

Original Research Article

Phytochemical composition, Antioxidant and Anti-inflammatory potential of bioactive fractions from extracts of three medicinal plants traditionally used to treat liver diseases in Burkina Faso.

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Abstract

Our aim in this study concerning the ethyl acetate and dichloromethane fractions was to provide a scientific basis for the treatment of hepatitis B in Burkina Faso of these three ethnomedicinal plants. As a result, we evaluated polyphenol content, antioxidant and anti-inflammatory evaluated by lipoxygenase (LOX) inhibitory and Xanthine Oxidase (XO) activities of aqueous acetone bioactive fractions from three species of Malvaceae (*Sida cordifolia*, *Sida rhombifolia*, *S. urens*). Folin-ciocalteu; AlCl₃ methods and tannic acid respectively were used for polyphenol content research. The antioxidant activity of the samples was evaluate using three separate methods, inhibition of free radical 2,2-diphenyl-1-picrylhydramzyl (DPPH), ABTS radical cation decolorization assay, Iron (III) to iron (II) reduction activity (FRAP). For anti-inflammatoty activity, lipoxygenase and xanthine oxidase inhibitory activities were used. Finally, in this study, the ethyl acetate fraction has shown the best results comparatively to the dichloromethane fraction.

Keywords: Polyphenol, Antioxidant, anti-inflammatory, Medicinal plants, hepatitis B.

Introduction

The uses of plants and plant preparation have been in existent since prehistory. There are several reports on the use of plants in traditional healing [1, 2] and consequently, the history of natural substances is identified partly with that of pharmacy. In fact, the biological properties of plants have long been known and have been used to increase production and shelf life of foods as well as human health [3]. The World Organisation (WHO) reported that about 80% of the world's population depend mainly on traditional medicine and the traditional treatment involve mainly the use of plant extracts [4]. This practice is commonly found in rural areas where synthetic drugs are not available or, where available, are too expensive to purchase [5]. In this way, the present study concerned *Sida cordifolia*, *Sida rhombifolia*, *S. urens* fractions some herbal plants. These Malvaceae are some veritable medicinal plants used in Africa particularly in Burkina Faso as some components of several primitive medicinal remedies against human diseases [5]. Ethnobotanical investigations in the central region of Burkina Faso have showed that about this Malvaceae specie is used frequently and widely in traditional medicine to treat various kinds of diseases such as malaria, fever, the treatment of human gastrointestinal infections, dermatitis, varicella, variola, anti-inflammatory and antibacterial properties, particularly hepatitis B [6]. The phytochemical screening of this Malvaceae specie revealed the presence of saponosides, cumarins, steroids, polyphenols/tannins and flavonoids [7]. The polyphenols presented toxicity against microbes [8]. Antioxidants are compounds that reduce the action of reactive oxygen species in tissue damage. The oxidation proceeds in lipids with polyunsaturated fatty acids, generating reactive oxygen species such as hydroxyl radicals and prevents many diseases as diabetes, hypertension and combating oxidative stress. However, recently studies have shown a link between polyphenols content and lipoxygenase inhibitory

activity [7]. We evaluated xanthine oxidase because, it is well know that xanthine oxidase is an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues that are involved in many pathological processes such as inflammation, cancer [9]. In this fact, the aim of the present study was to evaluate the phytochemical composition, antioxidant, lipoxygenase and xanthine oxidase inhibitions of fractions from Malvaceae specie for to provide the validity of remedies used for the treatment of hepatitis B in Burkina Faso.

Materials and methods

Chemicals

For evaluate phytochemical composition, antioxidant, lipoxygenase and xanthine oxidase inhibition for anti-inflammatory activity, we used solvents, enzymes and various classic reagents. All reagents and all other chemicals were of analytical grade. Folin-Ciocalteu reagent, carbonate de sodium (Na_2CO_3), gallic acid, quercetin, trichlorure d'ammonium (AlCl_3), lipoxygenase, xanthine oxidase, linoleic acid, tannic acid, xanthine, phosphate borate (1/15 M, pH 7.5), phosphate buffer (1/15 M, pH 7.5) and phosphate buffer (0.2M, pH 6.6) were purchased from Sigma-Aldrich chemie (Steinheim, Germany); ammonium ferric citrate (CAF), ammoniac, le potassium persulfate, DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka), 2,2'-azinobis (3-ethylbenzothiazoline-6-sulphonate) ABTS, acetone, methanol, ethanol hexane, dichloromethane (DCM), acetate of ethyl, n-butanol and trichloroacetic acid were supplied by Fluka chemie (Buchs, Switzerland); potassium hexacyanoferrate [$\text{K}_3\text{Fe}(\text{CN})_6$] was sourced from Proballo (Paris, France); ascorbic acid, tannic acid and ion trichloride were supplied by labosi (Paris, France).

Plants material

Sida cordifolia, *Sida rhombifolia*, *S. urens* were collected in August 2008 in Gampela, 25 Km east of Ouagadougou, capital of Burkina Faso. The plants were botanically identified by Prof. Millogo-Rasolodimby from the plants Biology Department of the University of Ouagadougou. A voucher specimen was deposited at the Herbarium of the Laboratoire de Biologie et d'Ecologie Végétale, UFR/SVT of University of Ouagadougou.

Preparation of fractions extracts

Fifty grams (50g) of powdered plant material were extracted with 80% aqueous acetone (500 ml) in 1/10 ratio (w/v) for 24 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature. After filtration, acetone was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavapor R-200, Switzerland) at approximately 40°C in order to obtain fractions. To do this, the concentrated extracts only water, will be extracted 3x 200mL with n-hexanne. Hexane extracts are pooled and concentrated under reduced pressure to form the rotation evaporator hexane fraction (HF). The extract depleted by petroleum ether is extracted 3x 200 mL were of analytical grade. The different residues are concentrated as before to give the fraction of dichloromethane (DCM). The extract will be exhausted by the dichloromethane extract 3x 200 mL of ethyl acetate. The various fractions will be aggregated to give the fraction of ethyl acetate (EA). The different fractions were freeze-dried by Telstar Cryodos 50 freeze-dryer. The fraction residues were packed in waterproof plastic flasks and stored at 4°C until use.

Polyphenols determination

Total phenolics content

Total polyphenols were determined by Folin-Ciocalteu method as described by [10]. Aliquots (125 µl) of solution of each fraction in methanol (10 mg/ml) were mixed with 625 µl Folin-Ciocalteu reagent (0.2 N). After 5 min, 500 µl of aqueous Na₂CO₃ (75 g/l) were added and the mixture was vortexed. After 2 h of incubation in the dark at room temperature, the absorbencies

were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu reagent + 1 ml Na₂CO₃) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid ($Y = 0.0289x - 0.0036$; $R^2 = 0.9998$). The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of fractions.

Total flavonoids content

The total flavonoids were estimated according to the Dowd method as adapted by [10]. 0.5 ml of methanolic AlCl₃ (2%, w/v) were mixed with 0.5 ml of methanolic fraction solution (0.1 mg/ml) of each plant. After 10 min, the absorbencies were measured at 415 nm against a blank (mixture of 0.5 ml methanolic fractions solution and 0.5 ml methanol) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and compared to quercetin calibration curve ($Y = 0.0289x - 0.0036$; $R^2 = 0.9998$). The data obtained were the means of three determinations. The amounts of flavonoids in plant fractions were expressed as mg of quercetin equivalents (QE)/100 mg of fractions.

Total Flavonols content

The contents of flavonols were determined as described by [11] method. Aliquots were prepared by mixing of 750 µl ethanolic fractions solution (0.1 mg/ml) of each plant and 750 µl aqueous AlCl₃ (20%, w/v). The absorptions were read at 425 nm after 10 min incubation against a blank (mixture of 750 µl ethanolic fraction solution of each plant and 750 µl ethanol) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). All determinations were carried out in triplicate. A standard calibration curve was plotted using quercetin (0-50 µg/ml). The results were expressed as mg of quercetin equivalents (QE)/100 mg of fractions.

Determination of tannins contents

Total tannin contents were determined as described by [7] using tannic acid as standard. In test tube, it was mixed 200 μ l aqueous fraction of each fraction, 1 ml distilled water, 200 μ l ammonium ferric citrate (3.5 g/l) and 200 μ l ammoniac (20%). After 10 min, the absorbencies of samples were measured at 525 nm against a blank (200 μ l aqueous fraction of each plant + 1.2 ml distilled water) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The data obtained was the mean of three determinations. The results were expressed as mg of tannic acid equivalents (TAE) per 100 mg of fraction (mg TAE/100 mg fractions).

Antioxidant activity determination

DPPH radical method

Radical scavenging activity of plant fractions against stable DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka) was determined with a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) at 517 nm as described by [7]. Fraction solutions were prepared by dissolving 10 mg of dry extract in 10 ml of methanol. The samples were homogenized in an ultrasonic bath. 0.5 ml of aliquots which were prepared at different concentrations from each sample of fraction was mixed with 1 ml of methanolic DPPH solution (20 mg/ml). After 15 min in the dark at room temperature, the decrease in absorption was measured. All experiments were performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of fraction ($Y = -16.815x + 6.8373$; $R^2 = 0.9976$). Quercetin, a reference was used as positive control.

ABTS radical cation decolorization assay

For ABTS radical cation decolorization assay, the procedure followed the method of [7]. ABTS was dissolved in water to a 7mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS stock solution with 2.45mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h before use. This mixture was

diluted with ethanol to give an absorbency of 0.7 ± 0.02 units at 734 nm using a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). For our study, we used 10 μ L of the diluted sample (1 mg mL^{-1} in methanol) which was allowed to react with 990 μ L of fresh $ABTS^{\cdot+}$ solution and the absorbance was taken 6 min exactly after initial mixing. Ascorbic acid was used as standard ($Y = -0.0342x + 0.634$; $R^2 = 0.9996$) and the capacity of free radical scavenging was expressed as mmol Ascorbic Acid Equivalent per g of fraction. Quercetin, a reference compound was used as positive control.

Iron (III) to iron (II) reduction activity (FRAP)

The FRAP assay was performed according to [13]. To summarise, 0.5 mL of each fraction (1 mg mL^{-1}) was mixed with 1.25 mL of phosphate buffer (0.2M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (1%). After 30 min incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at $2000 \times g$ for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625mL) and a freshly prepared $FeCl_3$ solution (0.125mL, 0.1%). Absorbencies were read at 700 nm on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and Ascorbic acid was used to produce the calibration curve ($Y = 0.008x - 0.0081$; $R^2 = 0.9999$). The iron (III) reducing activity determination was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of fractions. Troloc, a reference compound was used as positive control.

Anti-inflammatory activity

In vitro lipoxygenase inhibitory assay

Lipoxygenase inhibitory activity of plant fractions with linoneic acid as substrate was measured with a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) as described by [14] with some modifications. Fractions were screened for

lipoxygenase inhibitory activity at final concentration of 50 µg/ml. The mixture assay consisted of 150 µl phosphate borate (1/15 M, pH 7.5), 50 µl of each fraction solution and 50 µl enzyme solution (0.28 U/ml in phosphate borate). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in water). Enzymatic kinetic was recorded at 234 nm for 02 min. Negative control was prepared and contained 1% methanol solution without fraction solution. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase, calculated as (%) inhibition following equation 1: (%) inhibition = $(1 - B/A) \times 100$, where A is the change in absorbance of the assay without the fraction extracts ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$) and B is the change in absorbance of the assay with the fraction extracts ($\Delta\text{abs. with enzyme} - \Delta\text{abs. without enzyme}$).

***In vitro* xanthine oxidase inhibition assay**

Xanthine oxidase inhibition activity of our plants fractions and the xanthine was measured by a spectrophotometer (CECIL CE 2041, CECIL Instruments, England) as described by [15] with some modifications. Extracts were directly dissolved in phosphate buffer-MeOH (1%) and screened for xanthine oxidase inhibitory activity at final concentration of 50 µg/ml prepared. The mixture assay consisted of 150 µl phosphate buffer (1/15 M, pH 7.5), 50 µl fraction solution and 50 µl enzyme solution (0.28 U/ml in phosphate buffer). The reaction was initiated by adding 250 µl of substrate solution (0.15 mM in water). Enzymatic kinetic was recorded at 295 nm for 02 min. Negative control was prepared and contained 1% methanol solution without extract solution. Allopurinol a well known inhibitor of xanthine oxidase was used as a positive control at a final concentration of 50 µg/mL prepared. All experiments were performed in triplicate. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of xanthine oxidase, calculated as (%) inhibition following equation 1.

Statistical analysis

The data were expressed as Mean±Standard deviation (SD) of three determinations. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ and linear regression) was carried out with XLSTAT 7.1.

Results and discussion

The total phenolics content per 100 mg of *Sida cordifolia*, *Sida rhombifolia* and *S. urens* fractions ranged from 37.11±0.01mgGAE to 36.22±0.03mgGAE for *Sida cordifolia*, 32.45±0.01mgGAE to 32.13±0.15mgGAE for *Sida rhombifolia* and 30.35±0.01mgGAE to 30.19±0.20mgGAE for *S. urens*. The highest content of total phenolics was detected in EAF of *Sida cordifolia* with 37.11±0.01mgGAE following by *Sida rhombifolia* with 32.45±0.01mgGAE detected in EAF. The lowest total phenolics were obtained in *S. urens*.

The total flavonoids content per 100 mg of *Sida cordifolia*, *Sida rhombifolia* and *S. urens* fractions ranged from 3.78±0.00mgGAE to 3.43±0.04mgGAE for *Sida cordifolia*, 3.02±0.15mgGAE to 2.87±0.11mgGAE for *Sida rhombifolia* and 2.97±0.05mgGAE to 2.18±0.09mgGAE for *S. urens*. The highest content of total flavonoids was detected in EAF of *Sida cordifolia* with 3.78±0.00mgGAE following by *Sida rhombifolia* with 3.02±0.15mgGAE detected in EAF. The lowest total flavonoids were obtained in *S. urens*.

The total flavonols content per 100 mg of *Sida cordifolia*, *Sida rhombifolia* and *Sida urens* fractions ranged from 2.92±0.01mgGAE to 1.27±0.01mgGAE for *Sida cordifolia*, 1.02±0.15mg GAE to 0.96±0.10mgGAE for *Sida rhombifolia* and 1.33±0.13mgGAE to 1.12±0.00mgGAE for *S. urens*. The highest content of total flavonols in *Sida cordifolia* was detected in EAF with 2.92± 0.01mgGAE. For *Sida rhombifolia*, the highest content of total flavonols content was detected in EAF with 1.02±0.15mgGAE. For the three plants, the lowest total flavonols content were obtained in

Table 1: Polyphenols contents of *Sida cordifolia*, *Sida rhombifolia*, *S. urens* fractions

Fractions	Total phenolic (mgGAE/100mg fraction)	Total flavonoid (mg QE/100mg fraction)	Total flavonol (mgQE/100mg fraction)	Total tannin (mgTAE/100mg fraction)
DCMF/<i>S.cordifolia</i>	36.22±0.03 ^b	3.43±0.04 ^b	1.27±0.01 ^c	32.63±0.03 ^b
EAF/<i>S.cordifolia</i>	37.11±0.01 ^a	3.78±0.00 ^a	2.92±0.01 ^a	33.13±0.01 ^a
DCMF/<i>S.rhombifolia</i>	32.13±0.15 ^d	2.87±0.11 ^e	0.96±0.10 ^f	31.26±0.01 ^d
EAF/<i>S.rhombifolia</i>	32.45±0.01 ^c	3.02±0.15 ^c	1.02±0.15 ^e	30.87±0.11 ^c
DCMF/<i>S.urens</i>	30.19±0.20 ^f	2.18±0.09 ^f	1.12±0.00 ^d	28.65±0.02 ^f
EAF/<i>S.urens</i>	30.35±0.01 ^e	2.97±0.05 ^d	1.33±0.13 ^b	28.02±0.20 ^e

mg GAE/100mg fraction: mg equivalent Gallic acid for 100mg dried fraction

mgQE/100mg fraction : mg equivalent Quercetin for 100mg dried fraction

mgTAE/100mg fraction : mg equivalent Tanic acid for 100mg dried fraction

DCMF: dichloromethane fraction; EAF: ethyl acetate fraction.

Table 2: Antioxidant Properties of *Sida cordifolia*, *Sida rhombifolia*, *S. urens* fractions

Fractions	DPPH mmoL AAE/g fraction	FRAP mmoL AAE/g fraction	ABTS mmoL AAE/g fraction
DCMF/<i>S.cordifolia</i>	9.46±0.17 ^b	4.22±0.03 ^b	1.93±0.10 ^d
EAF/<i>S.cordifolia</i>	9.52±0.01 ^a	4.43±0.11 ^a	2.01±0.01 ^a
DCMF/<i>S.rhombifolia</i>	6.19±0.01 ^e	4.09±0.05 ^d	1.03±0.01 ^e
EAF/<i>S.rhombifolia</i>	6.52±0.22 ^c	4.17±0.04 ^c	1.97±0.04 ^b
DCMF/<i>S.urens</i>	5.89±0.03 ^f	3.97±0.11 ^f	0.98±0.10 ^f
EAF/<i>S.urens</i>	6.02±0.10 ^d	4.03±0.05 ^e	1.35±0.01 ^c
Quercetin	13.76±0.26	Not determined	7.81±0.21
Trolox	Not determined	7.46±3.38	Not determined

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

DCMF: dichloromethane fraction; EAF: ethyl acetate fraction;

Table 3: Lipoxygenase and xanthine oxidase inhibition activities of *Sida cordifolia*, *Sida rhombifolia*, *S. urens* fractions

Fractions	Lipoxygenase inhibition%	Xantine oxidase inhibition%
DCMF/ <i>S.cordifolia</i>	49.23±0.01 ^c	32.17±0.07 ^e
EAF/ <i>S.cordifolia</i>	53.13±0.07 ^b	37.20±0.02 ^c
DCMF/ <i>S.rhombifolia</i>	56.25±0.12 ^e	45.05±0.12 ^d
EAF/ <i>S.rhombifolia</i>	56.45±0.04 ^d	49.18±0.18 ^f
DCMF/ <i>S.urens</i>	53.66±0.08 ^f	40.11±0.03 ^b
EAF/ <i>S.urens</i>	54.61±0.11 ^a	43.22±0.41 ^a
Quercetin	52.74±1.72	Not determined
Allopurinol	Not determined	77.13±0.41

DCMF: dichloromethane fraction; EAF: ethyl acetate fraction;

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

DCMF fraction with 0.96±0.10mgGAE for *Sida rhombifolia*.

The total tannins content per 100 mg of *Sida cordifolia*, *Sida rhombifolia* and *Sida urens* fractions ranged from 33.13±0.01mgTAE to 32.66± 0.03mgTAE for *Sida cordifolia*, 31.26±0.01mgTAE to 30.87±0.11mgTAE for *Sida rhombifolia* and 28.65±0.02mgTAE to 28.02± 0.20mgTAE for *S. urens*. The highest content of total tannin in *Sida cordifolia* was detected in EAF with 33.13±0.01mgTAE following by *Sida rhombifolia* with 31.26±0.01mgTAE detected in DCMF. For the three plants, the lowest total tannin content was obtained in EAF with 28.02±0.02mgTAE for *S. urens*. The results are reported in the Table 1. These results of polyphenol content analysis show that EAF of *Sida cordifolia* exhibited the highest amount of polyphenol content than the

other plants. This fact could be explained by the fact that these compounds are more extractible by ethyl acetate. Also, we could add that, ethyl acetate extract abundantly especially phenolic compounds [6]. The abundance of this fraction in polyphenol content should also explained antioxidant activity results.

For the antioxidants properties, we have tested three methods for a best appreciation of our results; because a recent study demonstrates that there are differences between the test systems for the determination of the antioxidants properties [16].

Results are consigned in the Table 2. The reduction capacity of DPPH radicals was determined by the decrease of the absorbance induced by antioxidant at 517 nm, which is induced by antioxidant. The values of different concentrations of fractions varied respectively

from 9.52 ± 0.04 mmolAAE/g fractions to 9.46 ± 0.17 mmolAAE/g fractions for *Sida cordifolia*, *Sida rhombifolia* and *Sida urens*, 6.62 ± 0.22 mmolAAE/g fractions to 6.19 ± 0.01 mmolAAE/g fractions for *Sida rhombifolia* and 6.02 ± 0.10 mmolAAE/g fractions to 5.89 ± 0.03 mmolAAE/g fractions. Among the different fractions, the strongest DPPH activity was obtained by EAF with 9.52 ± 0.01 mmolAAE/g fractions for *Sida cordifolia* following by *Sida rhombifolia* with 6.62 ± 0.22 mmolAAE/g fractions. The lowest activity was obtained by DCMF with 5.89 ± 0.03 mmolAAE/g fractions for *Sida urens*. Control compound gave 13.76 ± 0.26 mmolAAE/g fraction for Quercetin.

For FRAP assay, the following values were varied respectively from 4.43 ± 0.11 mmolAAE/g fractions to 4.22 ± 0.03 mgAGE/100mg for *Sida cordifolia*, 4.17 ± 0.04 mmolAAE/g fractions to 4.09 ± 0.05 mgAGE/100mg for *Sida rhombifolia* and 4.03 ± 0.05 to 3.97 ± 0.11 mgAGE/100 mg for *Sida urens*. The strongest FRAP activity was obtained by EAF with 4.43 ± 0.11 mmolAAE/g fractions for *Sida cordifolia*. The lowest activity was obtained DCMF with 3.97 ± 0.11 mgAGE/100mg for *Sida urens*. Control compound gave 7.46 ± 3.38 mmolAAE/g fractions for Trolox. We remark also that, the fractions from *Sida cordifolia* have exerted a best antioxidant activity by FRAP method.

For ABTS radical cation decolourization assay, the different values were varied respectively from 2.01 ± 0.01 mmolAAE/g fractions to 1.93 ± 0.10 mmolAAE/g fractions for *Sida cordifolia*, 1.97 ± 0.04 mmolAAE/g fractions to 1.03 ± 0.01 mgAGE/100mg for *Sida rhombifolia* and 1.35 ± 0.01 to 0.98 ± 0.10 mgAGE/100 mg for *Sida urens*. The strongest ABTS activity was obtained by EAF with 2.01 ± 0.01 mmolAAE/g fractions for *Sida cordifolia*. The lowest activity was obtained in DCMF with 0.98 ± 0.10 mmolAAE/g fractions for *S.urens*. The reference compound is Quercetin 7.81 ± 0.21 mmolAAE/g fraction. We also note that, the fractions from *Sida cordifolia* have exerted a best antioxidant activity. We notice a relationship between total

phenolics and antioxidant activity. According certain study, total phenolics constitute one of the major groups of compounds antioxidants [17]. This good relationship between the results from total phenols analysis and the antioxidant activity has been previously reported by some studies in past [18]. But, comparatively to the reference compounds used, the antioxidant assay by ABTS method, is not interesting. The result may be due to the variety of the plant materials.

Anti-inflammatory activity was evaluated through the percentage of lipoxygenase inhibition and xantine oxidase inhibition. Results are consigned in the Table 3. Lipoxygenase inhibition with fractions varied from $53.13 \pm 0.07\%$ to $49.23 \pm 0.01\%$ for *Sida cordifolia*, $56.66 \pm 0.08\%$ to $56.25 \pm 0.12\%$ for *Sida rhombifolia* and $54.61 \pm 0.11\%$ to $52.74 \pm 1.72\%$ for *Sida urens*. The strongest inhibition was obtained by EAF with $56.45 \pm 0.04\%$ for *Sida rhombifolia* following by *Sida cordifolia* with $53.13 \pm 0.07\%$ obtained in EAF and with $50 \mu\text{g/ml}$ in the test solution. The low activity was obtained in DCMF with $49.23 \pm 0.01\%$ for *Sida cordifolia* and with $50 \mu\text{g/ml}$ in the test solution. We could remark that the fractions of *Sida rhombifolia* have a best inhibition with the lipoxygenase. For xanthine oxidase inhibition with fractions varied from $37.20 \pm 0.02\%$ to $32.17 \pm 0.07\%$ for *Sida cordifolia*, $49.18 \pm 0.18\%$ to $45.05 \pm 0.12\%$ for *Sida rhombifolia* and $43.22 \pm 0.41\%$ to $40.11 \pm 0.03\%$ for *Sida urens*. The strongest inhibition was obtained by EAF with $49.18 \pm 0.18\%$ for *Sida rhombifolia* and with $50 \mu\text{g/ml}$ in the test solution. The low activity was obtained in DCMF with $32.17 \pm 0.07\%$ for *Sida cordifolia* and with $50 \mu\text{g/ml}$ in the test solution. We could remark that the fractions of *Sida rhombifolia* have a best lipoxygenase inhibitory activity. This result could be explained by the abundance of the EAF in polyphenol content. So, the results showed a link between polyphenols content and lipoxygenase. This good relationship between polyphenol content and lipoxygenase inhibitory activity has been effectively reported by [7]. Regarding the xanthine oxidase, it is well know this enzyme is an important biological source of oxygen-derived

free radicals that contribute to oxidative damage to living tissues that are involved in many pathological processes such as inflammation, cancer [9]. But, unfortunately we haven't any interesting results with xanthine oxidase inhibition.

Conclusion

In conclusion, the fractions of ethyl acetate have the best results in polyphenol contents, antioxidants properties and anti-inflammatory activity. To achieve our objective, the fractions of ethyl acetate would be chosen for the isolation of bioactive molecules to be used as an alternative in the treatment of hepatitis B.

Contribution to authors

A. Souza, N.Barro and O.G.Nacoulma have contributed to improve the manuscript.

I.C.Dibala and Y.T.Kassi contributed by reading the article and also to correct the mistakes.

J.Millogo-Rasolodimby helped by the identification plants.

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