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In vitro Antioxidant, Lipoxygenase and Xanthine Oxidase Inhibitory Activities of Fractions from *Cienfuegosia digitata* Cav., *Sida alba* L. and *Sida acuta* Burn f. (Malvaceae)

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Abstract: In this study polyphenol content, antioxidant activity, lipoxygenase (LOX) and Xanthine Oxidase (XO) inhibitory effects of n-hexane, dichloromethane, ethyl acetate and n-butanol fractions of aqueous acetone extracts from *S. alba* L., *S. acuta* Burn f. and *Cienfuegosia digitata* Cav. were investigated. The total phenolics, flavonoids, flavonols and total tannins were determined by spectrophotometric methods using Folin-ciocalteu, $AlCl_3$ reagents and tannic acid, respectively. The antioxidant potential was evaluated using three methods: inhibition of free radical 2,2-diphenyl-1-picrylhydramzyl (DPPH), ABTS radical cation decolorization assay and Iron (III) to iron (II) reduction activity (FRAP). For enzymatic activity, lipoxygenase and xanthine oxidase inhibitory activities were used. This study shows a relationship between polyphenol contents, antioxidant and enzymatic activities. Present results showed that ethyl acetate and dichloromethane fractions elicit the highest polyphenol content, antioxidant and enzymatic activities.

Key words: Polyphenol, antioxidant, lipoxygenase, xanthine oxidase, malvaceae species, hepatitis B

INTRODUCTION

Traditional cures and plant-based remedies remain complementary and alternative solution to health problems in many developing countries (Azaizeh *et al*., 2003). The need to use folk medicine is explained by habit and customs, the poverty of populations, the lack of medical facilities and doctors and above all microbial resistance to some modern medicines (Adjanhoun *et al*., 1985). In addition, many people in developed countries are interested in having more autonomy over their medical care, so self-medication is commonplace (Eisenberg *et al*., 1993). In this fact, the present study concerned *Sida alba* L., *S.acuta* Burn f. and *Cienfuegosia digitata* Cav. fractions; those Malvaceae species are widely used in Africa particularly in Burkina Faso as a component of several primitive medicinal remedies against human diseases. Ethnobotanical investigations conducted in the central region of Burkina Faso have showed that those Malvaceae species are used frequently and widely in traditional medicine for the treatment of various kinds of diseases such as malaria, fever, gastrointestinal infections, dermatitis, varicella, variola, anti-inflammatory and antibacterial properties, particularly used to treat hepatitis B (Nacoulma, 1996). The phytochemical screening on those Malvaceae species revealed the presence of saponosides, cumarins, steroids, tannins, polyphenols and flavonoids (Nacoulma, 1996). It is well established that phenolic compounds are found to have effect on oxidation damage (Ribeiro *et al*., 2008). Antioxidants are compounds which 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 (Halliwell and Gutteridge, 1984) and prevented many diseases as diabetes, hypertension and combating oxidative stress (Cole *et al*., 2005). Several studies have shown a link between polyhenol content and lipoxygenase (Aquila *et al*., 2009). Then, it is know that xanthine oxidase is an important biological source of oxygen-derived free radicals that contribute to oxidative damage to living tissues involved in many pathological

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processes such as inflammation, cancer (Sweeney *et al*., 2001). In this fact, the objectives of this study were investigate the polyphenol content, antioxidant activity, lipoxygenase and xanthine oxidase inhibitions of fractions from those Malvaceae species. The choice of our investigated plants is based on two criteria: First, in this domain there is no study in Burkina Faso that deals with these plants and the second criterion is that these plants have ethnopharmacological data indicating their traditional utilization in the treatment of hepatitis B caused by an inflammation of the liver and due to a lack of antioxidants.

MATERIALS AND METHODS

Chemicals: For evaluate phytochemical composition, antioxidant, lipoxygenase and xanthine oxidase inhibition activities 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 (0.2 M, 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 [$K_3Fe(CN)_6$] was sourced from Probalo (Paris, France); ascorbic acid, tannic acid and ion trichloride were supplied by labosi (Paris, France).

Plants material: *Cienfuegosia digitata* Cav., *Sida alba* L. and *S. acuta* Burn f. 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.

Extraction and fractionation: Fifty grams (50 g) 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, Rotavopor R-200, Switzeland) at approximately 40°C. The aqueous extracts were subjected to sequential liquid-liquid extraction with n-hexane, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain n-hexane fraction (n-HF), dichloromethane fraction (DCMF), ethyl acetate fraction (EAF) and n-butanol fraction (n-BF). 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 phenolic content: Total polyphenols were determined by Folin-Ciocalteu method as described by (Lamien-Meda *et al*., 2008). Aliquots (125 µL) of solution from 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 Na_2CO_3 (75 g L^{-1}) 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 Na_2CO_3) 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 flavonoid content: The total flavonoids were estimated according to the Dowd method as adapted by (Lamien-Meda *et al*., 2008). The 0.5 mL of methanolic $AlCl_3$ (2%, w/v) were mixed with 0.5 mL of methanolic fraction solution (0.1 mg mL^{-1}) 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 a 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 Abarca *et al*. (2007) method. Aliquots were prepared by mixing of 750 µL ethanolic fractions solution (0.1 mg mL^{-1}) of each plant and 750 µL aqueous $AlCl_3$ (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^{-1}). The results were expressed as mg of quercetin equivalents (QE)/100 mg of fractions.

Determination of tannins contents: Total tannins contents were determined as described by European community in 2004, using tannic acid as a standard. In test tube, 200 μL aqueous fraction of each fraction, 1 mL distilled water, 200 μL ammonium ferric citrate (3.5 g L^{-1}) and 200 μL ammoniac (20%) were mixed. After 10 min, the absorbencies of samples were measured at 525 nm against a blank (200 μL 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 scavenging: 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 Lamien-Meda *et al.* (2008). 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 gram of fraction (Y = -16.815x+6.8373; R^2 = 0.9976). Quercetin was used as positive control.

ABTS radical cation decolorization assay: For ABTS radical cation decolorization assay, the procedure followed the method of Lamien-Meda *et al.* (2008). ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with 2.45 mM 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 present study, we used 10 μL of the diluted sample (1 mg mL^{-1} in methanol) which was allowed to react with 990 μL of fresh $ABTS^{\bullet+}$ 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 Hinneburg *et al.* (2006). The 0.5 mL of each fraction (1 mg mL^{-1}) was mixed with 1.25 mL of phosphate buffer (0.2 M, 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×g for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared $FeCl_3$ solution (0.125 mL, 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 gram of fractions. Troloc, a reference compound was used as positive control.

***In vitro* lipoxygenase inhibitory assay:** Lipoxygenase inhibiting activity of plant fractions with linoneic acid as a substrate was measured with a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) as described by Malterud and Rydland (2000) with some modifications. Fractions were screened for lipoxygenase inhibitory activity at a 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^{-1} 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 2 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 Eq. 1:

$$\text{Inhibition (\%)} = (1\text{-B/A})\times 100 \quad (1)$$

where, A is the change in absorbance of the assay without the fraction extracts (Δabs. with enzyme -Δabs. without enzyme) and B is the change in absorbance of the assay with the fraction extracts (Δabs. with enzyme -Δabs. 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 Filha *et al.* (2006) with some modifications. Extracts were directly dissolved in phosphate buffer-MeOH (1%) and screened for xanthine oxidase inhibitory activity at a final concentration of 50 μg mL^{-1}. 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^{-1} 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 2 min. The negative control was prepared and contained 1% methanol solution without extract solution. Allopurinol a well known inhibitor of xantine oxidase was used as a positive control at a final concentration of 50 μg mL^{-1} prepared. All experiments were performed in triplicate. Xanthine oxidase inhibitory activity was expressed as the percentage inhibition of xantine oxidase, calculated as (%) inhibition following Eq. 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

Polyphenols contents: The amount of total phenolics content varied in different fractions and ranged from 23.60±0.03 mg GAE to 61.23±0.12 mg GAE for *Cienfuegosia digitata* followed by 17.37±0.02 mg GAE to 35.16±0.04 mg GAE for *Sida alba* and 15.77±0.60 mg GAE to 33.98±0.20 mg GAE for *S. acuta.* The highest total phenolics levels have been detected in EAF and the lowest in n-HF.

The total flavonoids content per 100 mg of *Cienfuegosia digitata*, *Sida alba* and *S. acuta* fractions ranged from 3.83±0.07 mg QE to 10.83±0.02 mg QE for *Cienfuegosia digitata* followed by 3.25±0.04 mg QE to 5.79±0.01 mg QE for *Sida alba* and 3.08±0.07 mg QE to 6.22±0.07 mg QE for *S. acuta.* The highest content was detected in EAF and the lowest in n-HF.

The total flavonols content per 100 mg of *Cienfuegosia digitata*, *Sida alba* and *S.acuta* fractions ranged from 1.79±0.16 mg QE to 5.71±0.11 mg QE. The highest content of total flavonols was detected in EAF and the lowest in n-HF.

The amount of total tannin content ranged from 10.08±0.21 mg TAE to 53.62±0.03 mg TAE. The highest content was detected in EAF and the lowest in n-HF. It has been noted that amount of total phenolic compounds in EAF is higher following by DCMF; the results are reported in the (Table 1). This fact could be explained by the fact that these compounds are more extractible by dichloromethane and ethyl acetate. Also, we could add that, dichloromethane fraction abundantly alkaloids and certain flavonoids; on the other side, ethyl acetate fraction abundantly especially phenolic compounds (Nacoulma, 1996). Moreover, the presence of these metabolites in some Malvaceae species particularly *Sida acuta* has been already reported by Damintoti *et al.* (2005). The abundance of the two fractions in polyphenol content should also explained antioxidant activity results. It is well known that, total phenolics constitute one of the major groups of compounds antioxidants (Cakir *et al.*, 2003). The results of the present study confirmed these reports. However, the abundance of polyphenols could justify the use of our plants in traditional medicine because polyphenols show protective effects on brain degenerative processes (Conte *et al.*, 2003) and anti-inflammatory (Subbaramaiah *et al.*, 1998). We could note that many medicinal plants contain large amounts of polyphenols which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Many of these phytochemicals possess significant antioxidant capacities that are associated with lower occurrence and lower mortality rates of several human diseases (Djeridane *et al.*, 2006). The presence of the phenolic compounds in DCMF and EAF would be certainly justifying the popular use of these three Malvaceae species in traditional medicine in Burkina Faso.

Antioxidant activities: For the antioxidants properties, we have tested three methods because a recent study demonstrates that there are differences between the test systems for the determination of the antioxidants properties (Nsimba *et al.*, 2008). 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 5.00±0.09 mmol AAE/g fraction to 12.90±0.04 mmol AAE/g fractions. Among the different fractions, the strongest DPPH activity was obtained by EAF and the lowest activities were obtained by n-hexane fraction Control compound gave 13.76±0.26 mmol AAE/g fraction for Quercetin.

For FRAP assay, the following values were varied respectively from 1.93±0.10 mmol AAE/g fraction to 5.38±0.07 mmol AAE/g fractions. The strongest FRAP activity was obtained by EAF and the lowest activities

Table 1: Polyphenols contents of *Cienfuegosia digitata*, *Sida alba* and *S. acuta* fractions

Fractions	Total phenolic	Total flavonoid	Total flavonol	Total tannin
	----------(mgGAE/100mgfraction)----------			
n-HF/*C. digitata*	23.60±0.03^{g}	3.83±0.07^{h}	2.47±0.02^{f}	20.12±0.03^{h}
DCMF/*C. digitata*	57.27±0.07^{b}	8.55±0.08^{b}	4.36±0.03^{b}	49.45±0.04^{b}
EAF/*C. digitata*	61.23±0.12^{a}	10.83±0.02^{a}	5.71±0.11^{a}	53.62±0.03^{a}
n-BF/*C. digitata*	35.03±0.09(	5.07±0.05^{f}	2.83±0.07^{e}	27.17±0.07^{d}
n-HF/*S. alba*	17.37±0.02^{j}	3.25±0.04^{i}	1.94±0.06^{g}	12.13±0.03^{k}
DCMF/*S. alba*	35.16±0.04^{c}	6.88±0.05^{c}	3.55±0.08^{c}	28.88±0.05^{c}
EAF/*S. alba*	33.23±0.09^{e}	5.79±0.01^{e}	2.83±0.03^{e}	22.94±0.04^{g}
n-BF/*S. alba*	21.21±0.07^{h}	4.15±0.01^{g}	2.01±0.01^{g}	16.27±0.02^{i}
n-HF/*S. acuta*	15.77±0.6^{k}	3.08±0.07^{i}	1.79±0.16^{g}	10.08±0.11^{l}
DCMF/*S. acuta*	31.22±0.18^{f}	5.91±0.13^{i}	3.20±0.01^{d}	25.15±0.04^{f}
EAF/*S. acuta*	33.98±0.20^{d}	6.22±0.07^{d}	3.49±0.11^{c}	26.24±0.21^{e}
n-BF/*S. acuta*	19.98±0.20^{i}	4.03±0.09^{g}	1.85±0.16^{g}	14.00±0.15^{j}

mg GAE/100 mg fraction: mg equivalent Gallic acid for 100 mg dried fraction. mg QE/100 mg fraction: mg equivalent Quercetin for 100 mg dried fraction mg TAE/100 mg fraction: mg equivalent Tanic acid for 100 mg dried fraction. n-HF: n-hexane fraction; DCMF: Dichloromethane fraction; EAF: Ethyl acetate fraction; n-BF: n-butanol fraction. Values are Mean±SD (n = 3). Different letters in the same column indicate significant difference ($p<0.05$) for our different fractions

Table 2: Antioxidant Properties of *Cienfuegosia digitata*, *Sida alba* and *S. acuta* fractions

Fractions	DPPHmmol AAE/g fraction	FRAPmmol AAE/g fraction	ABTSmmol AAE/g fraction
n-HF/*C. digitata*	6.61±0.03^{e}	2.31±0.01^{g}	1.97±0.02^{f,g}
DCMF/*C. digitata*	12.65±0.12^{a}	4.78±0.02^{b}	3.5±0.15^{c}
EAF/*C. digitata*	12.90±0.04^{a}	5.38±0.07^{a,b}	4.16±0.03^{b}
n-BF/*C. digitata*	10.60±0.13^{b}	3.62±0.03^{d,e}	2.88±0.01^{e}
n-HF/*S. alba*	5.24±0.11^{g}	2.11±0.01^{g}	1.73±0.03^{g}
DCMF/*S. alba*	9.75±0.04^{c}	5.23±0.09^{a}	3.22±0.02^{c,d}
EAF/*S. alba*	8.27±0.41^{d}	4.24±0.03^{c}	2.96±0.04^{d,e}
n-BF/*S. alba*	6.3±0.19^{e,f}	3.31±0.01^{e,f}	2.16±0.03^{f}
n-HF/*S. acuta*	5.00±0.09^{g}	1.93±0.10^{g}	1.97±0.16^{f,g}
DCMF/*S. acuta*	8.05±0.12^{d}	3.67±0.07^{d,e}	4.12±0.13^{b}
EAF/*S.acuta*	10.03±0.05^{c}	4.10±0.12^{c,d}	6.23±0.20^{a}
n-BF/*S. acuta*	6.06±0.10^{f}	3.25±0.08^{f}	2.88±0.50^{c,d}
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. n-HF: n-hexane fraction; DCMF: Dichloromethane fraction; EAF: Ethyl acetate fraction; n-BF: n-butanol fraction. Values are Mean±D (n = 3). Different letters in the same column indicate significant difference ($p<0.05$) for our different fractions

were obtained n-hexane fraction. Control compound gave 7.46±3.38 mmol AAE/g fractions for Trolox. We remark also that, the fractions from *Cienfuegosia digitata* have exerted a best antioxidant activity by FRAP method.

For ABTS radical cation decolorization assay, the different values were varied respectively from 1.97±0.16 mmol AAE/g fraction to 6.23±0.20 mmol AAE/g fraction. The strongest ABTS activity was obtained by EAF and the lowest activities were obtained n-hexane fraction. The reference compound is Quercetin 7.81±0.21 mmol AAE/g fraction. We also note that, the fractions from *Cienfuegosia digitata* have exerted a best antioxidant activity. This good relationship between the results from total phenols analysis and the antioxidant activity has been previously reported by some studies in past (Zheng and Wang, 2001). Phenolic compounds are known as powerful chain breaking antioxidants (Shahidi *et al*., 1992) and may contribute directly to antioxidative action (Duh *et al*., 1999). But, comparatively to the reference compounds used and the results of research works reported by Damintoti *et al*. (2005), the antioxidant assay by ABTS method in this present study, is not interesting. However, the aqueous acetone extracts of *Sida acuta* showed a good relation between phenolic compounds contents and the antioxidant activities ($R^2 = 0.91$ with ABTS method) (Damintoti *et al*., 2005).The result may be due to the variety of the plant materials or the nature of the soil (Coulidiati *et al*., 2009). These results show the interest of our plants in the treatment of hepatitis B which is an immune deficiency disease, because natural antioxidants such as phenolic compounds possess the ability to reduce the oxidative damage associated with many diseases such as immune deficiency diseases (Pietta *et al*., 1998; Lee *et al*., 2000; Malterud and Rydland, 2000).

Enzymatic activities: Enzymatic activity was evaluated through the percentage of lipoxygenase inhibition and xantine oxidase inhibition. Results are consigned in the (Table 3), the amount of lipoxygenase inhibition varied from 19.68±0.57 to 93.17±0.02%. The strongest inhibition was obtained by EAF and the lowest activities were obtained n-hexane fraction.

Table 3: Lipoxygenase and xanthine oxidase inhibition activities of *Cienfuegosia digitata*, *Sida alba* and *S.acuta* fractions

Fractions	Lipoxygenase inhibition	Xantine oxidase inhibition
	(%)	
n-HF/*C. digitata*	29.20 ± 0.04^{i}	18.02 ± 0.14^{i}
DCMF/*C. digitata*	79.60 ± 0.56^{d}	52.92 ± 0.09^{d}
EAF/*C. digitata*	93.17 ± 0.02^{a}	67.22 ± 0.04^{a}
n-BF/*C. digitata*	34.68 ± 0.41^{h}	46.03 ± 0.10^{f}
n-HF/S. *alba*	20.87 ± 0.10^{j}	13.92 ± 0.07^{j}
DCMF/*S. alba*	76.57 ± 0.05^{e}	55.04 ± 0.11^{c}
EAF/*S. alba*	86.95 ± 0.04^{b}	49.95 ± 0.12^{e}
n-BF/*S. alba*	41.05 ± 0.12^{g}	30.88 ± 0.14^{h}
n-HF/*S. acuta*	19.68 ± 0.57^{j}	17.43 ± 1.95^{i}
DCMF/*S. acuta*	73.02 ± 0.16^{f}	58.13 ± 0.23^{b}
EAF/*S. acuta*	84.95 ± 0.13^{c}	59.98 ± 0.03^{b}
n-BF/*S. acuta*	39.92 ± 0.90^{g}	35.03 ± 0.05^{g}
Quercetin	52.74±1.72	Not determined
Ibuprofen	89.15±0.31	Not determined
Allopurinol	Not determined	77.13±0.41

n-HF: n-hexane fraction; DCMF: dichloromethane fraction; EAF: ethyl acetate fraction; n-BF: n-butanol fraction. Values are Mean±SD (n = 3). Different letters in the same column indicate significant difference ($p<0.05$) for our different fractions

Xanthine oxidase inhibition ranged from 17.43±1.95 to 67.22±0.04%. The strongest inhibition was obtained by EAF and the lowest activities were obtained n-hexane fraction.

We remark that DCMF and EAF have a best lipoxygenase and xanthine oxidase inhibitory activities comparatively to the other fractions. The results of these three fractions could explain by the abundance of the two fractions in polyphenol content. This good relationship between polyphenol content and lipoxygenase inhibitory activity has been reported by Aquila *et al.* (2009). We evaluated xanthine oxidase because, it 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 (Sweeney *et al.*, 2001). We also notice the present study, a good relationship between polyphenolic compounds and xanthine oxidase particularly flavonoids. Many research works reported that flavonoids are a group of polyphenolic compounds, which have been reported to possess xanthine oxidase inhibitory activity (Meda *et al.*, 2010). In this fact, lipoxygenase and xanthine oxidase inhibitory activities would be probably due to the phenolic compounds. Inhibition of these two liver enzymes by the fractions from three plants, particularly DCMF and EAF of *Cienfuegosia digitata* could explain the reasons of it most utilization in hepatitis B treatment.

CONCLUSION

In conclusion, the fractions of *Cienfuegosia digitata* have the best results in polyphenol contents, antioxidants properties and enzymatic activities than *Sida alba* and *S. acuta* fractions. Moreover, DCMF and EAF have the best results. The results of this study show that those Malvaceae species particularly *Cienfuegosia digitata* can be used as easily accessible source of natural antioxidants, natural lipoxygenase and xanthine oxidase inhibitories. However, the components responsible for the antioxidant potential, lipoxygenase and xanthine oxidase inhibitory activities are currently unclear. Therefore, it is suggested that further works should be performed on the isolation and identification of the news bioactive components in DCMF and EAF to better manage hepatitis B in Burkina Faso.

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