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Research Article

Evaluation of Antioxidant and Anti-inflammatory Activities of a Medicinal Plant Recipe from Burkina Faso

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Abstract

Background: Oxidative stress and chronic inflammation are crucial factors in the development of various diseases, such as hepatitis and cancer. In the case of hepatitis, persistent inflammation of the liver contributes to cellular damage and progression to more severe stages of the disease. *Objective:* The aim of this study is to evaluate the antioxidant and anti-inflammatory activity of a recipe, *Hepatib tiben*, traditionally used in the treatment of viral hepatitis B, and often associated with *Momordica charantia* in the treatment of liver cancer in Burkina Faso. *Material and Methods:* The recipe and associated plants were infused, macerated and the yields were evaluated and being freeze-dried. The extracts obtained were used to determine the total content of phenolic and flavonoid compounds by measuring the antioxidant activity through the FRAP, DPPH and ABTS methods and then the evaluation of the anti-inflammatory properties by inhibition of 15-LOX and COX 1 & 2. *Results:* The aqueous infusion of *Hepatib tiben* had the best yield, the highest content of phenolic compounds and flavonoids, an antioxidant activity comparable to that of Rutin, by the FRAP method, DPPH and ABTS method. But the best antioxidant activity by FRAP method was observed with the DCM fraction. It is on the other hand the hydroalcoholic extract which had the best anti-inflammatory activity by the inhibition of the 15-LOX. Both Aqueous and Hydroalcoholic Extracts inhibited COX 1 & 2. *Momortica charantia* had high phenolic content and significant antioxidant activity by FRAP, DPPH and ABTS methods. *Conclusion:* These observations suggest that the recipe of traditional medicinal plants used in Burkina Faso could be beneficial in the treatment of inflammatory conditions and oxidative stress. Further studies are needed to identify the active compounds responsible for these activities and to assess their safety and clinical effectiveness.

Keywords

Anti-inflammatory, Antioxidant, Recipe, *Momordica charantia*, Cancer, Hepatitis B

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1. Introduction

Inflammation and oxidative stress play a central role in the development and progression of many chronic diseases, including hepatitis and cancer [1]. The inflammatory process is often accompanied by an increase in oxidative stress, caused by an overproduction of free radicals and a decrease in natural antioxidants [2]. Oxidative stress contributes to the progression of hepatitis by promoting liver fibrosis, cirrhosis and potentially liver cancer [3]. For centuries, the use of medicinal plants has been the most accessible medical system in Africa. These plants contribute significantly to the life and social balance of rural African populations, especially those in the most disadvantaged areas [4]. They often offer natural compounds with anti-inflammatory and antioxidant properties. However, the effectiveness of these plants can vary and it is essential to test their ability to reduce inflammation and mitigate oxidative stress [5]. In Burkina Faso, the use of traditional medicinal plants continues to play a crucial role in the health and well-being of populations [6]. Phyllanthus amarus, Cassia nigricans, Balanites aegyptiaca and Momordica charantia are among the plants widely used in traditional medicine to treat various conditions, including inflammatory complications and oxidative stress disorders [7]. Phyllanthus amarus, known locally as Tchekeleni (Dioula); Tinguin garga (Mooré) is known for its hepatoprotective and anti-inflammatory properties [8]. Cassia nigricans, or «Zan-drekouka» in local Mooré language is used for its antioxidant, potential anti-inflammatory and analgesic effects [9]. Balanites aegyptiaca, called "Kyɛglga" in the Mooré language, is known for its antimicrobial, anti-inflammatory and analgesic properties [10, 11], while Momordica charantia is renowned for its hypoglycemic, anti-inflammatory and antitumor effects [12]. The evaluation of anti-inflammatory and antioxidant recipe activity based on the association of these medicinal plants is of increasing importance in the field of pharmaceutical research and traditional medicine. Indeed, the study and identification of bioactive compounds present in these plants and their therapeutic potential could provide a solid scientific basis for their use in traditional medicine and possibly for the development of new therapeutic agents.

Thus, this study aims to demonstrate the anti-inflammatory and antioxidant activity of a recipe composed of *Phyllanthus amarus*, *Cassia nigricans* and *Balanites aegyptiaca*, traditionally used in the treatment of viral hepatitis B, and often associated with *Momordica charantia* in the treatment of liver cancer in Burkina Faso.

2. Materials and Methods

2.1. Extraction

The plant material is a recipe, *Hepatib tiben*, composed of dried whole plants of *Phyllantus amarus* and *Cassia nigricans* and leaves of *Balanites aegyptia*, prepared and packaged in capsules often associated with whole plant of dried *Momordica charantia*. Harvesting, drying, powder reduction and packaging are carried out by the traditional health practitioner. Infusion and maceration were the processes used for the extraction of active ingredients.

2.1.1. Infusion

A test sample of 50 g of the vegetable drug of the recipe *Hepatib tiben* and the powder of *Momordica charantia*, was placed each in a glass jar with a lid. A volume of 500 mL of boiled distilled water was poured over the contents of each jar. The mixture of the plant material and the extractor solvent was homogenized by mechanical agitation using a spatula. The homogenized mixture was kept in contact for 1 hour. The infused extract was filtered by manual pressing on a fine mesh nylon fabric. The vegetable residue after pressing was dispersed in 250 mL of distilled water and then pressed again. The resulting filtrates were filtered a second time on the nylon fabric and then centrifuged at 2000 rpm for 10 min. the supernatant of each extract was collected and the volumes were measured to determine the extraction yields. Each extract was frozen for freeze-drying.

2.1.2. Maceration

1) The hydro-alcoholic maceration

Maceration is an extraction technique that consists of mixing a dry or fresh vegetable material with an extractor solvent and macerating for a given time. The procedure used for the maceration of the *Hepatib-tiben* recipe was the common one. Indeed, a test sample of 50 g of the vegetable drug of the recipe *Hepatib tiben* was placed in a glass jar with a lid. A volume of 500ml of a water and ethanol mixture in the proportions 30:70 (v/v) was poured over the contents of the jar. The mixture of the plant material and the extractor solvent was homogenized by mechanical agitation using a spatula. The homogenized mixture was held in contact for 48 hours. The macerated extract was filtered on Watman no 5 paper. The vegetable residue was leached by percolation with small portions of the extractor solvent until clear. The resulting filtrates were concentrated by evaporation under reduced pressure to the rotary evaporator to eliminate the ethanolic phase. The residual aqueous portion of the extract after concentration was frozen for freeze-drying.

2) Successive exhaustion extraction

Successive exhaustion extraction is an extraction technique that consists of extracting until exhaustion, a dry or fresh plant material with solvents of increasing polarity. The procedure used for the fractional extraction of the *Hepatib-tiben* recipe was the one adopted in the laboratory. Indeed, a test sample of 100g of the vegetable drug of the recipe *Hepatib-tiben* was placed in a glass jar with a lid. A volume of 500ml of analytical dichloromethane (DCM) was poured over the contents of each jar. The mixture of the plant material and the extractor solvent was homogenized by mechanical agitation using a

spatula. The homogenized mixture was maintained for 5 hours. The maceration was filtered on watman no 5 paper and then the vegetable residue was depleted with small portions of dichloromethane. The resulting filtrates were evaporated dry under reduced pressure using a BÜCHII rotary evaporator. The mass of the DCM dry extract obtained was determined. The vegetal residue after exhaustion with DCM was dried at laboratory room temperature and then infused with 500ml of distilled water scalded for 1 hour. The infused extract was filtered by manual pressing on a fine mesh nylon fabric. The vegetable residue after pressing was dispersed in 250ml of distilled water and then pressed again. The resulting filtrates were filtered a second time on the nylon fabric and then centrifuged at 2000 rpm for 10 min. the supernatant of the extract was collected and its volume was measured to determine the extraction yield (Rd). The infused was frozen for freeze-drying.

$$Rd\ (\%) = (M'/M) * 100$$

M: initial mass of the recipe

M': mass of dry extract after extraction and freeze-drying

3) Determination of Residual Moisture Content (THR)

The empty mass of the watch glasses was weighed and a gram of powder was added to it. The watch glasses were placed in an oven for two hours and 30 minutes. After cooling, they were weighed a second time. The Residual Moisture Content was calculated using the following formula:

$$THR\ (\%) = ((P\text{-}P') /P) *100$$

P: Initial weight (g) of plant material

P': Final weight (g) of plant material after parboiling

2.2. Phytochemical Determination of Extracts

2.2.1. Determination of Total Phenolic Content

The total phenolic content of Hepatib tiben and Momordica charantia extracts was determined using the method described by Singleton et al. [13] based on the oxidizability of phenolic compounds. The reagent used was a mixture of phosphomolybdate and sodium tungstate which is reduced during the oxidation of phenols in an alkaline medium in a mixture of tungsten blue and molybdenum. There is then a change in the colorimetric properties of the Folin-Ciocalteu reagent, which reacts with the OH function of phenol. For this study, 96-well microplates were used. In each well 50 µl of extract solution (1 mg/ml) were added 50 µl of FCR solution (0.2N) and 100 µl of sodium carbonate solution (Na_2CO_3) 20% successively. The mixture was incubated for 40 minutes in the dark, then the reading was carried out at an absorbance of 760 nm on the BioTek EPOCH 2 spectrophotometer. The results were expressed in mg Equivalent Gallic Acid per gram of dry extract (mg GAE/g ES), and calculated from the gallic acid calibration curve.

2.2.2. Determination of Total Flavonoid Content

The total flavonoid content of Hepatib tiben and Momor-dica charantia extracts was determined using the Kumaran method [14] with some modifications. Indeed, a volume of 100 µl of 2% aluminum chloride ($AlCl_3$) in methanol was mixed with a volume of 100 µl of extract (1 mg/ml) diluted in methanol. The same procedure was used for quercetin, with ranges of diluted concentrations of half (from 1 mg/ml) to establish the calibration curve. Absorption was measured using the BioTek EPOCH 2 spectrophotometer at 430 nm after 30 min of light-shielded incubation. The flavonoid concentration in the extracts was derived from a calibration line established with quercetin and expressed in mg quercetin equivalent per mg extract (mg QE/g) [15].

2.3. Antioxidant Activities

2.3.1. Fe^{3+} Ferric Ion Reduction Test (FRAP)

The ferric ion reduction test (FRAP test) is based on the ferric ion (Fe^{3+}) reduction in ferrous ion (Fe^{2+}). This method evaluates the reducing power of extracts. At low pH, when a ferric complex is reduced to the ferrous form (Fe^{2+}), an in-tense blue color develops with maximum absorption at 593 nm. The increase in absorbance in the reaction medium was proportional to the increase in iron reduction. The test was performed according to the method described by [16]. At 10 µl of extract (1mg/ml) distributed in a 96-well plate with a few wells used as blank containing 10 µl of buffer, 300 µl of FRAP Reagent was added: (1 volume of TPTZ (2,4,6-tripyridyl-j-triazine) + 10 volumes of CH_3COONa buffer + 1 volume of $FeCl_3$). Rutin was used as positive control. The reaction mixture was incubated for 30 minutes away from light and the absorbance was measured with the BioTek EPOCH 2 spectrophotometer. The absorbances were read against a standard ascorbic acid curve: equation: y = 0,8337x + 0,0499 (R2=0,9976) The results were expressed in millimole ascorbic acid equivalents per milligram (mmol EAA/mg) and calculated using the formula:

$$C= (c*D) /(M*Ci)$$

c: sample concentration (calculated from curved ascorbic acid)

D: Dilution factor

M: Molar mass of ascorbic acid (176.1g/mol)

Ci: Concentration of stock solution

The extracts and control samples were weighed and dissolved to obtain a concentration of 1 mg/ml. The test was then carried out in accordance with the established protocol for the extracts and control samples.

2.3.2. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) Anti-radical Activity

The free radical scavenging capacity will be determined

by the DPPH method [17]. Each extract was prepared at 1mg/ml. The extracts were initially diluted in cascades (1/2) in plates of 96 wells. Then, 20µl of extract was mixed with 200 µl of DPPH solution (4 mg/100ml in methanol, prepared away from light). White was 20µl methanol and 200µl DPPH solution. After 30 min incubation in the dark, absorbance was read at 490 nm using the BioTek EPOCH 2 spec-tropho-tometer. Quercetin was used as a positive control. The meas-ured absorbance was used to calculate the percentage inhibi-tion according to the following formula:

$$\% \text{ Inhibition} = [(\text{White Absorbance} - \text{Extract Absorbance}) / \text{White Absorbance}] \times 100$$

The results are expressed in µg of extracts/µg of DPPH. The extract concentration capable of trapping 50% of the DPPH radicals was calculated.

2.3.3. ABTS$^+$ (2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)) Antiradical Activity

Spectrophotometric analysis of the activity of ABTS+ rad-ical scavenging extracts was determined using the meth-od of Re et al., 1999 [18]. The ABTS+ solution was prepared by dissolving 10 mg of ABTS in 2.6 ml of distilled water. Then it was added 1.7212mg of potassium persulfate and the mix-ture was kept in the dark at room temperature for 12 hours. The mixture was then diluted with ethanol to obtain an ab-sorbance of 0.70 ±0.02 at 734 nm. In 96-well plates, 50 µl of extract solution was added to 100 µl of ABTS+ solution, with a blank composed of 50 µL of ethanol and 100 µL of ABTS solution. Mixing was carried out in 96-well plates, incubated in the dark at room temperature for 15 minutes and the concentration was read at 734 nm using the BioTek EPOCH 2 spectrophotometer. Each test was performed in triplet and the experiment was repeated three times. Inhibi-tion Percent-ages were calculated using the following for-mula:

$$\% \text{ Inhibition} = [(\text{White Absorbance} - \text{Extract Absorbance}) / \text{White Absorbance}] \times 100.$$

The inhibition percentages were then used to calculate the concentration allowing the inhibition of 50% ABTS+ (IC50). Trolox was used as a reference compound.

2.4. Anti-inflammatory Activity in Vitro

2.4.1. Inhibition of 15-lipoxygenase (15-LOX)

The inhibition of lipoxygenase by the extracts was done according to the method of Malterud and Rydland, 2000 [19]. In a 96-well plate, the following reaction mixtures were prepared: the enzyme control mixture consisted of 153.75 µl borate buffer plus 146.25 µl lipoxygenase (400 U/ml). The mixture for enzymatic activity was composed of 3.75 µl borate buffer plus 146.25 µl lipoxygenase and 150 µl linoleic acid (1.25 mmol). The mixture for extraction control was composed of 146.25 µl lipoxygenase, 3.75 µl extract and 150 µl borate buffer. The mixture for extract activity was com-posed of 146.25 µl lipoxygenase, 3.75 µl extract and 150 µl linoleic acid (substrate). Indomethacin was used as positive control. Each mixture was distributed in triplet in the wells of a 96-well plate. The plate was then read to the 234 nm absorbance spectrophotometer immediately after substrate addition. The percentage of lipoxygenase inhibition by the extracts was calculated as follows:

$$\text{Inhibition (\%)} = [(\text{Enzyme Absorbance} - \text{Sample Absorbance}) / \text{Enzyme Absorbance}] \times 100$$

Where,

Enzyme Absorbance = Absorbance of Enzyme Activity - Absorbance of Enzyme Blank; Sample Absorbance = Ab-sorbance of Sample Activity - Absorbance of Sample Blank

2.4.2. Inhibition of Cyclooxygenase (COX1 & 2)

COX activity inhibition tests were performed according to the manufacturer's procedure (Catalogue No. 560131, Cay-man Chemical, Ann Arbor, MI, USA). The tests were per-formed on a 96-well plate. The extracts were prepared at a final concentration of 100 µg/ml in the wells. A reaction mix-ture of 10 µl extract, 10 µl enzyme, 10 µl hemin and 150 µl diluted buffer was distributed in 96-well plate wells. The same mixture was made without the extract but with 10 µl of the solvent of dilution of the extracts and distributed in other wells. The blank consisted of 160 µl of diluted buffer, 10 µl of hemin and 10 µl of solvent for dilution of extracts. The plate was homogenized and incubated for 5 minutes. Then, 20 µl of sub-strate (arachidonic acid) and 20 µl of colorimetric substrate were added to the wells. The plate was read at 590 nm after two-minute homogenization and incubation. The reaction mixtures were analyzed in triplet. The percentage inhibition of COX 1 & 2 was calculated using the following formula:

$$\text{Inhibition (\%)} = [(\text{Enzyme Absorbance} - \text{Sample Absorbance}) / \text{Enzyme Absorbance}] \times 100$$

Where,

Enzyme Absorbance = Absorbance of Enzyme Activity - Absorbance of Enzyme Blank;

Sample absorbance = absorbance of sample activity - ab-sorbance of sample blank.

2.5. Data Analysis

All data were presented as mean standard deviation from three independent experiments. The data was entered in Excel version 2016. Statistical analyses were performed on IBM SPSS Statistics 25 for frequency calculation and one

way ANOVA testing from the Prism Graph Pad 8.02 software. The Dunett test was employed to calculate p-values and perform multiple comparisons. A p-value below 0.05 was used as a criterion of statistical significance.

3. Results

3.1. Phytochemical Determination of Extracts

After extraction of the active ingredients *Hepatib tiben* and *Momordica charantia*, their yield was determined in relation to 100 g of dry plant matter, expressed as percentage Rd (%) (Figure 1). The *Hepatib tiben* aqueous infused yielded the highest yield 31.13 ±0.52%, followed by its Infused-Aqueous Fraction 26.27 ±0.19% and Hydro-Alcoholic Macerated 21.44 ± 1.96%. The *Momordica charantia* aqueous infused yielded 16.95 ±0.42%. The lowest yield was observed with the DCM Fraction of *Hepatib tiben* (5.38 ± 0.03%). The residual moisture content (RH) was 4.50% and 4.99% respectively for the plant drugs *Hepatib tiben* and *Momordica charantia*.

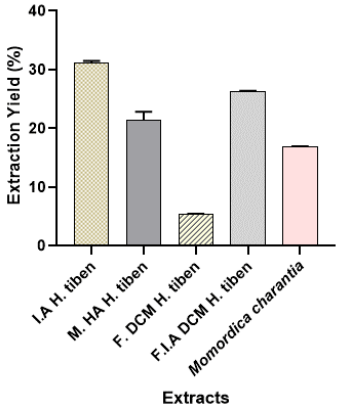

Figure 1. *Hepatib tiben and Momordica charantia extration yield.*

I.A *H. tiben*: Infused-aqueous of *Hepatib tiben*; M. HA *H. tiben*: Hydroalcoholic Macerated *Hepatib tiben*; F. DCM *H. tiben*: Dichloromethane fraction of *Hepatib tiben*; F.I.A DCM *H. tiben*: Dichloromethane-infused-aqueous fraction of *Hepatib tiben*.

3.2. Total Phenolic Compounds Content

Table 1. *Total phenolic and flavonoid content of extracts.*

Drugs	Extracts	TOTAL POLYPHENOL (mg EAG/g Es)	FLAVONOIDS (mg EQ/g Es)
Hepatib tiben	Aqueous infused	632.53 ±28.38[a]	2.18 ±1.69[d]
	Hydro-alcoholic macerate	609.88 ±36.57[a]	2.90 ±0.90[d]
	DCM Fraction	405.83 ±15.88[d]	77.30 ±2.03[a]
	Aqueous DCM Infused Fraction	562.05 ±18.59[b]	1.23 ±0.82[d]
Momordica charantia	Aqueous infused	513.04 ±32.33	2.18 ±0.72

a: non-significant difference between *Hepatib tiben* extracts; b: significant difference $p < 0.01$; d: significant difference $p < 0.0001$

The results of the total phenolic and flavonoid content of *Hepatib tiben* and *Momordica charantia* extracts are shown in Table 1. The aqueous infused extract of *Hepatib tiben* has the highest phenolic content 632.53 ± 28.38mg EAG/g Es, followed by its Hydro-alcoholic Macerate 609.88 ± 36.57 mg EAG/g Es, and its Aqueous Infused Fraction at DCM 562.05 ± 18.59 mg EAG/g Es. The DCM fraction had the lowest content of 405.83 ± 15.88 mg phenolic compounds EAG/g Es. There is a significant difference between its DCM fraction and the aqueous infused $p<0.0001$. On the other hand, the flavonoid content of the DCM fraction was highest 77.30 ± 2.03 mg EQ/g Es followed by Hydro-alcoholic Macerated 2.90 ± 0.90 mg EQ/g Es and aqueous infused 2.18 ± 1.69 mg EQ/g Es. The aqueous DCM-infused extract had the lowest content 1.23 ±0.82mg EQ/g Es with a very significant difference $p<0.0001$ between the DCM fraction and the rest of the *Hepatib tiben* extracts. *Momordica charantia* contained 513.04 ±32.33mg EAG/g Es and 2.18 ±0.72mg EQ/g Es.

3.3. Antioxidant Activity

The antioxidant activity of *Hepatib tiben* and *Momordica charantia* was measured by three methods including FRAP, DPPH and ABTS. The results are reported in Table 2. The ability to reduce ferric ions into ferrous ions of *Hepatib tiben* extracts was evaluated at 0.01 ±0.004mmol EAA/mg and 0,62 ±0.01mmol EAA/mg respectively for *Hepatib tiben* F. DCM and *Momordica charantia* extract IA compared to the reference compound (Rutin: 2.73 ± 0.09mmol EAA/mg) with which there is a significant difference $p<0.0001$ and $p=0.02$ for M. HA (3.44 ±0.01mmol EAA/mg). However, there is no differ-

ence between IA and F. IA DCM (3.58 ± 0.04mmol EAA/mg and 3.64 ± 0.02 mmol EAA/mg). The concentration of different *Hepatib tiben* and *Momordica charantia* extracts may inhibit 50% of $DPPH^+$ and $ABTS^+$ radicals, was evaluated and listed in Table 2. All *Hepatib tiben* and *Momordica charantia* extracts had superior DPPH radical inhibitory power, with the best I. A *Hepatib tiben* extract (0.002 ± 0.00001 µg/ml). There is a significant difference compared to the reference compound Quercetin (3.14 ± 0.02 µg/mL), p<0.0001. Regarding the inhibition of $ABTS^+$ cations, only F. DCM *Hepatib tiben* (220.49 ± 14.53 µg/ml) had a low inhibition. There is a significant difference, *p<0.0001*, between this extract and the reference compound Trolox (6.17 ± 0.20 µg/ml) used. The other *Hepatib tiben* extracts and *Momordica charantia* extract showed a rather interesting activity compared to the activity of the reference compound Trolox (6.17 ± 0.20 µg/ml).

Table 2. *Antioxidant activity of «Hepatib tiben» and Momordica charantia extracts.*

Drugs	Extracts	FRAP (mmol EAA/mg)	DPPH IC50 (µg/ml)	ABTS IC50 (µg/ml)
Hepatib tiben	Aqueous-infused	3.58 ±0.04	0.002 ±0.00001[d]	3.08 ±0.13[a]
	Hydro-alcoholic macerate	3.44 ±0.01[a]	0.004 ±0.0002[d]	1.28 ±0.08[a]
	DCM Fraction	0.01 ±0.004[d]	0.80 ±0.066[d]	220.49 ±14.53[d]
	Aqueous DCM Infused Fraction	3.64 ±0.02	0.003 ±0.0001[d]	5.19 ±0.19[a]
M. charantia	Aqueous-infused	0.62 ±0.01[d]	0.012 ±0.0004[d]	15.77 ±0.66[a]
	Quercetin	nd	3.14 ±0.02	nd
	Trolox	Nd	nd	6.17 ±0.20
	Rutin	2.73 ± 0.09	nd	nd

M. charantia: *Momordica charantia*; Antioxidant activity by the ferric ion reduction test Fe^{3+} and by inhibition of DPPH and $ABTS^+$ cation radicals with a: non-significant difference; d: significant difference, $p < 0.0001$; nd: not determined. One-Way ANOVA (and nonparametric or mixed): Dunnett's test

3.4. Anti-inflammatory Activity *in vitro*

The results of the inhibitory activity of 15-LOX and COX1 & 2 by the extracts of *Hepatib tiben* and *Momordica charantia* are recorded in Table 3. Comparing the values obtained with that of the reference compound Indometacin used, *Hepatib tiben* hydroalcoholic maceration is the one that most inhibits 15-LOX, with an inhibition percentage of 97,72 ± 1.46%. The aqueous infused and the hydroalcoholic macerated of the same recipe has an inhibitory activity on COX 1 & 2. The values are presented in average SD (n=3). As for *Momordica charantia*, it weakly inhibits 15-LOX.

Table 3. *Inhibition of 15-LOX and COX 1 & 2.*

Drugs	Extracts	% inhibition of 15-LOX	% inhibition of	
			COX 1	COX 2
Hepatib tiben	Aqueous-infused	45.91 ± 1.3[d]	38.31 ± 0.44[d]	30.19 ± 0.07[d]
	hydroalcoholic macerated	97.72 ± 1.46[b]	24.05 ± 0.014[d]	36.29 ± 3.74[d]
M.charantia	Aqueous-infused	16.66 ± 0.93[d]	nd	nd
	Indomethacin	92.25 ± 0.90	nd	nd

M. charantia: *Momordica charantia*; 100 µg/mL of extract was used for each test; %: percentage; c: significant difference $p < 0.001$; d: significant difference $p < 0.0001$; nd: not determined; Indomethacin: 100 µg/mL. One-Way ANOVA (and nonparametric or mixed): Dunnett's test

4. Discussion

African flora displays a very huge and an important bio-diversity which has been used as main source of medicines for millennia. In fact, more than 80% of the African population uses plants to treat from various ailments to important dis-eases due the accessibility of plants and the cultural values attached to them [20]. Traditional health practitioners also work in collaboration with modern medicine actors, ministries, to strengthen their capacity and to validate information and practices on the judicious use of herbal remedies [21]. Recipes are developed by traditional health practitioners to treat several pathologies including viral hepatitis B and cancer. It is also a recipe of Hepatib tiben plants used by a traditional health practitioner in Burkina Faso that was the subject of this study. This recipe composed of whole plants of Phyllanthus amarus and Cassia nigricans then leaves of Balanites aegyptiaca is used in the management of viral hepatitis B, and is associated in some cases with Momordica charantia to treat cases of liver cancer by this traditional health practitioner from Burkina Faso.

The aqueous infusion of Hepatib tiben had the highest ex-traction yield (31.13 ±0.52%) reflecting the high solubility of the active ingredients of Hepatib tiben in water than in other extraction solvents used. The residual moisture content (THR) of both plant drugs is less than 10%. This means that the plant material of both drugs was dried under acceptable conditions according to the standards of the European Pharmacopoeia (2010 edition), with a low risk of mold formation, signs of oxidation and deterioration of the active ingredients [15].

Plant phenolic compounds are one of the main groups of compounds that act as antioxidants and anti-inflammatories [22]. Phytochemical analysis of Hepatib tiben and Momor-dica charantia extracts revealed the presence of phenolic compounds in all extracts. The aqueous infused extract of Hepatib tiben had the highest content of total phenolic com-pounds (632.53 ± 28.38 mg EAG/g Es) and DCM Fraction in flavonoids (77.30 ± 2.03 mg EQ/g Es). The results obtained are similar to those of several authors [8, 11] who also showed the presence of the same compounds in the extracts of Phyl-lanthus amarus and Balanites aegyptiaca (L.) Delile, plants that make up the recipe Hepatib tiben. The work of [23] isolated flavonoids in the methanolic extract of Momordica charantia. The variation in the amount of these secondary metabolites observed between the different extracts of Hepatib tiben could be explained by the nature of the extraction solvents. Polyphenolic compounds are more soluble in high-polarity solvents such as water, while flavonoids are more soluble in low-polarity solvents such as dichloromethane [24, 25]. In one study, water was found to be the best solvent for the extraction of phenolic compounds from Phyllanthus amarus, resulting in a higher yield and total phenolic content than other solvents such as ethanol, methanol and dichloromethane [26]. The high flavonoid content for the DCM fraction could be explained by the presence of methoxylated and hydroxylated aglycones, which are low polarity compounds with an affinity for dichloromethane [27].

Oxidative stress, involved in many chronic diseases such as cardiovascular diseases, cancer, neurodegenerative diseases, refers to an imbalance between the production of free radicals and the body's ability to neutralize their harmful effects with antioxidants. Free radicals are highly reactive molecules that can damage cells, proteins and DNA if they are not effectively neutralized. The ability of Hepatib tiben and Momordica charantia extracts to prevent oxidation was tested by three complementary methods: the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}) (FRAP), the ability to trap DPPH radicals and ABTS+ cation radicals, in order to better appreciate the antioxidant activity of the extracts. All extracts showed a rather interesting antioxidant activity through the anti-radical activity by the three methods. For Momordica charantia, the results corroborate those of Yakubu et al., (2022) who found an IC50 = 20.63µg/ml for the radical ABTS+. The aqueous infusion Hepatib tiben showed the best antioxidant activity with the methods by DPPH, ABTS and FRAP which exceeded those of Quercetin, very close to that of Trolox and Rutin, used as reference compounds. F. DCM and Momordica charantia had the best antioxidant activities by the FRAP method. These interesting antioxidant activities of the extracts of Hepatib tiben and Momordica charantia in general could be explained by the fact that these drugs are a cocktail of plants containing phenolic compounds and fla-vonoids that are known for their antioxidant effect [8, 9, 11]. These effects are beneficial to health, including the man-agement of oxidative stress and the prevention of various related diseases.

15-lipoxygenase and cyclooxygenases 1 and 2 are enzymes involved in arachidonic acid cascade and eicosanoid biosynthesis, but catalyze different reactions and play distinct roles in regulating inflammatory response and other biological processes [28]. When comparing the results obtained with those of indomethacin which is the reference compound used, the Hepatib tiben Hydroalcoholic Macerate has a good ability to inhibit 15-LOX with inhibition percentages of 97.72 ± 1.46%. A study analyzed the ethanolic and aqueous extracts of Balanites aegyptiaca, one of the plants that make up Hepatib tiben, and showed moderate activity (41-70% inhibition) in the inhibition of 15-LOX [6]. Indeed, the hydroalcoholic maceration of Hepatib tiben has as high a content of phenolic compounds as the aqueous infused, and also has bioactive ingredients that inhibit 15-LOX [28]. This high content of phenolic compounds could justify here the best anti-inflammatory activity by inhibition of the 15-Lox of the hydroalcoholic extract of Hepatib tiben. Most of the plants that make up these recipes are traditionally used for their anti-inflammatory properties [7].

COX1 is naturally present in cyclooxygenases and main-tains tissue integrity, while COX2 is induced in response to inflammation [29]. The aqueous and hydroalcoholic infused extracts of Hepatib tiben show an inhibition of both

forms of COX. COX2 plays a crucial role in inflammation by producing prostaglandins that amplify the inflammatory response. However, simultaneous inhibition of both forms can not only reduce overall inflammation but also cause gastrointestinal side effects due to inhibition of COX1, which is associated with gastric disorders [30]. Indeed, the plants from the recipes Momordica charantia and Hepatib tiben are rich in phenolic and flavonoid compounds [6, 8, 31, 32] with anti-inflammatory properties that modulate the expression of COX and inhibit the release of pro-inflammatory mediators [30]. Studies have shown that methanolic extracts of Phyllanthus amarus significantly inhibit the production of pro-inflammatory mediators such as TNF-α, IL-1β and PGE2, as well as the expression of COX2 in human macrophages [33]. These results suggest that aqueous infusions of Hepatib tiben and Momordica charantia could be promising due to their high content of total phenolic compounds, known for their anti-inflammatory properties through inhibition of enzymes 15-LOX, COX1 & COX2. Indeed, authors have obtained similar results with extracts of Balanites aegyptiaca, a plant part of the recipe Hepatib tiben [6]. Given the link between inflammation and cancer, these results could justify the combined use of Hepatib tiben and Momordica charantia for the treatment of advanced chronic viral hepatitis and liver cancer by traditional health practitioners in Burkina Faso [6]. In light of these results, it would be highly advantageous to use Hepatib tiben due to its high polyphenol and flavonoid content. These secondary plant metabolites have demonstrated their antioxidant capacity through FRAP, DPPH, and ABTS methods, as well as their anti-inflammatory capacity through the inhibition of 15 lipoxygenase.

5. Conclusions

The study revealed that the *Hepatib tiben* recipe, composed of different traditional medicinal plants from Burkina Faso, has interesting anti-inflammatory and antioxidant properties. The results showed that the extracts of this recipe mainly aqueous infused, inhibited inflammatory processes in vitro and demonstrated an ability to neutralize free radicals. However, further studies are needed to better understand the mechanisms of action both in vitro and in vivo, assess long-term safety and determine optimal doses to achieve beneficial effects while minimizing potential adverse effects. These preliminary results nevertheless offer a promising basis for the *Hepatib tiben* recipe traditionally used in Burkina Faso by a traditional health practitioner in the management of viral hepatitis B. Furthermore, antioxidant and anti-inflammatory activities could be evaluated in a cell culture system or *in vivo*

Abbreviations

µg/mL	Microgram Per Milliliter
ABTS	2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid)
$AlCl_3$	Aluminum Chloride
CH_3COONa	Sodium Acetate
COX	Cyclooxygenase
DCM	Dichloromethane
DNA	Deoxyribonucleic Acid
DPPH	2,2-Diphenyl-1-Picrylhydrazyl
F. DCM *H. tiben*	Dichloromethane Fraction of *Hepatib tiben*
F.I.A DCM *H. tiben*	Dichloromethane Infused-Aqueous Fraction of *Hepatib tiben*
Fe^{2+}	Ferrous Ion
Fe^{3+}	Ferric Ion
$FeCl_3$	Ferric Chloride
FRAP	Ferric Reducing Antioxidant Power
I.A H. tiben	Aqueous Infusion of *Hepatib tiben*
IC50	Inhibitory Concentration
IL-1β	Interleukine 1 b êta
LOX	Lipoxygenase
M. HA *H. tiben*	Hydroalcoholic Macerated *Hepatib tiben*
M	Initial Mass of the Recipe
M'	Mass of Dry Extract After Extraction and Freeze-Drying
mg GAE/g ES	Milligram Equivalent Gallic Acid Per Gram of Dry Extract
mg QE/g	Milligram Quercetin Equivalent per Gram
mmol EAA/mg	Millimole Equivalent of Ascorbic Acid Per Milligram
Na_2CO_3	Sodium Carbonate
P	Initial Weight (g) of Plant Material
P'	Final Weight (g) of Plant Material After Parboiling
PGE2	Prostaglandin E2
Rd	Yield
SD	Standard Deviation
THR	Residual Moisture Content
TNF-α	Tumor Necrosis Factor-α
TPTZ	2,4,6-tripyridyl-j-triazine

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Conflicts of Interest

The authors declare no conflicts of interest.

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