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Bio-guided Fractionation: Optimization of Chemical Profiling, Antioxidant, Anti-inflammatory and Antibacterial Properties of *Vitex doniana* Fruits.

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Abstract

This study is an evaluation of the chemical profile of *vitex doniana* fruit as well as its antioxidant, anti-inflammatory and antibacterial properties *in vitro*. For this purpose, bio-guided fractionation was performed and the phytonutrients and antioxidant activity of these fractions were evaluated using standard methods. Then, the anti-inflammatory activity of the different fractions was evaluated by four inhibitory methods: protein denaturation, protienase, A5-LOX and xanthine oxidase. Finally, agar well diffusion and colorimetric microdilution methods were used to determine the antibacterial activity of the two best fractions on 11 reference bacterial strains. The results showed that the ethyl acetate (EAF) fraction presented the best chemical profile and correlated with interesting antioxidant, anti-inflammatory and antibacterial activities. To this end, bio-guided fractionation appears as a method to optimize the chemical profile and therapeutic activities.

KEYWORDS : Phytonutrients, Antibacterial; Anti-inflammatory ; Antioxidant; fruits of *Vitex doniana*

1. Introduction

The human immune system develops several mechanisms to fight infectious and inflammatory diseases (Behl *et al.,* 2021). For the effectiveness of the immune system in this task, antibiotic therapy is used to control microbial and bacterial activity (Valsamatzi *et al.,* 2021) while non-steroidal anti-inflammatory drugs (NSAIDs) are used to relieve fever and pain (Jiang *et al.,* 2018). However, the emergence of antimicrobial resistance, particularly bacterial resistance, is an imminent global threat (Allcock *et al.,* 2017). Indeed, some diseases such as pneumonia, tuberculosis, gonorrhoea and salmonellosis are becoming more difficult to treat with antibiotics and leading to inflammatory diseases (Sonter *et al.,* 2021). In addition, the inadequacy or absence of infection prevention and control methods play an important role in the emergence of antibiotic resistance and the occurrence of inflammatory diseases (Valsamatzi *et al.,* 2021). Also, it is worth noting that reactive oxygen species (ROS) resulting from oxidative stress have been shown to be a key driver of microbial resistance (Martelli and Giacomeini, 2018). To remedy this situation, new approaches are being initiated by scientists to discover and develop natural plant-based products, their analogues as potential therapeutic agents (Najmi *et al.,* 2022). Several studies have also shown a positive correlation between bioactive compounds, antioxidant and anti-inflammatory activities (Bendjedid, 2022). For this purpose, a plant of interest in the search for therapeutics and antimicrobial compounds is *Vitex doniana* (also known as black plum) (Njoku *et al.,* 2019). It is a valued plant for both food and medicinal use (Dadjo *et al.,* 2020). To further illustrate a basis for scientific validation of the medicinal use of *Vitex doniana* in traditional medicine, this study was undertaken to compare the efficacy of different fraction extracts of *Vitex doniana* to determine its phyto-constituents, antioxidant and antibacterial properties against selected microbial strains. To the best of our knowledge, this is the first comparative study on the pharmacological efficacy of different extracts of *Vitex doniana* from bio-guided fractionation.

2. Materials and Methods

2.1. Plant material

The plant material whose fruits of *Vitex doniana* were collected in August 2019 in the Hauts Bassins region, more precisely in Faramana (12°02′53" N and 4°40′02"W) located 120 km from Bobo-Dioulasso, Burkina Faso's economic capital. Dr Lassina TRAORE, botanist at the Laboratory of Biology and Ecology of the Joseph KI ZERBO University, has been working on the identification and authentication of the plant. Consequently, a reference number 20/08/2019/PRB has been deposited in the herbarium of the Life and Earth Sciences Unit of the Joseph KI ZERBO University.

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2.2. Fractionation of extracts

Fifty grams (50 g) of fruits were sprayed with 400 ml of acetone and 100 ml of distilled water, and the mixture was mechanically stirred for 48 hours at 37°C. After this stirring, the acetone evaporated in a rotavapor at 45°C.Hydroacetonic extracts were then subjected to liquid-liquid fractionation of increasing polarity with n-hexane (n-HF), dichloromethane (DCMF), ethyl acetate (EAF) and n-butanol (n-BF). Each fraction was concentrated and then lyophilized (Konaté *et al.,* 2010).

2.3. Phytonutrient analysis

2.3.1. Evaluation of total alkaloid contents

Total alkaloids contents (TAC) was determined by (Selvakumar *et al.,* 2019) with minor modifications. Thus, 1 ml of 1 mg/ml of the solution of each Fraction extract one summer mixed with 5 ml of green bromocresol solution and 5 ml of phosphate buffer of 4.7 pH. The mixture was vortexed pendant 3 to 5 minutes with 1; 2; 3 and 4 ml of chloroform. Afterwards, the whole mixture was collected in a 10 ml volumetric bottle. The atropine used as a standard, a summer used at the respective concentrations of: 20; 40; 60; 80 and 100 mg/ml. The reading was made at a wavelength of 470 nm. The experiments were carried out in triplicate. The TAC was expressed as mg Atropine Equivalent per 100 mg of dry fraction extract (mgAE/100 mgMS). Reagent blank was prepared in the same manner but without Fraction extract.

2.3.2. Evaluation of total phenolic contents

About this evaluation, the total phenolic contents was determined by using the Folin-Ciocalteu method and gallic acid as the standard as described by Belkacem et al. (2014) with minor modifications. An aliquot of 125µl solution of each methanolic fraction (10 mg/ml) solubilised in 1.5 ml of sodium carbonate solution (7.5%). After a 5 minute incubation, 1.25 ml of Folin-Ciocalteu (0.2 N) was added and vortexed for 2-3 minutes, incubated without light for one hour at 37°C. After this incubation period, the reading has been made at 760 nm against a blank on a UV/visible light spectrophotometer. The experiments were carried out in triplicate. The calibration curve was traced using gallic acid as a positive control. The results were expressed as mg Gallic Acid Equivalent per 100 mg of dry Fraction extract (mgGAE/100 mgMS).

2.3.3. Evaluation of total flavonoid contents

For the determination of total flavonoids contents, we used the method described by Elhanafi et al. (2020) with some modifications. Total flavonoids contents were identified using the aluminium chloride assay. 0.5 ml of methanol of each fraction solubilized (0.1 mg/ml) was mixed with 1.5 mL of $AlCl_3$ (2%) and incubated for 30 min at room temperature after this incubation period. The reading has been made spectrophotometrically on a blank at 415 nm. The experiments were carried out in triplicate. Quercetin was used as the reference standard compound. A standard curve was obtained using quercetin as a standard, and the results were expressed as in mg of equivalent per 100 mg of dry fraction extracted (mgEQ/100 mgMS).

2.4. In vitro Antioxidant activity determination of fraction extracts

2.4.1. Determination of DPPH radical scavenging assay

Radical analysis of DPPH (2, 2-diphenyl-1-picrylhydrazyl) trapping was performed according to Hifnawy et al. (2021) with some modifications. To determine the radical trapping behavior of fraction extract, a DPPH solution was prepared by dissolving 20 mg of DPPH in 100 ml of methanol. The absorbance of 3 ml of this solution was read as a control. For the preparation of stock solutions, 5 mg of each fraction was solubilised in 5 ml of methanol and then different serial dilutions (25; 50; 75 and 100 µL/ml) were prepared. Subsequently, 2 ml of each dilution was mixed with a 2 ml solution of DPPH and the IC_{50s} were determined. The percentage of DPPH free radical inhibition per fraction was determined by following the formula with some modifications.

$$\%\ \text{DPPH inhibition} = (1 - B/A) * 100 \quad (1)$$

Where, B is the absorbance of the sample, and A is the absorbance of the control. The scavenging activity of samples was expressed as IC_{50} value, which represented the inhibitory concentration of fraction/standard essential to scavenge 50% of DPPH radicals.

2.4.2. Determination of ABTS (2,2-azinobis (3-ethylbenzthiazoline) -6-sulfonic acid) free radicals scavenging activity

The antioxidant potential of the fractions was also assessed by ABTS free radical scavenging. An ABTS stock solution was prepared in methanol from ABTS (7 mM) and potassium persulphate (2.45 mM). Each fraction (300 µL) was evaluated in a 3 ml mixture of ABTS solution maintained at 25 °C for 15 min and the optical density was read at 734 nm (Dehimat *et al.,* 2021). The data were collected in triplicate, and the formula used to measure the percentage of ABTS free radical scavenging activity was as follows:

$$\%\text{ABTS scavenging inhibition} = (1 - D/C) * 100 \quad (2)$$

Where, C is the absorbance of the control, and D is the absorbance of the samples.

2.4.3. Determination of FRAP assay

The FRAP assay of fraction extract was studied according Jayanthi et Lalitha. (2011) in some minor modifications. Concentrations of fraction range from 0.2; 0.4; 0.6; 0.8 and 1 mg/ml and l-ascorbic acid at the same concentrations were then mixed with 2 ml of potassium ferricyanide ($K_3Fe(CN)_6$) and 2 ml of buffer phosphate (0.2 M, pH 6.6). The mixture was kept for 20 min at 50ºC. In addition, 2 ml of 10% trichloroacetic acid (TCA) was added to the mixture. The mixture was centrifuged for 10 min to 1000 revolutions per min (rpm). 2 ml of surnageant was sucked in and mixed with 1 ml of 0.1% iron chloride ($FeCl_3$) and 2 ml of distilled water. The reading absorbance was made at spectrophotometry at the wavelength of 700 nm. The median effective concentration (IC_{50}) of each fraction was determined.

2.4.4. Determination of RHS activity

The RHS (Radical Hydroxyl scavenging) was performed according to the method described by Klein et al. (1981) with minor modifications. The reaction medium

was made by adding 2.4 ml of phosphate buffer (pH 7.8), phenanthroline (90 mM), hydrogen peroxide (150 mM), iron (1 mM) and phytexpont (1.5 ml) and standard (l-ascorbic acid) at different concentrations (100%; 10%; 1%; 1% and 0.01%), except in the controls, and then incubated at room temperature for 5 min. The reading was taken at 560 nm, and the RHS activity was calculated using the following formula:

$$\%\,\text{RHS inhibition} = (1 - F/E) * 100 \quad (3)$$

Where F is the absorbance of the sample, and E is the absorbance of the control.

The half maximal inhibitory concentration (IC_{50}) of the fraction extracts was computed from a plot of percentage hydroxyl radical inhibition versus the fraction concentration.

2.5. In vitro anti-inflammatory test of fraction extracts

2.5.1. Evaluation of protein denaturation activity

Inhibition of protein denaturation was determined according to the method of Mizushima et Kobayashi. (1968) with some modifications. The reaction mixture contained the test fraction extracts at the final concentration of 200 µg/mL and 1% BSA (aqueous solution). The reaction mixture will be adjusted taking into account the pH. The fraction extracts will be subjected to different temperature variations, then finally cooled and the reading will be made to 600 nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows:

$$P\% = (1 - B/A) * 100 \quad (4)$$

With, A = The value of absorbance of control and B = The value of absorbance of test sample.

2.5.2. Evaluation of proteinase inhibitory activity

The reaction was initiated with trypsin (3 µg/mL), Tris HCl buffer (20 mM, pH=7.4), and 1 ml of each fraction (200µg/mL) was tested and incubated at 37°C for 5 min. Subsequently, 1 ml of 0.8% (w/v) casein was added and incubated for 20 min. 2 mL of perchloric acid (70%) was added again, and the cloudy suspension was centrifuged. The reading was taken at 210 nm (Oyedapo and Famurewa, 1995). The mean value of the three observations was noted. The percentage inhibition of PI activity was calculated as follows:

$$P\% = (1 - D/C) * 100 \quad (5)$$

With, D = The value of absorbance of control and C = The value of absorbance of test sample.

2.5.3. Evaluation of A5-LOX activity

A5-LOX (arachidonate 5-lipoxygenase) inhibitory activity of fractions was determined by spectrometric method (Dona *et al.,* 2018). For the reaction mixture, fraction extracts were used at the final concentration of 200µg/mL. Briefly, 10 µL of each fraction solubilised in methanol was added to sodium phosphate buffer (110 µL, 100 mM, pH 8.0) followed by A5-LOX solution (55 µL) and incubated for 10 min at 25 °C. To this reaction mixture, 25 µL of linoleic acid solution was added. The reading was made at 234 nm for a period of 10 min at 25 °C. Baicalein was used as the reference standard. Percentage inhibition of A5-LOX was determined by comparison of reaction rates of fraction relative to control using the formula :

$$P\% = (1 - E/F) * 100 \quad (6)$$

Where, P = Percent inhibition of A5-LOX was calculated as follows F and E are activities of the enzyme with and without fractions, respectively.

2.5.4. Evaluation of xanthine oxidase inhibitory activity

Xanthine oxidase inhibitory activity of fraction was determined by a kinetic method (Nagao *et al.,* 2014) with slight modifications. For the reaction mixture, fraction was used at the final concentration of 200µg/mL. Briefly, sodium phosphate buffer (150 µL, 50 mM, pH 7.4), fraction extract (10 µL) and xanthine oxidase solution (10 µL) were incubated at 25 °C for 10 min. The reaction was then initiated with the addition of xanthine solution (0.1 mM). The reading was made at 295 nm for a period of 10 min at 25 °C. Percentage inhibition of xanthine oxidase was calculated using the formula:

$$P\% = (1 - G/H) * 100 \quad (7)$$

Where, P = Percent inhibition of xanthine oxidase, G is the activity of enzyme without fraction extracts and H is the activity of enzyme with fractions. Allopurinol was used as the reference standard.

2.6. In vitro Antibacterial properties

2.6.1. Bacterial strains used

All microorganisms were provided by Food Technology Department (CNRST/IRSAT/DTA) and Center for Research in Biological, Food and Nutritional Sciences (CRSBAN) of Burkina Faso. Gram-positive ($Gram^+$) reference bacteria strains were : *Bacillus subtilis* ATCC 25923 ; *Bacillus cereus* 13569 ; *Micrococcus luteus* SKN 624 ; *Lysteria monocytogenesis* NCTC 9863; *Staphylococcus aureus* ATCC 2523 and *Bacillus subtilis spizinii* 6051. Gram-negative (Gram) bacterial strains were represented by *Escherichia coli* 25922 ; *Yersinia enterolitica* 8A30 SKN 601 ; *Pseudomonas aeruginosa* ATCC 9027; *shigella dysenteriae* SKN 557 and *Salmonella typhimurium* SKN 1152 were used in this research.

2.6.2. Preparation of inocula

We had used susceptibility tests by the MHA well diffusion method according to Ezoubeiri et al. (2005) with some modifications. Nutrient agar was used as a medium to grow the bacterial strains at 37°C for 24 hours and then the strains were suspended in saline (0.9%, w/v) NaCl and adjusted by a 0.5 standard Mac Farland turbidity test (10^8 CFU/ml). To obtain the inocula we are going to use, suspensions were diluted 100 times in MHB to give 10^6 colony-forming units (CFU)/ml.

2.6.3. Preparation of discs

The EAF and n-BF extracts were dissolved in distilled water containing 10% DMSO at a final concentration of 200 mg/L after a serial two-fold dilution. Each stock solution of the fractions was sterilised by filtration through a sterilising filter (0.22 µm). Sterile discs (6 mm) were impregnated with 10 µL of fraction extracts. Negative controls were prepared using discs impregnated with DMSO (10%) and commercially available antibiotic

diffusion discs used as positive controls for the experiment (Tambekar & Dahikar, 2011).

2.6.4. Determination of diameters of the inhibition zones (DIZ)

The Petri dishes (9 cm) were prepared with 20 ml of a base layer of MHA. Each Petri dish was inoculated with 15 µl of each bacterial suspension (10^6 CFU/ml. After drying in a sterile hood, 6 mm diameter discs soaked with 10 µl of the different solutions of fractions (EAF and n-BF) were then placed on the agar. The discs containing Gen.(30µg) and Cip.(25µg) were used as positive controls against DMSO (10%) as negative control. A 24 h incubation at 37°C of the petri dishes and at 44°C for *Escherichia coli* was performed. The DIZs were evaluated in millimetres. Fraction extracts (EAF and n-BF) inducing a zone of inhibition ≥ 3 mm around the disc were considered antibacterial. All tests were performed in triplicate, and the bacterial activity was expressed as the mean of DIZ (mm) produced (Deabes *et al.,* 2020).

2.6.5. Determination of Minimum Inhibition Concentration (MIC)

MIC was determined by the microdilution method according (Nigussie *et al.,* 2021) with some modifications. Eight twice-series dilutions of EAF and n-BF were prepared as described above, to obtain a final concentration range of 200 to 1.5625µg/ml. Microplates from 96 wells containing 100 µL of MHB were used. For each bacteria strain, three columns of eight wells to the micro-plate were used. Each well has getting: the culture medium + fractions (EAF or n-BF) + inoculum (10 µl of inocula) and INT (50 µl; 0.2 mg/ml). The plates were covered and incubated at 37°C and at 44°C for *Escherichia coli* for 24 h. All tests were performed in triplicate, and the bacterial activity was expressed as the mean of inhibitions produced. Inhibition of bacterial growth was judged by rose or yellow colour. The MIC was defined as the lowest concentration of fractions at which no colony was observed after incubation. So, the MIC was defined as the lowest concentration at which no visible growth was observed.

2.6.6. Determination of Minimum Bactericidal Concentration (MBC)

The lowest concentration of the fractions (EAF or n-BF) capable of neutralising 99.9% of the bacterial inocula after 24 hours of incubation at 37°C are considered MBC. For this purpose, MBCs were determined by inoculating 100 µl of bacterial suspension from the subculture showing no visible growth on nutrient agar. After incubation, MBC was determined with wells with concentrations ≥ MIC (Nigussie *et al.,* 2021). The MBC were determined in MHA medium.

2.6.7. Evaluation of bactericidal and bacteriostatic capacity

The MIC and MBC are the parameters that allow to assess the bacterial activity of a fraction on strains through the MBC/MIC ratio. Indeed, a MBC/MIC ratio equal to 1 or 2 has a bactericidal effect, whereas a MBC/MIC ratio = 4 or 16, the effect is bacteriostatic.

3. Statistical analysis

The R-Studio software version 4.1.3 was used for statistical processing of the data obtained through ANOVA, Tukeys test in order to evaluate the inhibitory effect of the extracts on the bacteria and to judge the efficiency of the different bioactive fractions.

4. Results

4.1. Total phytonutriment contents

Contents of total alkaloids, total phenolics and total flavonoids were determined respectively. Indeed, the phytonutrients showed significant differences for the various fractions. The EAF fraction showed the best contents of total alkaloids (7.28±1.73 mgAE/100 mgMS), total phenolics (48.34± 1.45 mg GAE/100 mgMS) and total flavonoids (14.18±1.01), while the lowest levels were noted for the hexanoic fraction (Figure 1).

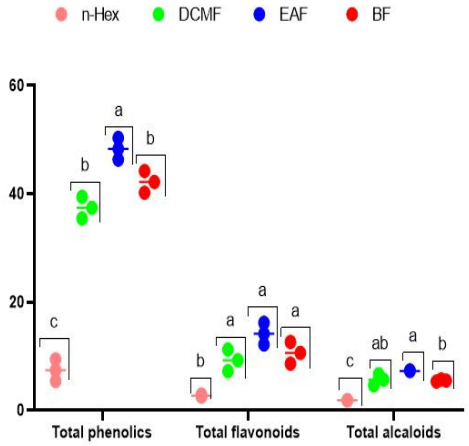

Figure 1. Phytonutrient contents of fraction extracts of fruits from *Vitex doniana*

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts.

4.2. In vitro Antioxidant activity of fraction extracts

Antioxidant activity was assessed by four different methods: Ferric Reducing Antioxidant Power (FRAP) test, DPPH radical scavenging activity, ABTS scavenging test and RHS test. For these different tests, we note that EAF presented the best scavenging activities for 50% of the radicals (IC_{50}): FRAP (70.82±1.71 ug/mL), DPPH (4.42 ± 1.01 µg/mL), RHS (184.12±1.21 µg/mL) and ABTS (53.2 ± 2.51 ug/mL) compared to the other fractions (figure 2). These results show that ethyl acetate extraction consequently optimized its antioxidant power but was slightly lower than the standard ascorbic acid.

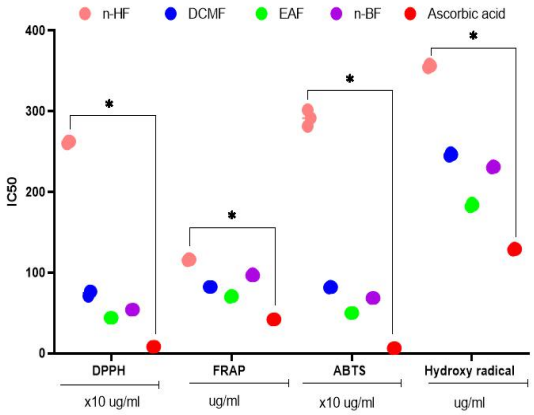

Figure 2. *In vitro* Antioxidant activities of fruits fractions of fruits from *Vitex doniana*

mmoL AAE/g extract: mmol equivalent Ascorbic Acid for 1g dried extracts

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for the different fractions and to the IC_{50} value of the standard (ascorbic acid) a significant difference with (P<0.001).

4.3. In vitro anti-inflammatory assay

The anti-inflammatory activity was evaluated by 4 inhibitory activities, namely: inhibition of protein denaturation; inhibition of proteinases; inhibition of arachidonate 5-lipoxygenase and inhibition of xanthine oxidase. For these four methods, it appears that the different fractions have an influence on the anti-inflammatory activities. Indeed, EAF showed the best anti-inflammatory activities including denaturation inhibition (70.12±1.02%), proteinase inhibition (69.93±2.00%), A5-LOX inhibition (70.60±1.54%) and xanthine oxidase inhibition (72.12±1.45) while n-HF showed the lowest activities (figure 3). Therefore, the EAF fraction will be used for *in vivo* testing.

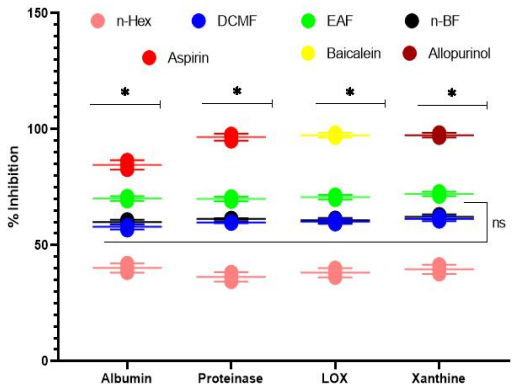

Figure 3. *In vitro* Anti-inflammatory of fraction extracts of fruits from *Vitex doniana.*

Values are Mean ±SD (n=3). Different in the same column indicate significant difference (P<0.05) for our different extracts.

4.4. Antibacterial activity

Antibacterial activities of two fractions (EAF and n-BF) of *Vitex doniana* were tested on 11 reference bacterial strains, of which six $Gram^+$ and five $Gram^-$ bacterial strains were used. In fact, the *in vitro* antibacterial activity was tested by the presence or absence of a zone of inhibition in diameter (DIZ), the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) against the reference antibacterial drugs (*gentamicin; cirpofloxacin*). In general, it was observed that both fractions showed anti-bacterial effects against all strains except *Salmonella typhimurium*. For the diameters of the inhibition zones, the EAF fraction showed the best activity on $Gram^+$ and $Gram^-$ bacterial strains similar to the standards. In particular, high bacterial activity of EAF was noted on *E.coli* (24.53±2.74 mm) and *Bacillus subtilis* (20.68 ±0.35 mm) (Table 1 and 2). MIC values ranged from 6.25 to 50 µg/mL for $Gram^+$ bacteria (Table 1), while 12.50 to 50 µg/mL for $Gram^-$ bacteria (Table 2). MBC values ranged from 25 to 50 µg/mL for $Gram^+$ bacteria (Table 1) and also from 25 to 50 µg/mL for $Gram^-$ bacteria (Table 2). These parameters confirm that fractionation has an influence on bacterial activity and, therefore, EAF is the best antibacterial fraction.

Table 1. *In vitro* Antibacterial activity of two fractions of fruits from *Vitex doniana* with Gram$^+$ bacterial strains

Activity	Fractions or Antibiotics	Gram$^+$ bacterial strains					
		B. subtilis	*B.cereus*	*M.luteus.*	*L.monocytogenesis*	*S.aureus*	*B.spizinii*
DIZ (mm)	EAF	20.68±0.35^a	19.58 ±3.09^a	18.77±0.70^a	15.60±0.65^a	20.73±0.25^a	13.30 ±1.25^{bc}
	n-BF	16.41±0.77^b	20.07±0.40^b	13.97±0.35^b	19.93±2.72^b	16.267±0.64^b	13.60 ±0.35^c
	Gen.	39.5± 2.18^a	34.38±1.71^a	39.26±1.10^a	34.96±0.65^a	19.37±0.32^a	34.73±1.62^a
	Cip.	40.83 ±1.75^a	27.67± 1.16^a	38.07±0.90^a	35.73±0.55^a	20.63±0.55^a	23.73±1.81^{ab}
MIC (µg/mL)	EAF	25	6.25	50	25	50	50
	n-BF	6.25	6.25	25	50	25	50
MBC(µg/mL)	EAF	25	12.50	25	50	50	50
	n-BF	12.50	12.50	50	50	50	50
MBC/MIC	EAF	+	+	+	+	+	+
	n-BF	+	+	+	+	+	+

DIZ includes diameter of discs (6 mm).

The results are the means of number of the colonies ± standard deviations.

+: bactericidal effect (MBC/MIC = 1 or 2) and –: bacteriostatic effect (MBC/MIC = 4 or 16).

Table 2: *In vitro* Antibacterial activity of two BF extracts of fruits from *Vitex doniana* with Gram$^-$ bacterial strains

Activity	Fractions or Antibiotics	Gram$^-$ bacterial strains				
		Escherichia coli;	*Yersinia enterolitica*	*Pseudomonas aeruginosa*	*Shigella dysenteriae*	*Salmonella typhimurium*
DIZ (mm)	EAF	24.53 ±2.74^a	16.30± 0.98^a	12.83 ±1.04^{ab}	13.47 ±0.41^b	Nd
	n-BF	16.17±1.25^b	13.37± 0.47^b	13.80± 1.05^b	15.30± 0.32^b	Nd
	Gen.	20.33±0.57^a	36.00±2.64^a	31.33±1.15^a	45.00±1.00^a	Nd
	Cip.	29.33 ±2.08^a	26.67± 1.52^a	26.33±1.52^a	41.00 ±1.00^a	Nd
MIC (µg/mL)	EAF	25	25	12.50	25	50
	n-BF	12.50	12.50	12.50	25	25
MBC(µg/mL)	EAF	50	50	50	50	25
	n-BF	50	50	50	50	25
MBC/MIC	EAF	+	+	-	+	+
	n-BF	-	-	-	+	+

DIZ includes diameter of discs (6 mm).

The results are the means of number of the colonies ± standard deviations.

+: bactericidal effect (MBC/MIC = 1 or 2) and –: bacteriostatic effect (MBC/MIC = 4 or 16).

5. Discussion

Regarding phytonutrient levels, the results obtained are interesting and as shown by the EAF with TPC (48.34 ± 1.45) and TFC (14.18 ± 1.01). Even higher levels were found by Traore et al. (2021) with total polyphenols (202.51 ±4.19 to 259.75 ± 2.81) and flavonoids (75.71 ± 1.03 to 145.55 ± 1.03). In addition, it is widely known that total phenolic compounds are one of the main groups of compounds acting as primary antioxidants or terminators of free radicals (Thakur *et al.,* 2021). Phenolics coumpounds is considered an important indicator of the antioxidant potential of plant extracts (Najmi *et al.,* 2022). Similar results show correlations between phenol content and antioxidant activities of plant extracts (El-chaghaby *et al.,* 2014). In this study, the radical inhibitory activity of the fraction (IC_{50} = 4.42 ± 1.01 µg/mL) was even more interesting than that of the EAF fraction obtained by Adjei et al. (2021) (99.35 ± 0.77µg/mL).

For anti-inflammatory activity *in vitro*, it should be noted that the fractions (EAF and n-BF) showed quite interesting results. When it comes to biological proteins in general, it is known that most biological proteins are denatured by the application of stress or chemicals and lead to the loss of their function. Proteins denaturation is a real and very important cause of inflammation (Flore *et al.,* 2019). Therefore, the anti-inflammatory potential of the extracts inhibited of proteins denaturation. It is well documented that serine proteinase contained in neutrophils play a fairly important role in inflammatory reactions during certain tissue damage, and proteinase inhibition is thought to have a more or less significant protective system (Leelaprakash *et al.,* 2011). In the present study, EAF inhibited protein denaturation and possessed anti-protease activity explaining their anti-inflammatory potential. With respect to the inhibitory activity of A5-LOX, EAF (200 mg/kg) showed very good A5-LOX inhibitory activity compared to that of control (baicalein). Xanthine oxidase also plays a vital role in the metabolic disease known to all and commonly called gout. It is closely linked to inflammation and other inflammatory-mediated diseases because of the formation of certain free radicals. Inhibition of this enzyme is considered to be a starting point for the management of diseases associated with oxidative stress and metabolic diseases (Yumita *et al.,* 2013). In the present study, the extracts studied showed significant inhibitory activity of xanthine oxidase ($P < 0.05$). EAF (200 mg/kg) showed the highest xanthine oxidase inhibitory activity compared to other fractions. In

addition, flavonoids, including quercetin, are recognized as a reference anti-inflammatory molecule, particularly in the treatment of acute inflammation (Li *et al.,* 2019). The EAF showed the highest anti-inflammatory potential, this may be related to the presence of certain secondary metabolites including triterpenes and flavonoids, saponins, tannins, and alkaloids (Qnais *et al.,* 2009). This statement is consistent with the work according to (Han and Bakovic, 2015) who suggest that triterpenoids are biologically active and possess anti-inflammatory properties.

Medicinal plants play a very important role in microbial defense mechanisms, thanks to the various secondary metabolites they contain (Anand *et al.,* 2019). The present study showed a good antibacterial potential of the plant fraction extracts on these different microorganisms studied. Indeed, the results show that the MICs obtained on *B. subtilis*, *S. aureus, P. aeroginosa and S. Typhi* bacteria are better to those obtained by Udeani et al. (2021) who obtained MICs for *B. subtilis* (28.15 mg/mL), *S. aureus* (8.90mg/mL), *P. aeroginosa* (3.84mg/L*) and S. Typhi* (3.80mg/mL) respectively. It is noted that anti-bacterial activity was found to be more pronounced against $Gram^+$ bacteria compared to $Gram^-$ bacteria. Our results would be similar to those obtained by Vlietinck et al. (1995) according to which, plant extracts have a better potential against $Gram^+$ bacteria than $Gram^-$. Indeed, $Gram^+$ and $Gram^-$ bacteria would be different in structure. The outer membrane that surrounds the cell wall is found only in $Gram^-$ bacteria and would be very impermeable for the passage of substances inside the bacterium unlike $Gram^+$ bacteria *(*Gibbons *et al.,* 2000). Studies already carried out show that the antimicrobial potential of plants is due to the different secondary metabolites namely phenols, flavones, flavanols, alkaloids and many others (Stavri *et al.,* 2007). The phytochemical characterisation of the different fractions used showed interesting contents of phenolic compounds, flavonoids and alkaloids. Moreover, flavonoids are known for their excellent antimicrobial properties. According to fairly recent literature, many flavonoids have anti-infectious properties by forming complexes with the different proteins of the walls of bacteria (Mahboubi *et al.,* 2015). In addition, it could be said that foods rich in polyphenols could greatly reduce health risks due to the anti-inflammatory, antioxidant and antibacterial properties that polyphenols possess (Abdel-Mawgoud *et al.,* 2019). Finally, it could also be said that the mechanism of toxicity of phenolic compounds against microorganisms could be explained by hydrolysis due to microbial enzymes including proteases and carbohydrolases or by other mechanisms, namely the action of the microbial andesis or that of transport proteins (Cowan, 1999).

6. Conclusion

The study showed that the EAF fraction of *Vitex doniana* would contain a considerable amount of phenolic compounds and possess interesting antioxidant, anti-inflammatory and antimicrobial properties. This study confirms a high availability of interesting nutrients in these plant extracts for pharmaceutical application. Therefore, chromatographic characterisation of individual compounds to elucidate their different biochemical mechanisms and clinical use. For this purpose, these isolated compounds should be scientifically evaluated using scientific animal models and clinical mechanisms of action in search of bioactive molecules. These results confirm the traditional use of the plant in inflammatory and infectious diseases in Burkina Faso.

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