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Evaluation of Enzymes Inhibition Activities of Medicinal Plant from Burkina Faso

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Abstract: The aim of the present study was to evaluate some enzymes inhibitory effects of 11 plant species belonging to 9 families from Burkina Faso. Methanolic extracts were used for their Glutathione-s-transferase (GST), Acetylcholinesterase (AChE), Carboxylesterase (CES) and Xanthine Oxidase (XO) inhibitory activities at final concentration of 100 µg mL^{-1}. The total phenolics, flavonoids and tannins were also determined spectrophotometrically using Folin-Ciocalteu, $AlCl_3$ and ammonium citrate iron reagents, respectively. Among the 11 species tested, the best inhibitory percentages were found with *Euphorbia hirta*, *Sclerocarya birrea* and *Scoparia dulcis* (inhibition>40%) followed by *Annona senegalensis*, *Annona squamosa*, *Polygala arenaria* and *Ceratotheca sesamoides* (inhibition>25%). The best total phenolic and tannin contents were found with *S. birrea* with 56.10 mg GAE/100 mg extract and 47.75 mg TAE/100 mg extract, respectively. *E hirta* presented the higher total flavonoids (9.96 mg QE/100 mg extract). It's was found that *Sclerocarya birrea* has inhibited all enzymes at more than 30% and this activity is correlated to total tannins contents. Contrary to *S. birrea*, the enzymatic activities of *E. hirta* and *S. dulcis* are correlated to total flavonoids contents. Present findings suggest that the methanolic extracts of those plant species are potential inhibitors of GST, AChE, CES and XO and confirm their traditional uses in the treatment of mental disorders, gout, painful inflammations and cardiovascular diseases.

Key words: Polyphenolic compounds, glutathione-s-transferase, acetylcholinesterase, carboxylesterase, xanthine oxidase

INTRODUCTION

The renewed interest in natural healing methods and the use of natural product treatments has led to a steadily growing interest in medicinal plants and the classical methods of plant extract preparations (Sweeney *et al*., 2001). Medicinal plants are well-known natural sources for the treatment of various diseases since antiquity. In Burkina Faso, medicinal plants are increasingly used by the tradipractitioners and herbalists for the treatment of various ailments i.e. nervous system diseases, nevralgy, rheumatism, arterial hypertension, mental disorder, antitumoral, gout, skin cancer and inflammation (Table 1). Some enzymes are known to be involved in these diseases. For example, Xanthine Oxidase (XO) which is responsible for gout, also serves as 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, atherosclerosis, cancer and aging (Wu and Ng, 2008; Havlik *et al*., 2010). Principal role of Acetylcholinesterase (AChE) is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of acetylcholine (Adsersen *et al*., 2007; Vinutha *et al*., 2007; Mukherjee *et al*., 2007; Bonesi *et al*., 2010). Carboxylesterases (CES) play a key biological role as they are able to hydrolyse numerous endogen and xenobiotic ester-containing substances and the over expression of Glutathione-S-Transferase (GST) in cancer results in resistance to chemotherapeutic agents. Thus, controlling the activity of these enzymes would be highly useful for the treatment of gout and Alzheimer's disease and for the management of the biological impact of ester compounds consumed by humans through different ways

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(Orhan *et al*., 2004; Sweeney *et al*., 2001; Mukherjee *et al*., 2007; Djeridane *et al*., 2008). Furthermore, the use of GST inhibitors as therapeutic agents has been proven to be useful in endeavours to modulate anticancer drug-resistance (Van-Zanden *et al*., 2003; Hayeshi *et al*., 2007).

In a search for new plant-derived biologically active extracts against those diseases, we have carried out the screening of medicinal plant from Burkina Faso for AChE, GST, XO and CES inhibitory activities *in vitro*. To obtain more accuracy data on these activities, the total phenolics, flavonoids and tannins contents were also estimated using spectrophotometric methods.

MATERIALS AND METHODS

Plant materials: Plant materials constituted of 11 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 La.B.E.V. (Laboratoire de Biologie et d'Ecologie Végétales) from the University of Ouagadougou.

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).

Preparation of plant extracts: The dried and powdered samples (10 g) of each plant were extracted with 3×100 mL of technical methanol by steeping over night. Each extract was filtered and concentrated to dryness in a rotary evaporator (BÜCHI Rotavapor R-200, Switzerland).

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 pH 8, 0.1% BSA buffer, 100 μL of extracts solution (final concentration: 100 $\mu g\ mL^{-1}$) 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 0.2 $\mu g\ mL^{-1}$ in the assay mixture. AChE inhibitory activity was expressed as:

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

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.

Table 1: Traditional uses of 11 medicinal plant species

Species	Traditional uses	Part used	Herbarium numbers
Scoparia dulcis L. (Scrophulariaceae)	Diabetes, hyperglycemia, fever, antispasmolytic (Nacoulma, 1996)	Stem-leaves	BK-sd2784
Euphorbia hirta L. (Euphorbiaceae)	Arterial hypertension, anti-inflammatory, anti-cancer, anti-leukemia (Nacoulma, 1996)	Stem-leaves	BK-eh2797
Sclerocarya birrea (A. Rich) Hochst (Anacardiaceae)	Anti-inflammatory, arterial hypertension, spleen and pancreas inflammation, (Nacoulma, 1996; Lamien-Meda *et al*., 2008)	Leaves	BK-sb2795
Calotropis procera Ait. F. (Asclepiadaceae)	Anti-inflammatory/anti-asthmatic, stimulant, rheumatism, headaches (Nacoulma, 1996)	Leaves, bark root	BK-calp2791
Ceratotheca sesamoides Endl. (Pedaliaceae)	Diabetes, spleen cancer, stimulant, anti-inflammatory (Nacoulma, 1996)	Stem-leaves	BK-cs2805
Annona senegalensis Pers (Annonaceae)	Anti-inflammatory, insecticidal, rheumatism, nevralgy (Nacoulma, 1996)	Leaves bark root	Bk-as2776
Annona squamosa L. (Annonaceae)	Fever, diarrhoea, spasmolytic (Nacoulma, 1996)	Leaves	BK-asq2086
Azadirachta indica A. Juss (Meliaceae)	Fever, insecticidal, anti-inflammatory (Nacoulma, 1996)	Leaves	BK-ai2794
Polygala arenaria Willd (Polygalaceae)	Anti-inflammatory, generalized pains (Nacoulma, 1996)	Stem-leaves	BK-pa2822
Securidaca longepednnculata Fresen (Polygalaceae)	Rheumatism, headache, antitumor (Nacoulma, 1996)	Root	BK-sl2777
Tribulus terristris L. (Zygophyllaceae)	Malaria, urinary infections, anti-inflammatory (Nacoulma, 1996)	Stem-leaves, seed	BK-tt2787

Inhibition of glutathione-S-transterase: GST inhibitory assay were conducted as described by Habdous *et al.* (2002). The reaction mixture was consisted of 200 μL of phosphate buffer 100 mM (pH 6) and respectively 100 μL of enzyme (1 U mL^{-1}) and 100 μL of extract (1 mg mL^{-1}). The reaction was initiated with 100 μL of GSH (5 mM) and 500 μL CDNB (1 mM). Enzyme preparation for each extract were assayed in triplicate. GST inhibitory activity was expressed as:

$$\text{Inhibition percentage of GST (\%)} = \frac{((A\text{-}B)\times 100)}{A}$$

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 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 was consisted of 100 μL of extracts, 300 μL of phosphate buffer 0.2 M (pH 9) 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 oxidase 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:

$$\text{Inhibition percentage of xanthine oxidase (\%)} = \frac{((A\text{-}B)\times 100)}{A}$$

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. Ascorbic acid (50 μg mL^{-1}) was used as reference. Test solution contained 400 μL of Tris-HCl 50 mM buffer (pH 8), 100 μL of plant extract at final concentration of 100 μg mL^{-1}, 100 μL of enzyme solution (0.027 U mL^{-1}) and 400 μL of 4-nitrophenyl (1 mM) was add after incubation at 3 min. The absorbance was readed at 414 nm. The results were expressed as:

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

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.

Determination of polyphenolics compounds

Determination of total phenolic content: The total phenolics of plant extract were determined by the Folin-Ciocalteu reagent method (Lamien-Meda *et al.*, 2008). The diluted solution of each extract (100 μg mL^{-1}) was mixed with Folin-Ciocalteu reagent method (0.2 N, 2.5 mL). This mixture was allowed to stand at room temperature for five min and then, 2 mL of sodium carbonate solution (75 g L^{-1}) was add. After 2 h incubation, the absorbancies were measured at 760 nm against water blank. A standard calibration curve was plotted using gallic acid (0-200 mg L^{-1}) ($Y = 0.0249x$; $R^2 = 0.9999$). The results were expressed in mg of gallic acid equivalents (mgGAE) per 100 mg extract.

Determination of tannins content: Tannins content was determined according to the European Commission (2000) method. One mililitter of plant extract (100 μg mL^{-1}) was mixed in mixer with: 5 mL of water, 1 mL of ammonium citrate iron (3, 5 g L^{-1}) old of 24 h and 1 mL of ammoniac (8 g L^{-1}). The absorbance was read at 525 nm after ten min against a blank consisting of: extract (1 mL), water (6 mL), ammoniac (1 mL). Tannic acid (25-350 mg L^{-1}) was used as reference to produce the standard curve ($Y = 0.0011x + 0.2236$; $R^2 = 0.9995$) and the results were expressed in mg tannic acid equivalents (mg TAE) per 100 mg extract.

Determination of flavonoids contents: The total flavonoids were estimated according to the Dowd method as adapted by Lamien-Meda *et al.* (2008). A diluted methanolic solution (2 mL) of each extract (100 μg mL^{-1}) was mixed with 2 mL of $AlCl_3$ (2%). The absorbance was read at 415 nm after 10 min against a blank consisting of 2 mL of methanol and 2 mL of extract (without $AlCl_3$). Quercetin (0-50 mg L^{-1}) was used as reference to produce the standard curve ($Y = 0.0148x$; $R^2 = 0.9997$) and the results were expressed in mg of quercetin equivalents (mgQE) per 100 mg extract.

Statistical analysis: The results are presented as 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 extracts bearing AChE inhibitory activities are shown in Table 2. The inhibitory percentages varied from 2.94 to 45.88%. The highest inhibition percentages were obtained with *S. dulcis* (45.88%) and *S. birrea* (41.12%) followed by *A. squamosa* (28.80%) and *P. arenaria* (25.17%). The lowest values were registered for *A. senegalensis* (2.94%) and *C. sesamoides* (4.71%). The results obtained with *S. dulcis* and *S. birrea* were moderate compared to Galanthamine (50.76±0.68%) at a final concentration 100 μg mL^{-1}. Recently, AChE inhibitory activity has been investigated on the dichloromethane and methanol fractions obtained from young stem of *S. birrea* (Moyo *et al*., 2010). The IC_{50} obtained was 150 μg mL^{-1} for dichloromethane fraction and 470 μg mL^{-1} for methanol fraction. Ones can speculate that the AChE inhibitors are more extractible by dichloromethane solvent.

GST inhibitory activity: Methanolic extract of each sample were assayed for GST inhibitory activity at a concentration of 100 μg mL^{-1} in the assay mixture, these results are shown in Table 2. Of the 11 plant samples used, it was evident that methanolic extract of *E. hirta* exhibited a greater inhibitory activity (47.87%) followed by *S. birrea* (44.34%) and *S. dulcis* (41.55%). The lowest inhibition were obtained for *P. arenaria* (3.46%) and *A. squamosa* (3.89%). Previous study have showed that overexpression of GST is associated with multidrug resistance of tumor cells (Hayeshi *et al*., 2007; Van-Zanden *et al*., 2004). The results of the present study suggest that *E. hirta*, *S. birrea* and *S. dulcis* might possess GST inhibitors potential to be used in the treatment of cancer diseases. This can justify their traditional use as indicated by Nacoulma (1996).

CES inhibitory activity: The CES inhibitory activity results were presented in Table 2. According to this table the inhibtion percentages were ranged between 1.93% and 34.06%. The best inhibition was found with *S. birrea* (34.06%) and *E. hirta* presented the lowest inhibition (1.93%). *A. senegalensis* and *C. sesamoides* presented a same inhibitory percentage (25.97%). Ascorbic acid was used as reference (56.72±0.85%) at a same concentration (100 μg mL^{-1}). CES are enzymes omnipresent (high levels in a large array of animal tissues) responsible to the detoxication to numerous endogen and xenobiotic. 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 suggest that extracts of *S. birrea* is good candidates to play that role under appropriate treatments.

XO inhibitory activities: Two extracts inhibited XO at a level higher than 25% (Table 2). The highest inhibition percentages were obtained with *E. hirta* and *S. Birrea* extract with 44.44 and 44.07%, respectively. The lowest values were obtained with *P. arenaria* (6.91%) and *S. longepedunculata* (5.93%). The results obtained in this study showed that the XO inhibitory activities of *E. hirta* and *S. birrea* are moderated, compared to allopurinol (96.38±0.59%) at the same concentration (100 μg mL^{-1}). Our results justify the traditional use of *S. Birrea* and *E. hirta* in the treatment of inflammatory diseases and gout.

Phytochemical analysis: Total phenolics of extracts ranged from 6.18 to 56.10 mg GAE/100 mg extract (Table 3). The highest value of total phenolic content was obtained with *S. birrea* (56.10 mg GAE/100 mg extract) followed by *C. sesamoides* (29.83 mg GAE/100 mg extract). The lowest contents of total phenolics were found in

Table 2: Enzymes inhibitory activities (%)

Species	AChE	GST	CES	XO
A. indica	15.65±0.55^{def}	6.32±0.79^{cd}	19.380±0.54^{cd}	ND
A. senegalensis	2.94±2.88^{f}	12.59±1.75^{bc}	25.970±3.83^{bc}	ND
A. squamosa	28.80±4.62^{bcd}	3.89±2.92^{cd}	8.530±1.98^{efg}	ND
C. procera	19.50±4.49^{def}	19.63±1.09^{b}	22.870±1.97^{c}	ND
C. sesamoides	4.71±3.46^{f}	19.09±3.96^{b}	25.970±0.54^{bc}	25.17±8.22^{c}
E. hirta	10.58±1.73^{def}	47.87±5.42^{a}	1.930±1.36^{g}	44.44±4.80^{b}
P. arenaria	25.17±13.51^{cd}	3.46±1.69^{cd}	12.020±1.09^{def}	6.91±0.88^{de}
S. birrea	41.12±2.90^{bc}	44.34±2.52^{a}	34.060±3.62^{b}	44.07±3.66^{b}
S. dulcis	45.88±2.92^{b}	41.55±0.87^{a}	17.390±2.05^{cde}	18.99± 0.00^{cd}
S. longepedunculata	6.95±2.36^{ef}	ND	7.730±4.48^{fg}	5.93±1.04^{e}
T. terristris	18.81±6.30d^{ef}	6.43±2.61 ^{cd}	5.800±3.55^{fg}	21.51±1.75^{c}
Galanthamine	50.76±068^{a}			
Ascorbic Acid			56.720±0.85^{a}	
Allopurinol				96.38±0.59^{a}

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

Table 3: Polyphenols contents

Species	TP	TAN	TF
A. indica	6.82 ± 0.47^{f}	0.60 ± 0.10^{h}	0.15 ± 0.02^{f}
A. senegalensis	17.77 ± 1.13^{d}	5.63 ± 0.52^{e}	9.19 ± 0.51^{ab}
A. squamosa	20.53 ± 0.76^{cd}	6.68 ± 0.25^{d}	5.86 ± 0.33^{d}
C. procera	10.83 ± 0.27^{e}	0.63 ± 0.09^{h}	7.88 ± 0.13^{c}
C. sesamoides	29.83 ± 0.73^{b}	18.70 ± 0.24^{b}	9.47 ± 0.46^{ab}
E. hirta	23.05 ± 1.67^{c}	15.13 ± 0.34^{c}	9.96 ± 0.62^{a}
P. arenaria	6.18 ± 0.78^{f}	2.33 ± 0.10^{fg}	3.81 ± 0.11^{e}
S. birrea	56.10 ± 1.16^{a}	47.75 ± 0.25^{a}	0.85 ± 0.15^{f}
S. dulcis	17.52 ± 1.96^{d}	2.73 ± 0.28^{f}	8.49 ± 0.34^{bc}
S. longepedunculata	12.10 ± 0.50^{e}	1.55 ± 0.10^{gh}	0.38 ± 0.01^{f}
T. terristris	11.15 ± 1.05^{e}	2.38 ± 0.13^{fg}	3.57 ± 0.11

[e]TP: Total phenolics (mg GAE/100 mg extract), TF: Total flavonoids (mg QE/100 mg extract), TAN: Tannins content (mg TAE/ 100 mg extract). Result within each column with different letters (a-h) differs significantly ($p<0.05$)

P. arenaria and *A. indica* with 6.18 and 6.82 mg GAE/100 mg extract, respectively. Previous phytochemical studies have been conducted on *S. birrea* and it was found that the methanolic extracts of young stem of this species contain total phenolics at a level of 14.15 mg GAE/g extract (Moyo *et al*., 2010). This result means that old stem barks contain more total phenolic than young stem bark. Among the 11 extracts of plants analyzed, *S. birrea* and *C. sesamoides* showed the highest tannin contents with 47.75 and 18.70 mg TAE/100 mg extract, respectively (Table 3). The lowest values were obtained with *A. indica* (0.60 mg TAE/100 mg extract) and *S. longepedunculata* (1.55 mg TAE/100 mg extract). Compared to total phenolic contents, our finding was that the tannins of *S. birrea* (85%), *E. hirta* (65%) and *C. sesamoides* (62%) are major compounds.

The total flavonoid contents varied from 0.15 to 30.43 mg QE/100mg extract (Table 3). *E. hirta*, *C. sesamoides* and *A. senegalensis* presented the high levels with 9.96, 9.47 and 9.15 mg QE/100 mg extract, respectively. The lowest total flavonoid contents were obtained with *A. indica* (0.15 mg QE/100 mg extract), *S. longepedunculata* (0.38 mg QE/100 mg extract) and *S. birrea* (0.85 mg QE/100 mg extract).

Plant secondary metabolites have also been reported to possess a range of pharmacological activities, including antimicrobial, anti-inflammatory, anti-diabetic and enzymes inhibitory effects. Ours founding was that *S. birrea* has inhibited all enzymes at more than 30% and this activity is correlated to total tannins contents. Contrary to *S. birrea*, the enzymatic activities of *E. hirta* and *S. dulcis* are correlated to total flavonoids contents. It's also demonstrated that hydroquinones and tannins are the inhibitors of XO and AChE (Owen and Johns, 1999; Wang *et al*., 2007; Pithayanukul *et al*., 2005). The inhibition of XO, GST, CES and AChE is correlated to the total flavonoid contents (Narayana *et al*., 2001; Van-Zanden *et al*., 2004; Senol *et al*., 2010; Meda *et al*., 2010). Hence, the presence of these phenolic compounds in methanolic extracts of these species would have contributed towards XO, GST, CES and AChE inhibition. *E. hirta*, *S. birrea* and *S. dulcis* could then contain bioactive substances useful in the treatment of gout or other xanthine oxidase induced diseases, Alzheimer's disease and cancer, justifying the popular use of these species in inflammatory and mental disorders in Burkina Faso folk medicine.

CONCLUSION

Methanolic extracts of 11 species were analyzed for their acetylcholinesterase, glutathione-S-transferase, carboxylesterase and xanthine oxidase inhibitory activities. The results obtained in this study showed that among these species, 7 are potential inhibitors of AChE, GST, CES and XO, which confirm the traditional uses of these plants in the treatment of mental disorders, gout, painful inflammations and cardiovascular diseases. *S. birrea* was effective inhibitors for all the four enzymes at 25%. The activities seem to be partially correlated to the flavonoid and tannin contents. *S. birrea* is indicated for the investigation of 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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