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Research Article

ANTIOXIDANT, ENZYME INHIBITION ACTIVITIES AND POLYPHENOL CONTENTS OF THREE ASTERACEAE SPECIES USED IN BURKINA FASO TRADITIONALLY MEDICINE

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ABSTRACT

Chrysanthellum americanum (L.) Vatke, *Eclipta alba* (L.) Hassk and *Vernonia colorata* (Willd.) Drake, three species of *Asteraceae* family are widely used in traditional medicine of Burkina Faso. The aim of the present study was to evaluate the antioxidant capacity, the Lipoxygenase and Acetylcholinesterase inhibition potentials and the phenolic content of these three species. The antioxidant activity of E. *alba extract* presented the best scavenging activity of DPPH radical (IC_{50} of 3.1 ± 0.07 µg/ mL) and the best activity of ferric ion reduction of (0.99 ± 0.01 mmol AAE/g). The extract of *C. americanum* presented the best activity for the cation radical $ABTS^{+}$ reducing activity (67.50 ± 3.88 mmol AAE/g). The extract of *E. alba* showed the best inhibitory activity of Acetylcholinesterase, enzyme involved in Alzheimer's disease with a inhibition percentage of 23.44 ± 0.96% and also the best value for the inhibition of Lipoxygenase (42.76 ± 2.45 %) at a concentration of 100µg/mL. The primary phytochemical tests allowed identifying the presence of secondary metabolites in the extracts. *E. alba* methanol extract showed the highest of total phenolic content (17.88 ±0.93 mg GAE/100mg of extract), of total flavonoids (8.02 ± 0.4 mg QE/100 mg of extract) and of total tannins content (6.33 ± 0.29 mg TAE/100mg of extract). *C. americanum* showed the best value of 0.96 ± 0.11 mg QE/100mg of extract for total flavonols content. This study showed that *E. alba* extract is a potential natural source for the treatment of neurodegenerative and oxidative stress related diseases.

Keywords: *C. americanum*; *E. alba*; *V. colorata*; Antioxidant activity; AChE; LOX, Phenolic content

INTRODUCTION

Since ancient times, plants have formed the basis of traditional medicinal systems, such as the Ayurvedic, Chinese and African medicine. In recent years, the interest in folk medicine from different cultures, also known as Traditional Medicine(TM), has increased significantly in industrialized countries, due to the fact that many prescription drugs worldwide have originated from the tropical flora[1].

The family of *Asteraceae* content more than 1000 genres and 20000 species which constitute 17 tribes gathered in two subfamilies (Lactucoïdeae and Asteroïdeae)[2]. In Burkina Faso, Asteraceae one of the greatest family of plants are using in traditional medicine. *Chrysanthellum americanum* (L.) Vatke, *Eclipta alba* (L.) Hassk. and *Vernonia colorata* (Willd.) Drake; three species of *Asteraceae* family are used for the treatment of various active diseases of metabolic diseases to bacterial diseases[3].

C. americanum is known for its antioxidant properties[4]; also recent studies in Burkina Faso showed that this plant was used in the treatment of kidney diseases which are due to several causes that contribute to oxidative stress[5, 6]. Studies showed that *E. alba* have hepatoprotector, antimycobacterial, antioxidant, anti-inflammatory, acetylcholine increaser activities…[7, 8, 9, 10]. In phytochemical studies, lactone and mineral compounds were isolated from this plant[11, 12, 13]. For the third plant, investigations were attributed the antimicrobial activities of the extracts to the presence of flavonoids, vernodaline and vernolide (lactone sesquiterpene compounds) and their derivatives[14, 15].

In the vision to bring our modest contribution to Burkina Faso traditional medicine, the aim of the present study was to evaluate the antioxidant capacity, the Lipoxygenase and Acetylcholinesterase inhibition potentials and the phenolic content of these three plants.

MATERIALS AND METHODS

Plants materials

Vegetable material of Asteraceae species: *Chrysanthellum americanum* (L.) Vatke, *Eclipta alba* (L.) Hassk. and *Vernonia colorata* (Willd.) Drake was collected in August 2008 in Loumlila, 15 Km north of Ouagadougou, capital of Burkina Faso. The plants were identified by Prof. Millogo- Rasolodimby from the plants Biology Department of the University of Ouagadougou. A voucher specimen (ID-10474, ID-10476 and ID-10478 respectively) was deposited at the Herbarium of the Laboratory of Vegetable Biology and Ecology, of the University of Ouagadougou.

Preparation of extracts

Leaves of *V. colorata* and whole-plants of *C. americanum* and *E. alba* were dried at room temperature and ground to fine powder. Twenty five grams of powdered plant material was extracted successively with 250 mL of chloroform and 250 mL of methanol by Soxhlet apparatus. After, extracts solution were concentrated under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) at approximately 40°C and dried at ambient temperature. The obtained extracts were weighed before packed in waterproof plastic flasks and stored at 4°C until use.

Chemical reagents:

To carry out our different activities, we used solvents, enzymes and various classic reagents. All reagents were of analytical grade. Folin-Ciocalteu reagent, Dragendorff's reagent, sodium carbonate (Na_2CO_3), sodium hydroxide, gallic acid, quercetin, aluminium trichloride ($AlCl_3$), hydrochloric acid, magnesium chloride, Bovine Serum Albumin (BSA), Acetylcholinesterase (EC 232. 559. 3. 3) (AChE) from electric eel, 15-lipoxygenase (EC 1. 13. 11. 12), acetylcholine iodide (ATCI), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), linoleic acid and Boric acid were purchased from Sigma Aldrich chemie (Steinheim, Germany); ammonium ferric citrate, potassium persulfate, DPPH (2, 2'-diphenyl-1-picrylhydrazyl), 2,2'-azinobis (3 ethylbenzothiazoline-6-sulphonate) ABTS and trichloroacetic acid were supplied by Fluka chemie (Buchs, Switzerland); sulfuric acid, acetic anhydride, ferric trichlorure, chloroform, ethanol, methanol, sodium tetraborate, and potassium hexacyanoferrate [$K_3Fe(CN)_6$] were sourced from Probalo (Paris, France); ascorbic acid and tannic acid were supplied by Labosi (Paris, France).

Biological studies

Antioxidant activities

DPPH radical Method

Radical scavenging activity of plant extracts against stable DPPH (20mg/mL) (2, 2'-diphenyl-1 picrylhydrazyl) was determined by

using UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England) at 517 nm by using the method described by Kadam et *al.*[16]. In each 0.5 mL of successively half dilution of methanol extracts (1mg/mL) added 1mL of DPPH solution. The absorbances were read at after 15 min dark incubation against a blank. DPPH reduction percentage of each dilution was obtained according to the following equation:

$$AAR(\%) = \frac{Abs(blk) - Abs(ext)}{Abs(blak)}.100$$

(Abs (blk), absorbance without extract; Abs (ext), absorbance with extract).

The concentration of extract reducing 50% of DPPH (IC_{50}) is obtained starting from the curve giving the AAR according to the concentration of extract. Quercetin, a reference compound was used as positive control.

ABTS Radical Cation Decolorization Assay

The ABTS radical cation decolorization assay according to the procedure[17] was used to determine the antioxidant activity of the extract. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting aqueous ABTS stock solution (7 mM) with 2.45mM potassium persulfate (final concentration). The mixture was put down in the dark at room temperature during 12h before use. This mixture was diluted with ethanol to give an absorbance of 0.7 ± 0.02 units at 734 nm using a spectrophotometer. 10 µL of the diluted sample (1g/mL in methanol) was added with 990 µL of fresh $ABTS^{\cdot+}$ solution and the absorbance was taken 6 min exactly after initial mixing. Ascorbic acid was used to produce the calibration curve (R^2 = 0.99) and the capacity of free radical scavenging of extract was expressed as mmol Ascorbic Acid Equivalent per g of extract

Iron (III) Reduction Activity (FRAP)

The FRAP assay was performed according to Lamien-Meda et *al.*[17]. 0.5 mL of each extract (1 mg/mL) 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 of incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000g during 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%). Absorbances were read at 700 nm on a spectrophotometer and Ascorbic acid was used to produce the calibration curve (R^2 = 0.99). The iron (III) reducing activity of the extracts was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract.

Enzyme Inhibition Assays

Lipoxygenase (LOX) Inhibition Assay

Lipoxygenase inhibition activity of extracts with linoneic acid as a substrate was measured as described by Coulibaly et *al.*[18]. Extracts were screened for lipoxygenase inhibitory activity at a final concentration of 100µg/mL. The mixture assay consisted of 50 µL of extract solution (dissolved in phosphate borate 1/15 M, pH 7.5 buffer) and 200 µL of enzyme solution (0.28 U/mL in phosphate borate buffer). The reaction was initiated 02 min after by adding 250 µL of substrate solution (0.15 mm in buffer). Enzymatic kinetic was recorded at 234 nm for 02 min. All experiments were performed in triplicate. Lipoxygenase inhibitory activity was expressed as the percentage inhibition of lipoxygenase, calculated as (%) inhibition following equation: (%) inhibition = (1 - B/A) × 100, where A is a change in absorbance of the assay without the extracts and B is the change in absorbance of the assay with the extracts.

Assay of Acetylcholinesterase (AChE) activity

Acetylcholinesterase inhibitory assay with inhibition kinetics analysis were conducted according to the protocol described by Kiendrebego et *al.*[19]. 200µLof Tris- HCl buffer (50Mm, pH8, 0.1% BSA) was added with 100µLof extract (final concentration of 100µg/mL dissolved in MeOH buffer 10%) and 100µLof AChE (0.22 U/mL). The mixture was incubated at room temperature for 2min before the addition of 500µLof DTNB (3mM) and 100µLof substrate (ATCI 15 mM). The developing yellow color was measured at 405nm after 4min on a spectrophotometer. Galanthamine was used as a positive control at a final concentration of 0.2µg/mL in the assay mixture. AChE inhibitory activity was expressed as inhibition percentage of AChE, calculated as (1-B/A) x 100, where A is a change in absorbance of the assay without the plant extract (Δabs. with enzyme- Δabs. without enzyme) and B is the change in absorbance of the assay with the plant extract (Δabs. with enzyme - Δabs. without enzyme).

Phytochemical investigation

Total Phenolics Content

Total polyphenols were determined by using Folin-Ciocalteu method as described by Singleton et *al.*[20] Aliquots (125 µL) of methanol extracts (100µg/mL) were mixed with 625 µL of Folin- Ciocalteu reagent (0.2 N). After 5 min, 500 µL of aqueous sodium carbonate (Na_2CO_3, 75 g/l) were added. After 2h of incubation in the dark at room temperature, the absorbances were measured at 760 nm against a blank (0.5 mL Folin-Ciocalteu reagent + 1 mL Na_2CO_3) by using spectrophotometer. The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (0-50 µg/mL) (R^2 = 0.99). The results were expressed as mg of gallic acid equivalents (GAE)/100 mg of extract.

Total Flavonoids Content

The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al.*[17]. 0.75 mL of methanolic $AlCl_3$ (2%) were mixed with 0.75 mL of methanolic extract solution (100µg/mL). After 10 min of incubation, the absorbances were measured at 415 nm against a blank (mixture of 0.75 mL methanolic extract solution and 0.75 mL methanol) by using spectrophotometer and compared to a quercetin (0-50 µg/mL) calibration curve (R^2 = 0.99). The data obtained were the means of three determinations. The amounts of flavonoids in plant extracts were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

Total Flavonols Content

The contents of flavonols were determined as described by Abarca et *al.*[21] method. Aliquots were prepared by mixing of 750 µL of methanolic extract (1 mg/mL) and 750 µL of ethanolic solution of $AlCl_3$ (20%). The absorbances were read at 425 nm after 10 min incubation against a blank (mixture of 750µL of extract and 750µL of ethanol) by using spectrophotometer. All determinations were carried out in triplicate. A standard calibration curve (R^2 = 0.99) was plotted using quercetin (0-50 µg/mL). The results were expressed as mg of quercetin equivalents (QE)/100 mg of extract.

Total Tannins Content

Total tannins contents were determined as described by Sombié et al.22 In eppendorff tube, 200µL of aqueous extract, 1mL of distilled water, 200µL of ammonium ferric citrate (3.5g/L) old of 24h and 200µL of ammoniac (20%) were mixed. After 10 min, the absorbances of samples were measured at 525 nm against a blank (200 µL aqueous extract, 200 µL ammoniac (20%) + 1mL distilled water) on a spectrophotometer and compared to tannic acid (0-50 µg/mL) calibration curve (R^2 = 0.99). The data obtained was the mean of three determinations. The results were expressed as mg of tannic acid equivalents (TAE) per 100 mg of extract (mg TAE/100 mg extracts).

Statistical Analysis

Results were expressed as mean ± standard deviations (SD); Tukey's test was used to determine level of significance of all results obtained on XLSTAT 7.1. Results were regarded as significant at $P < 0.05$.

RESULTS AND DISCUSSION

Biological studies

Antioxidant capacities

The important role of different oxidants on the beginning and the development of cardiovascular diseases such as atherosclerosis,

cancer, asthma, diabetes; neurodegenerative diseases were very well-known[23, 24, 25]. In this order, the finding of new antioxidants form traditional plant extracts is particularly welcome. The antiradical effects and the iron III reducing abilities of *C. americanum*, *E. alba*, *V. colorata* were presented in table 1. It was found that in FRAP as DPPH methods, *E. alba* has presented the best activity followed *C. americanum and V. colorata.* In radical ABTS inhibition capacity, the activity was decreased in the following order of: *C. americanum* < *E. alba* < *V. colorata.* This observation suggested that *E. alba extract*s possessed compounds with DPPH-scavenging activity and iron III reduction power. While plants extracts have demonstrated a lower antioxidants than reference namely quercetin which has 3.6±0.09 µg/mL as IC_{50}. These observations could partially justify the traditional uses of studied plant extract singurlay in inflammatory disease and diabetes.

Table 1: Antioxidant, AChE and LOX inhibitions activities

Species	DPPH (IC_{50}; µg/mL)	ABTS (µmol/g)	FRAP (mmol AAE/g)	AChE inhibition (%)	LOX inhibition (%)
C. americanum	10.10 ±0.72[b]	65.30 ±0.30[a]	0.99±0.01[b]	14.98 ±0.19[b]	22.88±2.38[c]
E. alba	3.10 ±0.07[c]	59.33 ±0.12[b]	1.37 ±0.07[a]	23.44 ±0.96[a]	42.76±2.45[a]
V. colorata	13.99 ±0.53[a]	54.37 ±1.31[c]	0.62 ±0.03[c]	13.80 ±0.38[b]	35.01±2.22[b]
Quercetin	3.6±0.09	Nd	Nd	Nd	Nd
Galanthamine	Nd	Nd	Nd	98.06 ±0.75	Nd

mmol AAE/g: mmol Ascorbic Acid Equivalent per g of extract; Nd: not determined;

Result within each column with different letters (a - c) differs significantly ($p < 0.05$).

This antioxidant activity is also interesting compared to those of quercetin reference compound. In other countries, authors showed that these plants had ABTS cation radical reduction and lipids peroxidation inhibition properties[4, 26].

Enzyme Inhibition Assay

LOX and AChE inhibition potentials of extracts

Lipoxygenase and Acetylcholinesterase activities played a main role in the inflammatory and neurodegenerative diseases[27, 28]. Additionally, it was demonstrated a possible interaction between these diseases[29]. The inhibition of these enzymes could be a ways for finding new compounds with anti-inflammatory and anti-neurodegenerative abilities. Plant extracts enzyme inhibition properties were showed in table 1. *E. alba* methanol extract presented the best inhibition of Lipoxygenase (42.76±2.45) and acetylcholinesterase (23.44±0.96%). The biggest sensitive of LOX to plant extracts could be explained by the presence of iron III in its structure that was reducible to iron II like in FRAP method (table 1). Galanthamine was 4-7 folds more active than plant extracts.

These activities could thus explained by the antioxidant activity of the plants and more specialy of the presence of the phenolic compounds [30, 31].

Phytochemical investigation

Preliminary screening

V. colorata presented a better extraction yield (Table 2); warranted by the fact which is an annual plant, therefore requiring to synthesize secondary metabolites enough to withstand climatic stress. A preliminary phytochemical screening allowed detecting the secondary metabolites such as polyphenols, tannins, alkaloids, flavonoids, coumarins, cardenolids, saponins and anthracenosids (table 2). For these preliminary tests carried out, *C. americanum*, *E. alba* and *V. colorata* were similarly phytochemical profiles. The presence of these compounds confirms their utility in traditional medicine[3]. This preliminary test allowed us to choose the methanol extracts for the remainder of our studies because chloroformic extracts practically did not contain phenolic compounds.

Table 2: preliminary phytochemical screening

Tested material	Extraction yield (%)	Positive tests	Negatives tests
C. americanum	19.66	Flavonoids, Coumarins, Saponins Anthracenosids, Tannins and/or Polyphenols, cardenolids	Alkaloids
E. alba	17.44	Flavonoids, Coumarins, Alkaloids, Saponins, Tannins and/or Polyphenols, cardenolids	Anthracenosids
V. colorata	20.44	Flavonoids, Coumarins, Alkaloids, Saponins, Tannins and/or Polyphenols, cardenolids	Anthracenosids

Polyphenols contents

The total phenolics, total flavonoids, total flavonols and total tannins contents of the methanolics extracts of the three species of *Asteraceae* are shown in Table 3.

The methanol extract of *E. alba* contains the highest in total phenolics and total flavonoids content followed by *C. americanum* extract and that of *V. colorata*. The best content of 0.96±0.11 was obtained for *C. americanum* in total flavonols, followed by 0.88 ± 0.11 for *E. alba* and 0.32±0.07 for *V. colorata*. The total flavonols of *C. americanum* extract content is not significantly different from that of *E. alba*. The highest concentration of total tannins was recorded for *E. alba* (6.33±0.29 mg TAE/100 mg of extract) followed by *V. colorata* (4.62±0.08 mg TAE/100 mg of extract). The level of tannins was comparatively 2–3 times lower for *C. americanum* extracts (2.60 ± 0.05 mg TAE/100 mg of extract).

Table 3: polyphenols contents of three Asteraceae species

Species	Total phenolics (mgGAE/100mg extract)	Total flavonoids (mgQE/100mg extract)	Total flavonols (mgQE/100mg extract)	Total tannins (mgTAE/100mg extract)
C. americanum	13.2 ± 0.1[b]	4.22 ± 0.06[b]	0.96 ± 0.11[a]	2.60 ± 0.05[c]
E. alba	17.88 ± 0.93[a]	8.02 ± 0.4[a]	0.88 ± 0.11[a]	6.33 ± 0.29[a]
V. colorata	10.97 ± 0.5[c]	2.01 ± 0.05[c]	0.32 ± 0.07[b]	4.62 ± 0.08[b]

mg GAE/100mg of extracts: mg Equivalent Gallic Acid for 100mg of dried extracts;

mg QE/100mg extract: mg Equivalent Quercetin for 100 mg of dried extracts;

mg TAE/100mg of extract: mg Equivalent Tannic Acid for 100 mg of dried extracts;

mmol AAE/g of extract: mmol Equivalent Ascorbic Acid for 1g of dried extract;

Result within each column with different letters (a - c) differs significantly ($p < 0.05$).

The presence of these phenolic compounds in these extracts justifies their antioxidant activities [32, 33]. The inhibition of LOX and AChE is undoubtedly due to the antioxidant activity of the extracts. It is in this logic that studies of correlation showed a good correlation between antioxidant activity and anti-inflammatory drug activity[34, 35]. AChE and LOX inhibition would be also due to the presence of the phenolic compounds endowed with antioxidant activities[30, 36].

CONCLUSION

In this study; antioxidant activities and capacities to inhibit the 15-lipoxygenase, the acetylcholinesterase and finally phenolic compounds content of extracts of the three plants were evaluated. Force is to note that enzyme inhibitions effect is in the same direction as the antioxidant capacity of our extracts; antioxidant capacity is also in the same order with phenolic content of extracts.

C. americanum is used much in decoction form in the treatment of lithiasis in Burkina Faso as elsewhere that would be explained by its very low toxicity, antioxidant and enzymatic properties. The weak antioxidant and enzymatic activities obtained and also the former results of the antimicrobial activity of *V. colorata* would partly justify the traditional use of this plant for antimicrobial needs. The vegetable specie *E. alba* as for it presented for the major part of the tests carried out the best activity, it is thus necessary to continue the studies on this one in order to lead at the stage molecule-biological activity. In Burkina Faso, this species is especially used against fungal in children; it could also be exploited in traditional medicine for its antioxidant properties. It is a way to isolate new bioactive compounds in Burkina Faso future research.

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