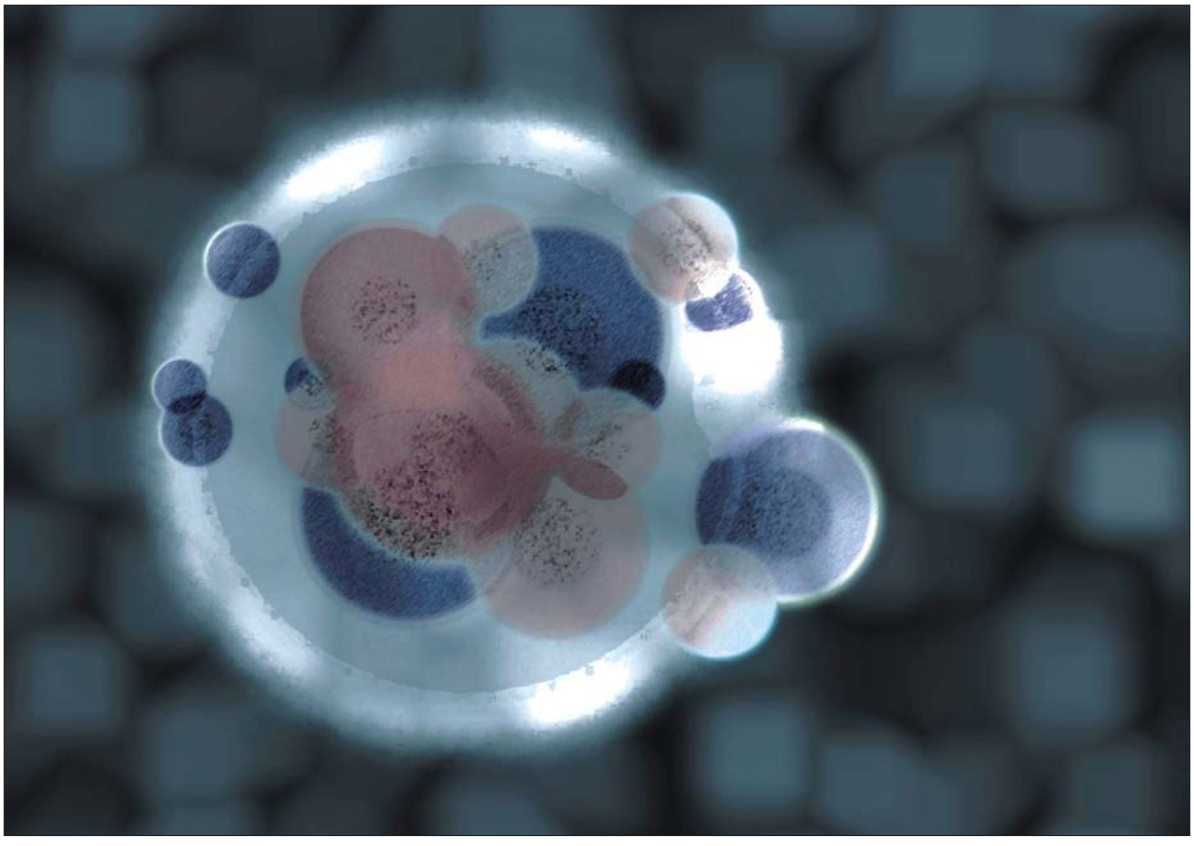

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Enzyme Inhibition Effect and Polyphenolic Content of Medicinal Plant Extracts from Burkina Faso

[1]Mindiédiba Jean Bangou, [1]Martin Kiendrebeogo, [1]Moussa Compaoré, [1]Ahmed Yacouba Coulibaly, [1]Nâg-Tiero Roland Meda, [2]Norma Almaraz Abarca, [3]Boukaré Zeba, [4]Jeanne Millogo-Rasolodimby and [1]Odile Germaine Nacoulma
[1]Laboratoire de Biochimie et de Chimie Appliquées (LA.BIO.C.A), U.F.R/S.V.T, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso
[2]Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional, Instituto Politécnico Nacional, Unidad Durango. Becaria COFAA; Sigma 119, Fraccionamiento 20 de Noviembre II, Durango, Dgo., México
[3]Laboratoire d'Enzymologie de la Chimio-Résistance Bactérienne (L.E.CR.B) U.F.R/S.V.T, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso,
[4]Laboratoire de Biologie et d'Ecologie Végétales, U.F.R/S.V.T, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso

Abstract: In the present study, 36 plant extracts, belonging to 6 families from Burkina Faso were used to evaluate their glutathione-S-transferase (GST), acetylcholinesterase (AChE), carboxylesterase (CES) and xanthine oxidase (XO) inhibitory activities and their phenolic, tannin and flavonoids contents by using spectrophotometrical methods. At 100 μg mL^{-1}, *Lippia chevalieri*, *Eclipta prostrata, Lantana camara* and *Indigofera pulchra* extracts showed the best percentage of inhibition by regulating GST, AChE, CES and XO activities, respectively. The phytochemical investigations showed that all plant extracts were rich in biological compounds, namely phenolic, tannin and flavonoids. Particularly *Cassia mimosoides* extract presented the best phenolic, tannin and flavonoid contents. This result indicated that phenolic from Ceasalpiniaceae, flavonoids from Combretaceae and tannin from Verbenaceae contribute significantly to the inactivation of CES, AchE and GST, respectively. However, no significant correlation was found between polyphenolic compounds content and XO inhibitory activity. Present findings could partially justify the traditional uses of these plants in the treatment of mental disorders, gout, painful inflammations and cardiovascular diseases.

Key words: Medicinal plant, phenolic content, tannin content, flavonoid content, enzymes inhibition

INTRODUCTION

Glutathione-s-transferase (GST), Acetylcholinesterase (AChE) and Carboxylesterase (CES) are known for their participation on the development of Alzheimer's disease (AD), gout, cancer and cardiovascular diseases (Djeridane *et al*., 2008; Hayeshi *et al*., 2007). These diseases are becoming now-a-days a threat to public health (Orhan *et al*., 2004). Some previous investigations showed that the inhibition of these enzymes was a good way for biological molecules research against those diseases (Orhan *et al*., 2004; Trumbeckaite *et al*., 2006; Wu and Ng, 2008; Havlik *et al*., 2010). Furthermore, it is well demonstrated that medicinal plants were a promising way for obtaining biological molecules (Djeridane *et al*., 2006). In Burkina Faso, some ethnobotanical investigations showed the popular use of medicinal plants in the treatment of cardiovascular diseases, gout and cancer (Nacoulma, 1996). Table 1 shows the traditional uses of 36 well-known plants from Asteraceae, Caesalpiniaceae, Combretaceae, Fabaceae, Lamiaceae and Verbenaceae families.

Previous biological investigations showed that *E. prostrata* and *L. camara* extracts possess AChE inhibitory activities (Vinutha *et al*., 2007). Nevertheless, the phytochemical studies demonstrated that flavonoids, tannins, polyphenolic were founds in *T. indica*, *Indigofera* species, *E. prostrata* and *O. americanum* extracts (Paarakh, 2010; Lamien-Meda *et al*., 2008; Bakasso *et al*., 2008; Gopiesh and Kannabiran, 2007; Vieira *et al*., 2003). Other previous results have shown the secondary metabolite such as flavonoids and tannins

Corresponding Author: Mindiédiba Jean Bangou, Laboratoire de Biochimie et de Chimie Appliquées (LA.BIO.C.A), U.F.R/S.V.T, Université de Ouagadougou, 09 BP 848 Ouagadougou 09, Burkina Faso

Table 1: Traditional use, part used and herbarium numbers of plants

Family/species	Traditional use	Parts used	Herbarium numbers
Asteraceae			
Acanthospermum hispidum DC	Stem, stem leaves: nevralgy, stomach aches, colic, headaches, rheumatism	WP	BK-ah2796
Ageratum conyzoïdes L.	Leaves: anti-inflammatory, ulcer, snakes bites and headaches	WP	BK-ac2800
Chrysanthellum americanum L.		L	BK-cra2782
Bidens engleri O. E. Shulz	Stem leaves: antibiotic, fever, snakes bites, colic, skin inflammation	WP	BK-be2802
Dicoma tomentosa Cass	Stem leaves: anti-inflammatory, rheumatism	L	BK-dt2819
Eclipta prostrata L.		L	BK-ep2801
Tridax procumbens L.	Whole plant: icterus, arterial hypertension and hepatitis	L	BK-tp2789
Vernonia colorata (Willd) Drake	Leaves and flower: fever, diarrhoea, jaundice, antibiotic and anti-biotic	L	BK-vc2798
Caesalpinaceae			
Cassia absus L.		SL	BK-cab2809
Cassia alata L.	Leaves: mental disorder, headache, anti-inflammatory, rheumatism, arterial hypertension,	SL	BK-cal2807
Cassia mimosoides L.	Stem leaves: anti-inflammatory, stimulant	WP	BK-cam2812
Cassia nigricans Vahl	Stem leaves: uterus tumors	WP	BK-cn2780
Cassia obtusifolia L.	Stem leaves: headache, junction ache, hepatitis	SL	BK-co2792
Cassia occidentalis L.	Leaves: anti-rheumatism, diarrhoea, headaches,	SL	BK-coc2799
Cassia singueana Del	Skin ulcers, stomach aches,	SL	BK-case2808
Cassia italica (Mill)	Stem leaves, root: skin ulcers, teeth aches, venereal disease	SL	BK-it2813
Tamarindus indica L.	Bark: cardiac weakness, diabetes, hypotension	SL	BK-ti2804
Combretaceae			
Combretum aculeatum Vent	Stem leaves, root: arterial hypertension, fever, teeth aches	SL	BK-ca2779
Combretum adenogonium Stend	Leaves, stem: arterial hypertension, hepatitis, anti-inflammatory, urinary disease	L	BK-cad2820
Combretum crotonoides Hutch	Stem leaves, mistletoe: hepatitis, rheumatism	SL	BK-ccr2821
Combretum micranthum G. Don	Leaves, mistletoe: hepatitis, fever, mental disorder, antibiotic	SL	BK-cm2778
Combretum paniculatum Vent	Flower, root: anti-inflammatory, antitumoral	SL	BK-cp2785
Fabaceae			
Indigofera colutea (Burm) Merr	Whole plant: stomach aches	SL	BK-ico2814
Indigofera macrocalyx L.	Whole plant, stem leaves: tireness, stimulant	SL	BK-im2788
Indigofera nigritana Hook F.	Stem leaves: stomach aches, malaria, stimulant	SL	BK-in2815
Indigofera pulchra L.		SL	BK-ip2816
Indigofera tinctoria Var. A. Benth	Leaves: nervous system diseases, fever, sexual transmitted diseases, colic	SL	BK-it2790
Lamiaceae			
Hyptis spicigera Lam	Stem leaves/flower: fever, headaches, uterus diseases, cephalic	SL	BK-2811
Hyptis suaveoleus (L.) Poit	Stem leaves: insecticidal, icterus, antispasmodic and stimulant	WP	BK-hs2781
Leucas martinicensis Jacq R. Br.	Leaves: fever, headaches and stimulant; Flower: gout	SL	BK-lm2803
Ocimum americanum L.	Leaves: spleen inflammation, stomach tumor, malaria, stimulant	SL	BK-oca2783
Ocimum basilicum L.	leaves: fever, mental disorder and stimulant	SL	BK-oba2817
Verbenaceae			
Lantana camara L.	Stem-leaves, anti-rheumatic, and antidiabetic	SL	BK-lc2793
Lantana rhodesiensis Moldenke	Stem leaves: arterial hypertension, stimulant	SL	BK-lar2810
Lippia chevalieri Moldenke	Stem leaves/ stem leaves flower: liver pathologies (hepatitis, inflammation, jaundice)	WP	BK-la2775
Lippia multiflora Moldenke	Stem leaves/ stem leaves flower: liver pathologies, anti-inflammatory	WP	BK-lmu2818

L: Leaves, SL: Stem leaves, WP: Whole plant

presented some interesting enzyme inhibition activities (Senol *et al*., 2010). Flavonoids for example are known to inhibit a number of enzymes such as xanthine oxidase, acetylcholinesterase, glutathione-s-transferase and carboxylesterase (Bonesi *et al*., 2010; Iswantini *et al*., 2009; Senol *et al*., 2010; Soeksmanto *et al*., 2010). But, according to present knowledge, there is little scientific information about the enzyme inhibition properties of most of these plants. In the present study, 36 medicinal plant extracts, according to their traditional utilizations, were used to assess: (1) their AChE, GST, CES and XO inhibitory capacities and (2) their phenolic, tannin and flavonoid content.

MATERIALS AND METHODS

Chemicals: Reagents come from Sigma Aldrich Chemie GmbH, Germany: L-Glutathione reduced (GSH), glutathione-S-transferase (GST) from rate liver, 1-chloro-2,4-dinitrobenzene (CDNB), Albumin from bovine serum (BSA), potassium phosphate monobasic (KH_2PO_4) and dibasic (K_2HPO_4). Acethylcholinesterase (AChE) from electric eel, acethylcholine iodide (ATCI), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), tannic acid, gallic acid, quercetin were provided from Sigma-Germany. HCl and sodium carbonate were from Labosi-France. Folin-Ciocalteu reagent was from Sigma-USA. Carboxylesterase from pig liver, Xanthine oxidase, DMSO and Tween were purchased from Sigma-Aldrich Chemie GmbH (Germany). Aluminum trichloride ($AlCl_3$), Na_2HPO_4 and NaH_2PO_4 were purchased from Sigma-Aldrich Chemie (Steinheim, Germany).

Plant materials: Plant materials constituted of 36 medicinal plants from interior of Burkina Faso were collected at Ouagadougou in July 2006. The

plants were botanically identified by Professor Millogo-Rasolodimby from Ecology Laboratory of the University of Ouagadougou. Voucher specimens (Table 1) were deposited in the herbarium of the Laboratory of Biology and Vegetal Ecology, UFR/SVT of the University of Ouagadougou.

Preparation of plant extracts: Tissue samples (leaves, stems-leaves and whole plant) of each plant were dried at room temperature and ground to fine powder; using a grinder. The extraction was processed using ten gram of each sample in 3×100 mL by technical methanol steeping during one night. The extracts were filtered and evaporated until they dry.

BIOLOGICAL ACTIVITY

Acetylcholinesterase activity: The AChE inhibition was conducted according to the protocol described by Lopez *et al.* (2002) with some modifications. Briefly described, the assay mixture consisted of 200 µL of Tris-HCl (50 mM pH8), 0.1% BSA buffer, 100 µL of extracts solution (final concentration: 100 µg mL^{-1}) and 100 µL of AChE (0.22 U mL^{-1}). The mixture was incubated at room temperature for 2 min before adding 500 µL of DTNB (3 mM) and 100 µL of substrate (ATCI 15 mM). The developing yellow color was measured at 405 nm after 4 min (Cecil CE 2041, England). Galanthamine was used as a positive control at a final concentration of 100 µg mL^{-1} in the assay mixture. AChE inhibitory activity was expressed as percent inhibition of AChE, calculated as:

$$\text{Inhibition percentage of AChE} = ((A - B)\times 100)/A$$

where, A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract.

Inhibition of glutathione-S-transferase: GST inhibitory assays were conducted as Habdous *et al.* (2002).

Assay of xanthine oxidase activity: The XO inhibitory activities were measured spectrophotometrically by using Filha *et al.* (2006) procedure with some modifications. The extracts were directly dissolved in phosphate buffer-MeOH (1%) and screened for XO inhibitory activity at final concentration of 100 µg mL^{-1}. The assay mixture consisted of 100 µL of extracts, 300 µL of phosphate buffer (0.2 M pH9) and 100 µL enzyme solution (0.28 U mL^{-1} in phosphate buffer). The mixture was incubated at room temperature for 2 min. Then, the reaction was initiated by adding 500 µL of xanthine solution (0.15 mM in phosphate buffer) and the change in absorbance was recorded at 295 nm for 2 min at room temperature. Allopurinol was used as a positive control at a final concentration of 100 µg mL^{-1}. The results were expressed as percent inhibition of xanthine oxidase, calculated as:

$$\text{Inhibition percentage of XO} = ((A - B)\times 100)/A$$

where, A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract.

Assay of carboxylesterase activity: The method of Djeridane *et al.* (2008) was used with some modifications. Test solution contained 400 µL of Tris-HCl (50 mM pH8) buffer, 100 µL of plant extract at final concentration of 100 µg mL^{-1}. One hundred of enzyme solution (0.027 U mL^{-1}) and 400 µL of 4-nitrophenyl (1 mM) was added after incubation at 3 min. The absorbance was read at 414 nm. Ascorbic acid (50 µg mL^{-1}) was used as reference. The results were expressed as percent inhibition of CES, calculated as:

$$\text{Inhibition percentage of CES} = \frac{A - B}{A}\times 100$$

where, A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract.

Determination of polyphenolics compounds

- **Determination of total phenolic content:** The total phenolics of plant extract were determined by the Folin-Ciocalteu method (Lamien-Meda *et al.*, 2008)
- **Determination of tannins content:** Tannins content was determined according to the European Commission (2000)
- **Determination of flavonoids contents:** The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al.* (2008)

Statistical analysis: The data are expressed as the Means±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

Biological investigations

AChE inhibitory activity: The highest inhibition activities were obtained with *L. chevalieri, C. mimosoides, C. nigricans* and *C. singueana* extracts. The lowest inhibitory effects were obtained with *C. absus*,

Table 2: Acetylcholinesterase (AChE), glutathione-S-transferase (GST), carboxylesterase (CES) and xanthine oxidase (XO) Percentage Inhibition Activities

Species	AChE	GST	CES	XO
Asteraceae				
A. conyzoides	24.71±1.44^{bc}	4.96±3.04^{mno}	21 97±1.93^{ij}	27.69±5.75^{gh}
A. hispidum	12.55±3.85^{fgh}	19.34±5.07^{fgh}	16.29±3.75^{klm}	20.00±2.51^{jkl}
B. engleri	4.70±1.04^{ij}	17.68±5.42^{hi}	15.89±1.45^{klmn}	ND
C. americanum	22.00±2.80^{cd}	15.76±3.84^{hij}	25.97±0.54^{fgh}	30.82±0.88^{gh}
D. tomentosa	8.04±2.64^{hij}	34.73±3.00^{bc}	19.77±0.94^{ijk}	67.93±3.08^{e}
E. prostrata	9.22±2.21^{hi}	61.89±5.71^{a}	27.27±0.92^{fgh}	26.72±3.23^{hij}
T. procumbens	19.22±1.26^{cde}	10.92±2.74^{jkl}	9.09±1.60^{opq}	7.88±1.71^{mno}
V. colorata	4.12±2.09^{ij}	38.46±1.49^{abc}	15.91±2.78^{klm}	8.65±0.95^{mno}
Ceasalpiniaceae				
C. absns	1.76±2.99^{k}	22.46±1.52^{def}	11.83±2.01^{mno}	72.11±2.54^{bc}
C. alata	17.59±5.40^{fg}	15.39±3,23^{hij}	24.73±4.02^{gh}	35.50±0.61^{fg}
C. italica	27.21±6.54^{bcd}	3.33±0.80^{no}	18.99±1.97^{ijk}	56.60±1.54^{d}
C. mimosoides	39.12±1.42^{b}	20.04±3.30^{fg}	49.76±2.98^{ab}	41.56±4.24^{e}
C. nigricans	37.42±4.33^{b}	27.60±2.12^{cd}	15.05±2.74^{lmn}	18.18±1.83^{kl}
C. obtusifolia	14.90±4.7^{fg}	7.46±1,23^{lm}	9.14±0.76^{opq}	16.02±3.06^{lm}
C. occidentalis	ND	20.98±2.64^{fg}	9.18±1.81^{opq}	25.97±1.06^{ij}
C. singueana	35.30±2.99^{bc}	8.86±1.61^{lm}	48.93±2.01^{ab}	34.63±2.20^{fgh}
T. indica	14.51±1.43^{fg}	0.66±0.08^{o}	40.58±1.18^{cd}	7.33±3.30^{mno}
Combretaceae				
C. paniculatum	3.33±1.00^{ijk}	26.11±6.25^{cde}	2.27±0.92^{rs}	13.08±2.60^{lm}
C. aculeatum	12.47±2.52^{fgh}	23.94±0.75^{def}	2.27±1.60^{m}	3.82±1.64^{o}
C. adenogonium	10.39±0.27^{fgh}	28.16±1.87^{cd}	6.06±2.14^{qr}	3.56±1.29^{o}
C. crotonoides	22.45±3.45^{cd}	16.21±1.14^{hij}	47.35±1.93^{bc}	64.82±4.53^{cd}
C. micranthum	19.22±4.66^{cde}	4.92±1.97^{mno}	ND	6.33±1.79^{no}
Fabaceae				
I. colutea	10.78±4.73^{gh}	5.40±2.60^{mn}	10.45±1.21^{nop}	37.95±1.91^{ef}
I. macrocalyx	20.39±1.46^{cde}	4.88±3.10^{mno}	21.39±3.06^{ij}	72.82±1.45^{bc}
I. nigritana	6.47±3.36^{ijk}	11.86±6.69^{ijk}	11.94±2.43^{mno}	36.41±3.83^{efg}
I. pulchra	17.06±5.41^{ef}	21.01±1.13^{ef}	10.49±2.43^{nop}	77.44±0.72^{b}
I. tinctoria	20.78±12.37^{cd}	5.28±3.10^{lmn}	8.46±2.81^{pq}	31.80±0.72^{gh}
Lamiaceae				
H. spicigera	7.25±1.00^{hij}	27.41±0.96^{cd}	0.48±1.81^{s}	ND
H. suaveoleus	21.32±4.17^{cd}	33.31±3.72^{bc}	11.11±1.81^{no}	3.58±1.01^{o}
L. martinicensis	12.70±6.63^{fgh}	11.78±3.88^{ijk}	9.66±0.68^{opq}	ND
O. americanum	9.62±2.32^{h}	24.15±3.21^{de}	ND	ND
O. basilicum	5.88±6.30^{ij}	22.46±5.11^{def}	17.83±0.55^{klm}	25.85±0.96^{ij}
Verbenaceae				
L. camara	18.59±5.25^{def}	21.83±1.62^{ef}	56.20±3.05^{a}	20.76±2.67^{jkl}
L. chevalieri	39.26±2.18^{b}	42.99±3.03^{ab}	37.68±1.18^{de}	14.00±2.16^{lm}
L. multiflora	17.59±2.29^{ef}	21.03±0.36^{ef}	33.82±0.68^{def}	14.67±0.47^{lm}
L. rhodesiensis	17.65±6.35^{ef}	39.76±2.41^{ab}	32.37±1.81^{efg}	ND
Reference compound				
Galanthamine	50.76±0.68^{a}			
Ascorbic acid			56.72±0.85^{a}	
Allopurinol				96.38±0.59^{a}

ND: not determined; Result within each column with different letters (a-s) differs significantly ($p< 0.05$).

C. paniculatum, V. colorata and *B. engleri* extracts. All extracts of AChE inhibitory activities were less than Galanthamine inhibitory effect. Previous studies showed *E. prostrata* and *L. camara* extracts AChE inhibitory activity (Vinutha *et al*., 2007). According to the cholinergic hypothesis memory impairment in patients suffering from Alzheimer's disease is a result of decreased levels of the neurotransmitter acethylcholine (ACh) in the cortex. In the healthy brain AChE is the most important enzyme regulating the ACh level (Ahmad *et al*., 2003; Adsersen *et al*., 2007). In this way, it would be relevant to search for substance capable of inhibiting AChE activity in Alzheimer's disease patients to increase their ACh levels such as Galanthamine that is used in this treatment.

Table 2 showed the 36 extracts AChE inhibitory activities that were compared with Galanthamine activity. Present result could probably justify the plant traditional uses in cancer treatment. Among the five species, which inhibited AChE at more than 25%, four are included in Caesalpiniaceae family. In this way, Caesalpiniaceae is most indicated to search acetylcholinesterase inhibitors.

GST inhibitory activity: Table 2 showed 36 extracts GST inhibitory activities. The best activities were obtained with *E. prostrata, L. chevalieri, L. rhodesiensis* and *V. colorata* extracts. The lowest inhibition were obtained with *T. indica, C. italica* and *C. macrocalyx* extracts. Previous investigations showed that GST was implicated

Table 3: Result of polyphenolic quantification

Species	TP (mgGAE/100mg)	TF (mgQE/100mg)	TN (mgTAE/100mg)
Asteraceae			
A. conyzoides	21.10±1.17^{fgh}	6.50±0.27^{k}	2.90±0.22^{no}
A. hispidum	12.18±0.37^{op}	2.78±0.15^{no}	2.27±0.22^{op}
B. engleri	15.83±0.60^{kl}	6.55±0.07^{k}	9.08±0.06^{g}
C. americanum	10.15±0.37^{pq}	6.31±0.10^{k}	4.38±0.14^{klm}
D. tomentosa	12.08±0.82^{op}	9.14±0.10^{gh}	5.57±0.16^{ijk}
E prostrata	27.90±0.70^{de}	18.93±0.13^{b}	14.45±0.18^{d}
T. procumbens	17.40±0.85^{ijk}	0.29±0.01^{p}	1.27±0.10^{pq}
V. colorata	13.62±0.43^{no}	1.81±0.32^{o}	5.15±0.31^{kl}
Ceasalpiniaceae			
C. absns	17.23±0.19^{ijk}	15.88±0.53^{c}	4.47±0.17^{klm}
C. alata	14.68±0.93^{mno}	11.23±0.25^{e}	6.37±0.51^{j}
C. italica	15.48±0.67^{lmn}	9.37±0.09^{gh}	4.68±0.29^{klm}
C. mimosoides	51.3±0.49^{a}	30.43±0.28^{a}	33.60±0.57^{a}
C. nigricans	23.82±0.98^{fgh}	9.03±0.08^{ghi}	20.73±0.53^{b}
C. obtusifolia	14.52±1.41^{mno}	10.66±0.04^{f}	3.78±0.09^{mn}
C. occidentalis	13.58±1.02^{no}	11.29±0.15^{e}	1.25±0.07^{pq}
C. singueana	43.68±1.30^{b}	11.15±0.37^{f}	15.67±0.93^{c}
T. indica	36.33±1.47^{c}	3.41±0.05^{n}	17.30±0.41^{b}
Combretaceae			
C. aculeatum	11.68±0.08^{op}	5.75±0.56^{kl}	4.73±0.06^{klm}
C. adenogonium	25.08±2.79^{def}	6.62±0.06^{k}	12.30±0.21^{e}
C. micranthum	45.50±0.30^{b}	5.09±0.13^{l}	2.08±0.02^{op}
C. crotonoides	37.28±0.23^{c}	4.64±0.03^{lm}	2.29±0.06^{op}
C. paniculatum	20.67±2.4^{gh}	8.74±0.15^{hi}	8.88±0.38^{gh}
Fabaceae			
I. colutea	19.27±0.56^{ijk}	3.66±0.16^{mn}	11.95±0.12^{ef}
I. macrocalyx	30.05±2.76^{d}	13.39±0.09^{d}	5.17±0.13^{kl}
I. nigritana	29.70±2.94^{d}	11.53±0.24^{e}	12.12±0.02^{e}
I. pulchra	19.17±0.5^{ijk}	18.88±0.82^{b}	4.73±0.18^{klm}
I. tinctoria	21.63±3.02^{fgh}	1.76±0.09^{o}	4.18±0.05^{kl}
Lamiaceae			
H. spicigera	14.98±0.12^{mno}	7.91±0.22^{ij}	9.13±0.37^{g}
H. suaveoleus	12.17±0.90^{op}	11.15±0.28^{f}	4.48±0.27^{klm}
L. martinicensis	12.68±0.26^{nop}	2.72±0.25^{no}	2.72±0.15^{no}
O. americanum	12.72±0.94^{nop}	10.61±0.34^{f}	5.55±0.12^{ijk}
O. basilicum	17.15±0.79^{ijk}	7.77±0.68^{j}	7.73±0.33^{hi}
Verbenaceae			
L. camara	10.03±0.75^{pq}	8.28±0.77^{hi}	0.48±0.09^{q}
L. chevalieri	17.88±0.90^{ijk}	3.19±0.15^{n}	7.62±0.37^{i}
L. multiflora	20.20±0.75^{ghi}	10.90±0.11^{f}	10.9±0.09^{f}
L. rhodesiensis	21.55±0.75^{fgh}	5.09±0.19^{l}	14.52±0.27^{cd}

TP: Total phenolic content TF: Total flavonoid content TN: Tannins content. Result within each column with different letters differs significantly ($p<0.05$)

Table 4: Correlative study between polyphenolics componnds and the enzymes inhibition

	Phenolic			Tannin			Flavonoid		
Enzymes	R^2	p-value	Families	R^2	p-value	Families	R^2	p-value	Families
AchE				0.89	0.015	Fabaceae	0.79	0.041	Lamiaceae
				0.61	0.037	Caesalpiniaceae	0.92	0.008	Combretaceae
CES	0.99	0.0008	Verbenaceae	0.91	0.044	Verbenaceae	0.60	0.022	Asteraceae
	0.87	0.0009	Caesalpiniaceae	0.54	0.036	Caesalpiniaceae			
GST				0.62	0.02	Asteraceae	0.98	0.006	Verbenaceae

in tumor cell resistance to antitumoral drug treatment (Hayeshi *et al*., 2007). So, the inhibition of GST activity become a promising way to develop antitumoral drugs, particularly, drugs from medicinal plant. Present results demonstrated that these extracts contained some compounds with GST inhibitory activities singularly in *E. prostrata* extract. These observations could be partially supported by the plant traditional use in cancer treatment indicated in Nacoulma (1996) ethnobotanical investigations. Between the six families which were studied Fabaceae species have not presented any inhibition for the enzymes.

CES inhibitory activity: The CES inhibitory effect of extracts were shown in Table 2. The best inhibition activity was found with *C. mimosoides, C. crotonoides*, *C. singueana* and *L. camara* extracts. Interestingly, ascorbic acid and *L. camara* extract have shown similar

CES inhibition activities. Verbenaceae was the most CES inhibitor among the families. CES are enzymes omnipresent (high levels in a large array of animal tissues) responsible of the detoxication to numerous endogen and xenobiotic. Now-a-days, their biological role are not clearly delimited. For example CES also hydrolyse aspirin and some anti-cancerous such as chemotherapeutic agents (Djeridane *et al*., 2008). In this way, their inhibition can contribute to strengthen these drug effects (Crow *et al*., 2008; Rodinbo *et al*., 2003). Present results indicated that different extracts contain some molecules which were able to inhibit this enzyme, particularly in *L. camara* extract, while species from Lamiaceae and Fabaceae seem to be poor in CES compound inhibitor. Plant extract CES inhibitory properties evaluated in the first time could partially justify the traditional uses found in Burkina Faso (Nacoulma, 1996). In the CES inhibition, some extracts of plants show that they possess interesting activities as compared to ascorbic acid (56.72±0.85). *L. camara* (Verbenaceae) which has the best inhibition activity, inhibited CES at 56.20%.

XO inhibitory activities: The extracts XO inhibitory activities compared to allopurinol effect were shown in Table 2. Sixteen extracts inhibited XO at a level higher than 25%. The highest inhibitions were obtained with *C. absus*, *I. macrocalyx* and *I. pulchra* extracts (72.11±2.55, 72.82±1.45 and 77.44±0.73%, respectively) and the lowest are obtained with *C. adenogonium*, *H. suaveolens, C. aculeatum*. The Indigofera family presented a greater XO inhibitory effect than other families. But, all extracts have not reached the reference compound in XO inhibitory activity, i.e., allopurinol with 96.38±0.58%. The role of xanthine oxidase is to catalyze the oxidation of hypoxanthine to xanthine and generates uric acid, hydrogen peroxide and superoxide anion (Wu and Ng, 2008). Clinical reports have shown that uric acid is the key factor of risk of gout and cardiovascular disorders, nephrolithiasis and diabetes (Havlik *et al*., 2010; Iswantini *et al*., 2009; Gagliardi *et al*., 2009). Thus, xanthine oxidase inhibition is useful in the prevention and/or treatment of hepatic diseases and gout, and also for the reduction of harmful hydrogen peroxide and superoxide anion productions. Present results suggest that respective extracts contained XO inhibitors compounds. These observations could justify the traditional use of particularly *I. pulchra* (cellulites), *C. crotonoides* and *D. tomentosa* (rheumatism). No species of Verbenaceae family inhibited XO but all species of Fabaceae family inhibited at more than 30%.

Phytochemical analysis: The total tannin and phenolic content of the extracts were determined spectrophotometrically (Table 3), because polyphenols present in vegetable and fruit are responsible for many biological activities (Arct and Pytkowska, 2008; Soobrattee *et al*., 2005). Total phenolics of extracts were ranging from 51.3±0.49 to 10.03 ± 0.75 mg GAE/100 mg. The highest values of total phenolics were in the following order: *C. mimosoides*> *C. micranthum* = *C. singueana*. The lowest ones were found in *L. camara* and *C. americanum* with 10.03±0.75 to 10.15±0.37 mg GAE/100 mg extract plant, respectively. Among the 36 extracts of plants analyzed, *C. mimosoides* (33.60±0.57 mg TAE/100 mg extract) and *C. nigricans* (20.73±0.53 mg TAE/100 mg extract) showed the highest tannin contents. The lowest values were found in *L. camara* and *C. occidentalis* extracts. Previously, the polyphenolic contents were evaluated in Fabaceae family extracts (Bakasso *et al*., 2008), *T. indica* extracts (Lamien-Meda *et al*., 2008) and the tannin content in *E. prostrata* extract (Gopiesh and Kannabiran, 2007).

Many flavonoids possess anti-tumor activity against various human cancer cell lines and xenograft systems of human tumors, suggesting that they may be promising anticancer agents (Zeng *et al*., 2009; Paarakh, 2010; Soeksmanto *et al*., 2010). In this way, flavonoids contents were estimate in 36 plant extracts (Table 3). Total flavonoid contents varied from 30.43±0.28 to 0.29±0.01 mg QE/100 mg plant extract. The best flavonoids contents were obtained with *I. pulchra*, *E. prostrata, C. mimosoides* extracts and the lowest contents with *A. hispidum, L. martinicensis, V. colorata, T. procumbens* and extracts. In previous phytochemical investigations, Bakasso *et al*. (2008), Lamien-Meda *et al*. (2008), Gopiesh and Kannabiran (2007) and Vieira *et al*. (2003) have found the flavonoids in *Indigofera* species, *T. indica*, *O. americanum* and *E. prostrata* extracts, respectively.

Relationship between enzyme activities, total phenolic, tannin and flavonoids contents: In the present study we were interested by the evaluation of the phenolic, flavonoid and tannin of extracts capacities to inhibit the different enzymes (Table 4). Tannin from Fabaceae, Caesalpiniaceae, Verbenaceae and Asteraceae families extracts contributed significantly to inhibit AChE, CES and GST, respectively. The same observation was notified concerning the flavonoids from Lamiaceae, Combretaceae, Asteraceae and Verbenaceae on these enzyme inhibition activities. Only the phenolic from Caesalpiniaceae and Verbenaceae families extracts presented a significant correlation to CES inhibitory activities. Nevertheless any correlation was found concerning the XO activity and the metabolites from different family extracts, although this enzyme was inhibited, interestingly, by *indogofera* species (Fabaceae) extracts. These results indicated the enzyme inhibition did not only depend on the metabolite

quantities. Consequently, *L. camara* extract presented little phenolic as tannin contents (10.03 mg GAE/100 mg and 0.48 mgTA/100mg) with interesting AChE, GST and CES inhibitory activities. Also *C. absus* extract was a good XO inhibitor with little phenolic and flavonoids contents.

These are several reasons to explain the ambiguous relationship between the inhibitory potency and the phenolic, tannins and flavonoids. The total phenolics content did not include all the possible inhibitors; the synergism among the inhibitors in the mixture accounted for the inhibition but was not only dependent of the concentration of individual inhibitors but also on the structure and interaction among them. Previous studies showed the structure relationship of flavonoids in CES (Stocker *et al*., 2004), GST (Van Zanden *et al*., 2004), AChE (Ji and Zhang, 2006) and XO (Nagao *et al*., 1999) inhibitory activities. On the other hand, the method used to quantify the flavonoids was limited to flavone and flavonol (Meda *et al*., 2004). A detailed examination of phenolic of different plant extracts is necessary for a comprehensive assessment of the individual compounds enzyme inhibitory ability.

CONCLUSION

This study has showed that the methanolic extracts of 28 on 36 species are potential inhibitors of AChE, GST, CES and XO which could support the traditional uses of these plants in the treatment of mental disorders, gout, painful inflammations and cardiovascular diseases. Fabaceae family is particularly important as a source of natural XO inhibitors. Considering the correlation analysis, Combretaceae and Caesalpinaceae could also be relevant sources of inhibitors. Some plant extracts were effective inhibitors for three of the four enzymes at 25% such as *E. prostrata, L. chevalieri, C. nigricans* and *C. mimosoides*. Those activities seem to be partially correlated to the flavonoid and tannin contents. Those plant species were indicated for new molecules to relieve the diseases, which involved these enzymes. Future studies aim to isolate and identify these active constituents that exhibit significant AChE, GST, CES and XO inhibitory activity through bioassay-guided fractionation.

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