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Original Research Article

In-vitro study of antiradical activity, phospholipase A_2 and 15-lipoxygenase inhibitory activity of eight malian medicinal plants used by traditional healers to treat inflammatory diseases

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ABSTRACT

Pro-inflammatory enzymes play a key role in inflammatory processes and are potential targets of anti-inflammatory drugs. In this study, the objective was to evaluate the antiradical activity of the extracts and their ability to inhibit phospholipase A_2 and 15-lipoxygenase activity. The hydroethanolic extract of *T. macroptera* revealed the strongest reducing power with an IC_{50} of 7.29 ± 0.61 μg/mL. The three most active extracts on PLA_2 were hydroethanolic extracts of *C. tinctorium* (IC_{50} of 21.43 ± 0.66 μg/mL), *T. macroptera* (IC_{50} of 23.72 ± 0.71 μg/mL), *X. americana* (IC_{50} of 24.49 ± 0.39 μg/mL). The best inhibitory activity on 15-lipoxigenase was obtained with aqueous and hydroethanolic extracts of *X. americana* with IC_{50} of 18.35 ± 1.26; 18.47 ± 1.12 μg/mL respectively. The present study showed that extracts of leaves of *T. macroptera* and roots of *X. americana* have antioxidant activity and effectively inhibit $sPLA_2$, and 15-LOX pro-inflammatory enzymes *in vitro.*

1. Introduction

Inflammation is a non-specific adaptive response triggered by harmful stimuli, due to endogenous or exogenous tissue attack, the purpose of which is the recovery of tissue damage.[1,2] It contributes to all pathological processes including infectious and non-infectious diseases.[3] The mechanism of injury due to inflammation is partly attributed to the release of reactive oxygen species by activated neutrophils and macrophages.[4,5] Inflammation is mainly mediated by secretory phospholipase A_2 ($sPLA_2$), which plays a key role in releasing free arachidonic acid, which is the precursor of pro-inflammatory lipid mediator and ROS production. Cyclooxygenase-1/2 and lipoxygenase catalyze the synthesis of pro-inflammatory mediators such as prostaglandins, thromboxanes and leukotrienes. Moreover, the arachidonic acid cascade is considered as an important source of ROS such as O^{2-} and OH. The generation of free radicals, if it persists, leads to a more deleterious complication. ROS play an essential role in inflammation, which itself is a component and or manifestation of infectious and chronic diseases.[6,7] In Mali, ethnopharmacological surveys have identified that the species of *Ximenia americana* L., *Strychnos spinosa* Lam, *Cola cordifolia* (Cav.) R.Br., *Vitellaria paradoxa* C.F.Gaertn. *Saba senegalensis* (A. DG.) Pichon, *Cochlospermum tinctorium* Perrier ex

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A. Rich, *Terminalia macroptera* (Guill. & Perr.) and *Leptadenia hastata* Vatke are indicated in the traditional treatment of diseases with inflammatory component such as, infections, arthritis, pain, tuberculosis, hepatitis, leprosy.[8–10] The mentioned plants have high levels of total polyphenols, flavonoids, and hydrolyzable and condensed tannins.[11] They have already been the subject of several pharmacological studies, including antimycobacterial, hepatocurative, immunomodulatory, anti-inflammatory and antiradical properties.[9–12] Therefore, the evaluation of the pharmacological properties of these plants commonly used in Malian traditional medicine has attracted considerable interest due to their therapeutic effects. The study of the mechanisms by which plant extracts demonstrate their anti-inflammatory and antioxidant activities in biological systems is complex.[13] Models for the preliminary evaluation of anti-inflammatory and antioxidant activities use *in vitro* screening assays, inhibitors of secretory phospholipase A_2, 15-lipoxygenase for inhibitory activity of pro-inflammatory enzymes, and the free radical scavenging test for scavenging activity Chemical constituents that can inhibit inflammation may also serve as appropriate agents to inhibit lipid oxidation.[14,15] In this study, the objective was to evaluate the antiradical activity of the extracts and their ability to inhibit phospholipase A_2 and 15-lipoxygenase. xczc.

2. Material and Methods

2.1. Plant collection and authentification

The leaves of *Leptadenia hastata* Vatke and the roots of *Cochlospermum tinctorium* Perrier ex A. Rich., *Ximenia americana* L. and *Strychnos spinosa* Lam. were harvested in Kati. The leaves of *Cola cordifolia* (Cav.) R.Br., of *Saba senegalensis* (A.DG.) Pichon, of *Terminalia macroptera* Guill.& Perr. and *Vitellaria paradoxa* C.F.Gaertn. were harvested in Samé. The samples were authenticated at the Department of Traditional Medicine (DMT) of the National Institute for Public Health Research in Mali and a herbarium of each plant was deposited with reference numbers 1481; 0048; 0027; 0150; 3024; 3005; 2468; 2792 respectively.

2.2. Chemicals

All solvents and reagents used were of analytical grade. $sPLA_2$ (Type V) Inhibitor Screening Assay Kit Item No. 10004883 and Lipoxygenase Inhibitor Screening Assay Kit Item No. 760700 from Cayman Chemical Co. (MI, USA).

2.3. Preparation of aqueous and hydroethanolic extracts

After drying, the plant parts were pulverized. On each powder, aqueous decoction and 70% hydroethanolic maceration methods were used as previously described.[11]

2.4. ABTS (2, 2'-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid] Radical Scavenging

The capacity of extracts to scavenge the ABTS radical cation was determined according to the procedure described by Boualam K.[16] A stock solution of ABTS (7 mM) was mixed with 2.45 mM of potassium persulfate ($K_2S_2O_8$). The mixture was incubated for 12-16 hours in the dark. The $ABTS^{\cdot+}$ solution (7 mM) was diluted with distilled water to achieve an absorbance of 0.7 ± 0.02 at 734 nm. 950 μL of this solution was added to 300 μL of extract solution at different concentrations (0.1-100 μg/mL). After incubation for 7 min at room temperature, the absorbance was measured at 734 nm. The blank was prepared by replacing the extract solution with methanol. The test was performed in triplicate and the percentage inhibition was determined by the following equation:

$$\% \text{ Inhibition} = (A_b - A_s) / A_b \times 100$$

A_b: Absorbance of blank; A_S: Absorbance of sample/reference compound.

IC_{50} was estimated using graph of free radical scavenging against the extract and standard concentrations.

2.5. Enzyme inhibitory activity

2.5.1. Phospholipase A_2 inhibition assay

Type V $sPLA_2$ (phospholipase A_2) has been shown to be involved in eicosanoids formation in inflammatory cells, such as macrophages and mast cells.[17] Cayman's $sPLA_2$ Inhibitor Assay allows the Screening Type V $sPLA_2$ inhibitors. Thus, the assay of this enzyme was performed using the method described by Cayman Chemical Co. (MI, USA) in catalog No. 10004883. For this purpose, the extracts dissolved in ethanol were used so that their final concentrations in the wells ranged from 3.03 to 151.52 μg/mL. The assay utilizes the 1.2-dithio analog of diheptanoyl phosphatidylcholine as a substrate. Upon hydrolysis by $sPLA_2$, free thiols are detected using DTNB (5,5 dithio bis-(2-nitrobenzoic acid). Briefly, add 10 μL of $sPLA_2$ to all wells except the background wells and 10 μL of ethanol or Thioetheramide-PC or extracts to the 100% initial activity wells, positive control inhibitor wells and inhibitor wells respectively. Then dispense 200 μL of Diheptanoyl Thio-PC solution followed by 10 μL of DTNB into all wells. The plate was carefully shaken for 10 seconds and then incubated for 15 minutes at 25°C. The test was performed in duplicate and 96-well microplates were used. The absorbance was read at the wavelength of 405 nm. The percentage inhibition of $sPLA_2$ was calculated by the formula:

$$\% \text{ Inhibition} = [(AEA - AIA) / AEA] \times 100$$

1. AEA: Activity enzyme test absorbance - background wells absorbance.

2. AIA : Activity inhibition test Absorbance - background wells absorbance.

2.5.2. 15-Lipoxygenase inhibition assay

The lipoxygenase inhibition test was performed using the Lipoxygenase Inhibitor Screening Assay (LISA) kit, Cayman Chemical Co. (MI, USA) in catalog No.760700 according to a previously reported method.[13] A solution of 15-lipoxygenase (90 μL), extract (10 μL) and assay buffer (100 μL) were placed in the test wells and incubated for five minutes at 25°C. The reaction was initiated by adding arachidonic acid solution (10 μL) as substrate to each test well. All test wells were covered and placed on a shaker for 10 minutes. Chromogen (100 μL) was added to each well to stop enzymatic catalysis and prevent further development of the reaction and placed the plate on a shaker for five minutes. The levels of hydroperoxides (HP) produced were measured at 490 nm absorbance. Concentrations ranging from 4.76 to 238.09 μg/mL of each extait as well as nordihydroguaiaretic acid (NDGA), a lipoxygenase inhibitor standard, were tested for lipoxygenase inhibitory activity. The whole test was performed in duplicate. The inhibitory capacity was determined according to the following formula:

% Inhibition = [(AEA - AIA) / AEA] X 100

AEA : Activity enzyme test absorbance - background wells absorbance.

AIA : Activity inhibition test Absorbance - background wells absorbance.

IC_{50} value (concentration at which there was 50% inhibition) was deduced from graph representing the percentage inhibition of the enzyme activity.

2.6. Statistical analysis and expression of results

The results were expressed as mean ± SD. The statistical analysis was carried out using One-way ANOVA followed by the Tukey test about Graph Pad Prism® version 5.03. Differences were considered statistically significant, very significant and highly significant when p <0.05 (*), <0.00l (**), <0.000l (***) respectively.

3. Results and Discussion

A total of 16 extracts of 8 plants, 8 obtained by hydroethanolic maceration and 8 by aqueous decoction were screened for ABTS antiradical activity, type V $sPLA_2$ inhibition and 15-lipoxigenase inhibition.

3.1. ABTS Radical Scavenging activity

On each sample of the 8 plants in the study, two different extraction methods were performed. Eight (8) extract were obtained by hydroethanolic maceration and eight (8) by aqueous decoction. One of the most commonly used antioxidant methods is $ABTS^{\cdot+}$. It is characterized by excellent reproducibility under certain assay conditions. The $ABTS^{+}$ radical must be generated by enzymes or chemical reactions. $ABTS^{+}$ can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in the samples.[18] Other authors also report that antioxidant substances can prevent and delay lipid oxidation by acting on the reduction of ROS and the production of other free radicals.[19] The antiradical activity of the extracts varied between 7.29 ± 0.61 and 43.31 ± 0.25 μg/mL and 2.69 ± 0.25 μg/mL for gallic acid. Recurrently, the hydroethanolic extracts gave the best antiradical activities. The hydroethanol extract of T. macroptera revealed the strongest reducing power with an IC_{50}= 7.29 ± 0.61 μg/mL. Compared to the other extracts, no significant difference was observed with the hydroethanolic extract of *C. tinctorium* (IC_{50}= 7.93 ± 0.48 μg/mL), *X. americana* (IC_{50}= 8.14 ± 0.39 μg/mL) and with the aqueous extract of *T. macroptera* (IC_{50}= 9.23 ± 0.77 μg/mL). A significant difference ($p < 0.0001$) was noted with both extracts of *L. hastata, C. cordifolia, S. spinosa* and *S. senegalensis* (Table 1). Previously, similar results showed, strong antiradical activity with *T. macroptera, X. americana, C. tinctorium* and *S. senegalensis* were reported.[20–23]

3.2. Phospholipase A_2 inhibition

This study demonstrated an inhibitory effect of these Malian plants on $sPLA_2$, 15-LOX. The inhibitors of these enzymes could become key compounds in the development of new anti-inflammatory drugs. Table 2 shows the inhibitory effect of hydroethanolic and aqueous extracts on phospholipase A_2. The effect on phospholipase A_2 enzymatic activity of the hydroethanolic extracts was greater than that of the aqueous extracts. Molander *et al.*[15] and Ouedraogo *et al.*[24] found similar results with higher percent inhibition for hydroethanolic extracts. Hydroethanolic extracts of *C. tinctorium, T. macroptera, X. americana* and Thioetheramide-PC (reference compound) inhibited with inhibitory concentration 50 (IC_{50}) of 21.43 ± 0.66; 23.72 ± 0.71; 24.49 ± 0.39 and 15.23 ± 0.4 μg/mL respectively. These results confirm the inhibitory capacity of these plants on $sPLA_2$ demonstrated by Molander *et al.*[15] and Bernard *et al.*[25] Previous studies have reported high levels of polyphenols and flavonoids in these plants. These phytoconstituents are known for their anti-inflammatory properties.[11,26,27]. The hydroethanolic

Table 1: Effects (IC_{50} ± S.D. μg/mL) of the aqueous and hydroethanolic extracts on scavenging of ABTS radical.

Plantes	L. hastata	C. cordifolia	S. spinosa	C. tinctorium	X. americana	V. paradoxa	T. macroptera	S. senegalensis	Gallic acid
ABTSAq	43.31 ± 0.25***	15.03 ± 0.91***	22.24 ± 0.66***	18.87 ± 0.56***	9.38 ± 0.17*	18.55 ± 0.82***	9.23 ± 0.77	11.70 ± 0.68***	2.69 ± 0.25
ABTSHe	39.61 ± 0.58***	33.79 ± 0.65***	21.80 ± 0.56***	7.93 ± 0.48	8.14 ± 0.39	11.03 ± 0.41***	7.29 ± 0.61	18.16 ± 1.12***	

ABTS: 2, 2'-azinobis- 3-ethylbenzothiazoline-6-sulfonic acid; Aq: aqueous; He: hydroethanolic

Table 2: Inhibitory effects (IC_{50} ± S.D. μg/mL) of aqueous and hydroethanolic extracts on $sPLA_2$.

Plantes	L. hastata	C. cordifolia	S. spinosa	C. tinctorium	X. americana	V. paradoxa	T. macroptera	S. senegalensis	TPC
sPLA2 (Aq)	NA	NA	NA	NA	39.9 ± 0.57***	41.94 ± 0.37***	25.73 ± 0.40**	38.97 ± 0.82***	15.23 ± 0.4
sPLA2 (He)	NA	25.84 ± 0.82**	NA	21.43 ± 0.66	24.49 ± 0.39	31.66 ± 0.55***	23.72 ± 0.71	30.45 ± 2.02***	

$sPLA_2$: Secretory phospholipase A_2; TPC: Thioetheramide-PC; Aq: aqueous; He: hydroethanolic; NA: Not active.

Table 3: Inhibitory effects (IC_{50} ± S.D. μg/mL) of aqueous and hydroethanolic extracts on 15-LOX.

Plantes	L. hastata	C. cordifolia	S. spinosa	C. tinctorium	X. americana	V. paradoxa	T. macroptera	S. senegalensis	NDGA
15-LOX (Aq)	NA	NA	NA	NA	18.35 ± 1.26	34.75 ± 0.65***	19.45 ± 0.25	32.88 ± 1.14***	1.86 ± 0.36
15-LOX (He)	NA	22.87 ± 1.2**	39.87 ± 0.67***	92.58 ± 0.95***	18.47 ± 1.12	74.49 ± 0.55***	19.3 ± 0.50	37.42 ± 0.63***	

15-LOX: 15-lipoxygenase; NDGA: nordihydroguaiaretic acid; Aq: aqueous; He: hydroethanolic; NA: Not active.

extracts of *C. tinctorium* showed significant inhibitory activity ($p < 0.0001$) compared to the aqueous extract of *X. americana* and both extracts of *V. paradoxa* and *S. senegalensis*[11,26,28] The hydroethanolic extracts of C. tinctorium showed significant inhibitory activity ($p < 0.0001$) compared to the aqueous extract of X. americana and both extracts of V. paradoxa and S. senegalensis (Table 2). The non-activity of the extracts of *L. hastata* and *S. spinosa* was observed.

3.3. 15-lipoxigenase inhibition

The best inhibitory activity on 15-lipoxigenase was obtained with aqueous and hydroethanolic extracts of *X. americana* with IC_{50} of 18.35 ± 1.26; 18.47 ± 1.12 μg/mL respectively. Nordihydroguaiaretic acid (reference compound) had an IC_{50} of 1.86 μg/mL. No significant difference was observed with the aqueous and hydroethanolic extracts of *T. macroptera* with IC_{50} of 19.45 ± 0.25; 19.3 ± 0.50 μg/mL respectively. Previous studies demonstrated strong inhibition of 15-LOX (IC_{50}= 23.2 ± 0.5 μg/mL) by leaves of *T. macroptera.*[29] A significant difference ($p < 0.0001$) was observed when to compared the extracts of *X. americana* to with the both extracts of *V. paradoxa* and *S. senegalensis* and hydroethanolic extracts of *S. spinosa* and *C. tinctorium*. However, the two extracts of *L. hastata* and the aqueous extracts of *C. cordifolia, S. spinosa and C. tinctorium* were not active on 15-lipoxigenase (Table 3). Isa *et al.*[27] showed the inhibitory activity of Leptadenia hastata extracts different from our extracts on 5- lipoxygenase. This proves that the activity depends on the compounds dissolved in the extract. Previous studies have reported anti-inflammatory activity of these plants in vivo and/or in vitro.[22,29–32] The non-inhibitory activity of plants on $sPLA_2$ and 15-LOX may be explained by traditional uses that may include some inappropriate applications or, the presence of several biological targets could also be responsible for the anti-inflammatory properties of these plants.[25]

4. Conclusion

The present study showed that extracts of leaves of *T. macroptera* and roots of *X. americana* have antioxidant activity and effectively inhibit $sPLA_2$, and 15-LOX pro-inflammatory enzymes *in vitro.* While *in vivo* studies are required, *T. macroptera* and *X. americana* may be a potential resource of $sPLA_2$ and 15-LO that may be useful for the prevention or treatment of inflammation, and our results may therefore justify its use against diseases with an inflammatory component by traditional healers in Mali.

5. Statement of Ethical Approval

Our study protocol was approved by the Ethical Committee at the University of Sciences, Techniques and Technologie of Bamako with a protocol approuval #

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7. Source of Funding

None.

8. Conflict of Interest

None.

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