 µg/mL according to the work of Subhaswaraj *et al.* [47]. Similarly, the work of Ghasemzadeh *et al.* [9] showed a lower activity of *Gingiber officinale* than the recipe, with a reducing power of 680.68 ± 18.38 µmol. The presence of flavonoids could explain the antioxidant activity of the extracts, as flavonoids are free radical scavengers, which gives them antioxidant properties [45, 48]. The results of the inhibition of lipoxygenase activity show that the extracts in the formulation had an inhibitory effect on this activity. The percentage inhibition values obtained were 87.43%, 59.72% and 42.24% for the hydroalcoholic macerate, aqueous macerate and aqueous decoction, respectively. The hydroalcoholic macerate showed a higher activity. These results could be explained by the presence of polar and apolar compounds with antioxidant properties in the hydroalcoholic extract, whereas our aqueous extract is mainly composed of polar compounds. These results show that the formulation has a strong anti-inflammatory effect. This justifies the use of the plants that make up the formula in the treatment of respiratory infections. The presence of flavonoids and tannins could explain the high antioxidant activity of the extracts. Flavonoids and tannins are free radical scavengers, which gives them antioxidant properties [49]. Anti-free radical activity (DPPH) shows that the extracts have the ability to scavenge the DPPH radical. The ability to scavenge the DPPH radical reflects the ability to eliminate free radicals formed in the body. The IC_{50} of the hydroalcoholic macerated, aqueous macerated and aqueous decoction extracts of the formulation are 6.50 µg/mL, 13.08 µg/mL and 12.61 µg/mL respectively. Trolox tested under the same conditions gave an IC_{50} of 6.3 µg/mL. The IC_{50} values indicate a strong anti-free radical activity of the extracts. The hydroalcoholic macerate showed the best anti-free radical activity close to that of Trolox compared to the aqueous extracts. The anti-free radical activity of the extracts was better than that of *Acacia nilotica*, whose IC50 was 45 µg/mL [40]. According to the work of Namkona *et al.*, the activity of *Zanthoxylum zanthoxyloides* is very low compared to that of the extracts, with an IC50 = 3.44 mg/mL [43]. This high activity of the formulation is probably due to the synergistic effect of the activity of these plants. This activity could be attributed to certain phytochemical groups detected in the formula, such as flavonoids, saponins and tannins, which are potent free radical scavengers [50]. The results of the antibacterial activity showed that the extract inhibited the growth of all 3 bacterial strains from the concentration of 1 mg/mL, with a bactericidal effect at the maximum concentration of 4 mg/mL. All 3 bacterial strains showed sensitivity to the aqueous extract of ACAZY at 10 mg/mL, with inhibition diameters greater than 10 mm. These results demonstrated that the extract had effective antimicrobial activity against *Streptococcus pneumoniae* 49619-ATCC, *Staphylococcus aureus* MRSA-43300-ATCC and *Staphylococcus aureus* MSSA-6538-ATCC. Chloramphenicol, used as a control, was more effective than the extract. The study conducted by Dewi and Manik on the activity of Zingiber officinale rhizomes against *Staphylococcus aureus* which showed a MIC of 1mg/mL [51]. The antibacterial activity of the formula is thought to be related to the presence of phytochemical groups in the plants used in the formula, such as flavonoids, tannins, alkaloids and saponins. Several studies on these secondary metabolites show their antibacterial properties. Rauha *et al.* reported that flavonoids such as quercetin and naringenin are active against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis*, *Micrococcus luteus*, *Escherichia coli* and *Pseudomonas aeruginosa* [52]. Akiyama *et al.* investigated the antibacterial effect of various tannins on plasma clotting by S. aureus. They observed that tannic acid inhibited the growth of all bacteria tested [53]. Okoro *et al.* demonstrated the antibacterial activity of alkaloids on several bacteria, namely *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 53154, *Staphylococcus carmonum* LMG 13567, Bacillus cereus LMG 13569, Listeria offensive LMG 13568, Enterococcus faecalis CIP 103907, Shigella dysentery CIP 54051 and *Escherichia coli* CIP 105182 [54]. The chemical groups identified, sterols and saponins, flavonoids and tannins are known to have anti-inflammatory and antibacterial properties [16, 55]. This may justify the potential anti-inflammatory and antibacterial properties of the recipe extracts.

5. CONCLUSION

The results of this study show that extracts from the ACAZY formula have anti-inflammatory properties *in vitro*. The aqueous decoction (AD), the form used in traditional medicine, also showed very good

antibacterial activity *in vitro* against *Streptococcus pneumoniae* and *Staphylococcus aureus*, the bacteria responsible for acute respiratory infections. The chemical groups identified as tannins, flavonoids, sterols, triterpenes and saponins could be responsible for the anti-inflammatory and antibacterial properties of the extracts in the ACAZY formulation. The use of this formula in traditional medicine for acute respiratory infections seems justified. The results of our study indicate the possibility of evaluating the acute and sub-acute general toxicity of the extracts, assessing the *in vivo* anti-inflammatory activity of the extracts, and performing bioguided fractionation to isolate and identify the molecule(s) responsible for the activities against respiratory infections.

AUTHOR'S CONTRIBUTION

MBB: conceptualization, investigation, analysis, interpretation of data, and wrote the manuscript. BB: project administration and review. AGLB, MAO, ARB, BY and FB: conducted experiment, formal analysis. PN, J-MC and PD: plant collect and recipe formulation. CD, MK, DI, CBS and MTZ: methodology, performed literature survey. MO, AT, NO, FM, RS and SO: validation, supervision and revising manuscript. JS and IPG: funding acquisition, editing and supervision.

ETHICS STATEMENT

The experimental protocol was carried out in accordance with international standardized protocols [guidelines established by the European Union on the protection of the environment]. international [European Union guidelines on the protection of animals (CEC Council 86/609)] and adopted by the Laboratoire de Recherche Developpement de Phytomédicaments et Médicaments (LR-D/PM) of the IRSS, Burkina Faso. The sections of this report concur with the ARRIVE guidelines for reporting animal research.

ACKNOWLEDGEMENTS

We are grateful to the "Fond National de la Recherche et de l'Innovation pour le Développement (FONRID)" which has kindly agreed to finance the project known as AAP N˚0086 FONRID/AAP8/NCP/PC/2021 FONRID. Thanks to AAP rapide COVID-19_mala infect_1-03 FONRID, which helped us to carry out this work.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest regarding the publication of this paper.

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