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In vitro inhibition of cyclooxygenases, anti-denaturation and antioxidant activities of Malian medicinal plants

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Cyclooxygenase 2 (COX-2) is involved in the production of prostaglandins that sustain the inflammatory process. Inflammatory cells release a number of reactive species. Furthermore, reactive oxygen species can initiate intracellular signaling cascades and enhance the expression of pro-inflammatory genes. The objective was to study the cyclooxygenase and protein denaturation inhibition and antioxidant activity of ten extracts from five plants. Cyclooxygenases inhibitory activity of the extracts was measured using the Cayman Kit method and the protein denaturation inhibitory activity using the bovine serum albumin. Ferric reducing antioxidant power and hydrogen peroxide scavenging activity were used to evaluate the antioxidant capacity of aqueous and hydroethanolic extracts. COX-2 inhibition was more important with the hydroethanolic extract of *X. americana* (IC_{50} = 11.13 ± 1.24 µg/ml) and *T. macroptera* (IC_{50} = 12.79 ± 0.56 µg/ml). COX-1 was strongly inhibited with the hydroethanolic extract of *S. senegalensis*. The hydroethanolic extracts of *C. tinctorium* and *X. americana* showed the strongest inhibitory activities of protein denaturation with 86.61 ± 1.22% and 84.5 ± 0.56% respectively. The greatest effects on iron reduction were observed with the hydroethanolic extracts of *X. americana* (R^2 = 0.996; IC_{50} = 29 µg/ml) and *T. macropteria* (R^2 = 0.990; IC_{50} = 35.46 µg/ml). The hydrogen peroxide scavenging activity of the extracts varied from 39.31 ± 3.6 to 77.37 ± 2.16 µg/ml. The present study concluded to an important antioxidant activity, protein denaturation and COX-2 inhibitory activities of the hydroethanolic extracts of *X. americana, T. macroptera* and *C. tinctorium.* Further studies are needed to confirm the anti-inflammatory effect of these extracts *in vivo.*

Key words: *X. Americana, T. macroptera*, cyclooxygenases, antioxidant, protein denaturation.

INTRODUCTION

Inflammation is a complex process associated with pain and involves phenomena such as increased vascular

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permeability, protein denaturation and membrane destruction. It is a complex biological response of vascular tissue to a range of stimuli. It has been shown that inflammation-related injured or damaged cells release arachidonic acid from the cell membrane through phospholipase A_2 activity (Bhutia, 2020; Naz et al., 2020). The arachidonic acid is then treated by two pathways, the cyclooxygenase (COX) and the lipoxygenase pathways (LOX). The products of these two pathways are implicated in the pathogenesis of many inflammatory diseases (Dannhardt and Kiefer, 2001; Yedgar et al., 2007). It is commonly assumed that cyclooxygenase (COX) is the most important enzyme among mediators of pro-inflammation. It possesses two isoforms: COX-1, constitutively expressed and COX-2, an inducible isoform (Eldeen and Van Staden, 2008; Ramadhan, 2020). COX is directly inhibited by the non-steroidal anti-inflammatory drug (NSAID) family, many of these drugs being non-selective. Most adverse effects of these drugs, including burns, stomach ulcer, asthma attack are related to COX-1 inhibition (Yougbaré-Ziébrou et al., 2016; Karim et al., 2019). COX-2 is expressed in response to inflammatory stimuli and NSAIDs that inhibit only COX-2 has fewer side effects (Oniga et al., 2017). Medicinal plants have been extensively studied as anti-inflammatory agents and are known to be very well tolerated compared to widely used synthetic products (Naz et al., 2020). However, the selectivity of these plants towards the inducible isoform (COX-2), is poorly explored (Baek et al., 2021). Previous studies have confirmed the anti-inflammatory activity of traditional Malian medicine plants by inhibiting phospholipase A_2 and 15-lipoxygenase (Ballo et al., 2022). In addition, high levels of polyphenols, flavonoids and tannin in extracts of these plants have been reported (Ballo et al., 2021). Therefore, it is great interest to focus the search for new anti-inflammatory agents on medicinal plants, which constitute a promising source of natural anti-inflammatory molecules with minimal side effects. In a previous studies, the anti-inflammatory activity of 8 traditional Malian medicine plants was shown/confirmed. Indeed, extracts from those plants have shown an inhibitory activity on phospholipase A_2 and 15-lipoxygenase enzymes (Ballo et al., 2022). In addition, the presence of high levels of polyphenols, flavonoids and tannin in extracts from these plants (Ballo et al., 2021) was highlighted). This study is aimed at evaluating the antioxidant activity and inhibitory effects of the extracts on COX-1, COX-2 and protein denaturation.

MATERIALS AND METHODS

Chemicals

All solvents and reagents used were of analytical grade. COX (ovine) Colorimetric Inhibitor Screening Assay Kit Item No. 760111 was from Cayman Chemical Co.,MI, (USA), Lipid Rich Bovine Serum Albumin from AlbuMax II, (New Zealand); Trichloroacetic acid from PROLABO, (France) and Potassium ferricyanide $K_3Fe(CN)_6$; Hydrogen peroxide; Iron chloride from PROLABO, (Belgium).

Plants materials and extraction

The roots of *Cochlospermum tinctorium* Perrier ex A. Rich., *Ximenia americana* L., the leaves of *Saba senegalensis* (A.DG.) Pichon, of *Terminalia macroptera* Guill & Perr, and *Vitellaria paradoxa* C.F.Gaertn were harvested. These specimens have been certified by the Department of Traditional Medicine (DMT) of the National Institute of Public Health of Mali and the herbarium of each plant is kept with reference number 0048, 0027, 3005, 2468 and 2792, respectively. On each harvested plant organ, two extraction methods, aqueous decoction and 70% hydroethanolic maceration were used as described previously (Ballo et al., 2022).

In vitro anti-inflammatory activity

Cyclooxygenase (COX-1 and COX-2) inhibition test

COX-1 (ovine) and COX-2 (recombinant ovine) extract inhibition test was performed using COX (ovine) Colorimetric Inhibitor Screening Assay Kit item No. 760111, that measures cyclooxygenase peroxidase activity by monitoring the appearance of oxidized N,N,N',N'-tetra-methyl-p-phenylenediamine (TMPD) at 590 nm as described previously (Ilic et al., 2014). These tests were performed according to the manufacturer's instructions. Briefly, it consisted of the addition of assay buffer (150 µl), hemin (10 µl) and enzyme (10 µl) (either COX-1 or COX-2); and 10 µl of extract, reference drug into the inhibitor wells.10 µl of ethanol (used as diluent for the extracts) were used for the 100% initial activity and background wells, which contained only test buffer (150 µl) and hemin (10 µl). The plate was incubated for five minutes at 25°C. 20 µl of the colorimetric substrate solution were added, followed by 20 µl of arachidonic acid to all wells. The plate was then incubated for another two minutes at 25°C and the absorbance was read at 590 nm. Test products, extracts, diclofenac and celecoxib were used so that their final well concentrations ranged from 0.45 to 113.64 µg/ml. The test was carried out in duplicate. The inhibitory capacity was determined according to the following formula:

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

AEA: Activity enzyme test absorbance - background wells absorbance; AIA: Activity inhibition test Absorbance - background wells absorbance. The results were expressed in concentrations that inhibit 50% (IC_{50}) of the enzyme activity.

Inhibition of protein denaturation

In vitro anti-inflammatory activity of crude extracts, expressed as protein denaturation was performed according to previously used methods (Deshpande et al., 2009; Ghosh et al., 2015; Rahman et al., 2015). The test solutions consisted of mixtures of different concentrations (100, 250 and 500 µg/ml) of extracts (50 µl) or standard drug sodium diclofenac (50 µl) with 0.5% w/v bovine albumin serum (BSA) (450 µl). A mixture of distilled water (450 µl) and different extracts (50 µl) with various concentrations (100, 250 and 500 µg/ml) served as product control solution. BSA 0.5% w/v (450 µl) aqueous solution and distilled water (50 µl) was used as a control solution. The preparations were incubated at 37°C for 20 min, followed by a temperature increase to 57°C for 3 min. After cooling, 2.5 ml of saline buffer phosphate solution (pH 6.3) was added to all solutions. The absorbance was read by UV-visible

spectrophotometer at 660 nm. The control represented 100% protein denaturation. The percentage inhibition of protein denaturation was calculated using the following formula:

Percentage inhibition = 100 - [{(optical density of test solutions - optical density of product control) / optical density of test control} × 100].

In vitro antioxidant activity

Ferric reducing antioxidant power

The ferric reducing antioxidant power was assessed according to an experimental protocol previously used by the authors (Abu-Serie et al., 2018; El-Haci and Bekkara, 2016). Briefly, 500 µl of extracts at different concentrations (0.1 to 200 µg/ml) is mixed with 1.25 ml of 0.2 M phosphate buffer solution (pH 6.6) and 1.25 ml of a 1% potassium ferricyanide ($K_3Fe(CN)_6$) solution. The preparation was incubated at 50°C for 20 min, and cooled down to room temperature. 1.25 ml 10% trichloroacetic acid was then added. Afterward, the tubes were centrifuged at 3000 rpm for 10 min. Finally, 2 ml of the supernatant were added to 2 ml of distilled water and 400 µl of a 0.1% freshly prepared iron chloride ($FeCl_3$) solution were added to the mixture. The absorbance was read against a blank at 700 nm. Ascorbic acid was used as a positive control and was treated under the same experimental conditions as the extracts. An increase in absorbance indicates greater reducing power. The results were expressed as IC_{50} values which translate into the concentration of antioxidant used to obtain an absorbance of 0.5.

Hydrogen peroxide scavenging activity

The hydrogen peroxide (H_2O_2) scavenging activity of the extract was determined according to previously described methods (Bhatti et al., 2015; Nabavi et al., 2008). Briefly, 2 ml of extract at concentrations ranging from 0.1 to 100 µg/ml was mixed with 0.6 ml of 40 mM H_20_2 solution. Ascorbic acid was used as positive control.

Statistical analysis

Results are expressed as mean ± SD. Multiple groups were compared using one-way ANOVA test followed by the Tukey test. Differences were considered statistically significant when $p < 0.05$ (*), very significant when p <0.00l (**) and highly significant when p <0.000l (***).

RESULTS AND DISCUSSION

In vitro anti-inflammatory activity

Using different biochemical *in vitro* methods, we investigated the anti-inflammatory properties of the selected plant extracts.

Cyclooxygenase (COX-1 and COX-2) inhibitory activity

Prostaglandins are inflammatory mediators produced from arachidonic acid by the action of cyclooxygenase enzymes. The constitutive form (COX-1) is expressed throughout the body and performs a number of homeostatic functions such as maintaining normal gastric mucosa and influencing renal blood flow and aggregation (Parente and Perretti, 2003). In contrast, the inducible form (COX-2) is expressed in response to inflammatory and physiological stimuli, and is involved in the production of prostaglandins that mediate pain and support the inflammatory process (Lamon et al., 2010). The inhibitory effect of the selected plant extracts on constitutive COX-1, along with their inhibitory effects on the inducible COX-2 was examined. The results of COX-1 and COX-2 inhibition were expressed as IC_{50} values (Figures 1 and 2), which is very important to determine the relationship between concentration and effect of a product. The literature shows a insufficiency of data expressed in IC50 values. From these results, a number of interesting observations can be made (Elgorashi and McGaw, 2019). First, a general observation is that the inhibitory effect of hydroethanolic extracts was greater than that of aqueous extracts, and this difference could be observed both in the cases of COX-1 and COX-2 inhibition. Indeed, all IC_{50} values of hydroethanolic extracts are significantly to highly significantly higher than those of aqueous extracts, both for COX-1 and COX-2 inhibitory effect ($p<0.05$ to $p<0.0001$). The only exception was observed in the case of extracts of *C. tinctorium* for which the IC_{50} value of the hydroethanolic extract is not significantly different than the IC_{50} value of the aqueous extract (Figure 1, $p>0.05$). Secondly, the investigated plant extracts appeared to be more active in inhibiting COX-2 activity than COX-1. Thus, except hydroethanolic extract of *V. paradoxa*, all hydroethanolic extracts exhibited an IC_{50} value lower than 30 ug/ml (though those values remain ~4 times less active than the reference drug celecoxib) for COX-2 activity inhibition, the most active extract in that regard being hydroethanolic extract of *X. americana*. All extracts, except hydroethanolic extract of *C. tinctorium*, *V. paradoxa* and *S. senegalensis* had IC_{50} values higher than 30 ug/ml on COX-1 activity. Subject to further studies this particular difference could be a point of interest in the context of selectivity research for existing anti-inflammatory drugs. Indeed, non-selective AINS are generally known to have important gastrointestinal and renal adverse effects, while studies on selective COX-1 inhibitors have shown that COX-1 inhibition is responsible for these adverse effects (Parente and Perretti, 2003). Comparatively, COX-2 inhibitors appear to have more selective therapeutic effects on pain, inflammation, arthritis (Gautam et al., 2010). In this regard, the extraction products of aqueous decoction of *C. tinctorium*, both extracts of *X. americana* and *T. macroptera* exhibit a striking profile, not only for their significant potential anti-inflammatory properties but also and more importantly because of the apparent

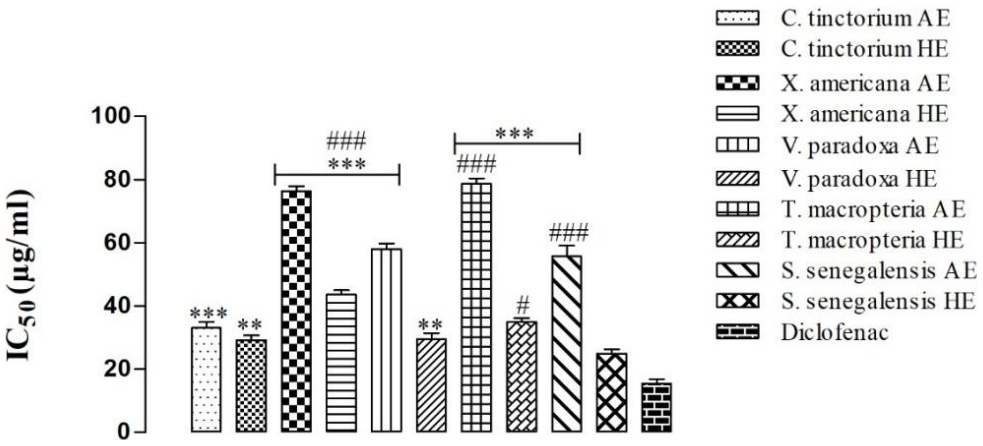

Figure 1. *In vitro* COX-1 inhibitory effect of the aqueous (AE) and hydroethanolic (HE) extracts, diclofenac was used as reference standard. Differences were considered statistically significant when p<0.05 (*) or (#) very significant when p<0.001 (**) or (##) and highly significant when p<0.0001 (***) or (###) compared all extracts to Diclofenac or *S. senegalensis* HE, respectively.
Source: GraphPad Prism 5.03, Ballo et al., 2022

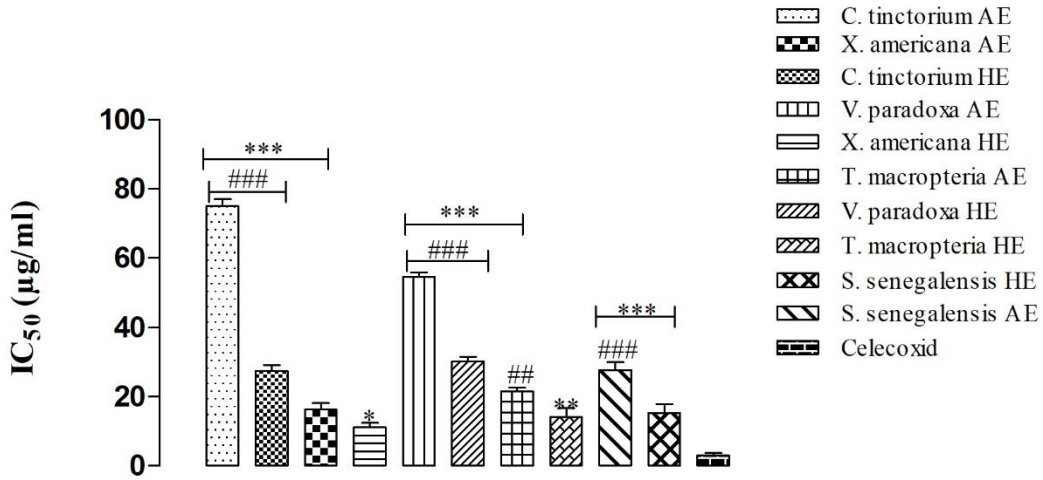

Figure 2. *In vitro* and COX-2 inhibitory effect of the aqueous (AE) and hydroethanolic (HE) extracts and celecoxib was used as reference standard. Differences were considered statistically significant when p <0.05 (*) or (#) very significant when p <0.001 (**) or (##) and highly significant when p <0.0001 (***) or (###) compared all extracts to celecoxib or *X. americana* HE, respectively.
Source: GraphPad Prism 5.03, Ballo et al., 2022

selectivity for COX-2 enzyme that they show. Moreover, comparative chemical analysis of the extracts, including between genera and species of plants, as well as between types of extracts, could give more information on the chemical aspect of the inhibitory action of the extracts. As is shown in Figures 1 and 2, striking differences in COX inhibition activity are apparent, not only between the plant species but also between the extracts for the same plant, at least for number of them. Thus, in the case of *C. tinctorium*, aqueous and hydroethanolic extracts appear to have the same degree of COX-1 inhibition ($p>0.05$) while the hydroethanolic extracts showed 2.5 times more activity than the aqueous extracts on COX-2 inhibition (Figure 2). Studies have shown that the aqueous extract of *Cochlospermum tinctorium* contain high levels of polysaccharides, while the hydroethanolic fraction appears to be richer in polyphenols. A quiet opposite profile is apparent for *X. americana* and *T. macroptera*, to a lesser extend however (Ballo et al., 2021; Nergard et al., 2005).

Although more pharmaco-chemical analyses are needed, it is tempting to hypothesize that inhibition of

COX-2 could more related to the presence of polyphenols while inhibition of COX-1 could be more associated with the presence of polysaccharides. Moreover, the hydroethanolic extract of *S. senegalensis* is known to contain higher levels of total flavonoids than its aqueous extract (Ballo et al., 2021). That could confirm or explain importance of polyphenols in the inhibition of COX-2. Finally, it should be noted that despite the remarkable inhibitory effects of most the extracts on COX activity, none of them equaled the effectiveness of the reference drugs diclofenac for COX-1 inhibition or celecoxib for COX-2 inhibition. Thus, COX-1 activity was significantly inhibited by the positive control (diclofenac with IC_{50} = 15.4 ± 1.80 µg/ml) and none of the tested plant extracts (IC_{50} ranged from 24.9 ± 1.85 to 78.6 ± 2.45 µg/ml) was more effective than diclofenac ($p>0.05$). The most effective extract in inhibiting COX-1 was the hydroethanolic extract of S. senegalensis with an IC_{50} value (IC_{50} = 24.9 ± 1.85 µg/ml) was the only extract that showed no significant difference with diclofenac (Figure 1). In normal conditions, activation of COX-1 enzyme initiates the production of beneficial prostaglandins responsible for the maintenance and protection of the gastric mucosal layer (Dennis and Norris, 2015). Therefore anti-inflammatory substances (plant extracts) with the least inhibitory activity on COX-1 are sought due to lesser side effects associated with COX-1 inhibition (Suleyman et al., 2007). In that regard, the plant extracts, when effective in reducing inflammation through inhibition of COX-2, would potentially be better choices. Interestingly, the selected plant extracts also elicited significant inhibitory effect on COX-2 with IC_{50} values ranging from 11.13 ± 1.24 to 75.09 ± 2.04 µg/ml. The most effective extract in inhibiting COX-2 enzyme was the hydroethanolic extract of *X. americana* (IC_{50} = 11.13 ± 1.24 µg/ml) followed by the extract of *T. macroptera* (IC_{50} = 14.15 ± 2.49 µg/ml) and *S. senegalensis* (IC50 = 15.28 ± 2.6 µg/ml). However, these extracts remained significantly less effective than the reference drug (Celecoxib, IC_{50} = 2.96 ± 0.81, $p < 0.05$, $p < 0.001$, $p < 0.001$ respectively for *X. americana*, *T. macroptera* and *S. senegalensis*) All other extracts were largely less effective that the reference drug celecoxib ($p < 0.0001$) (Figure 2). These results are in accordance with previousreport from Dias et al. (2018) who found that catechin from *X. americana* significantly inhibited COX-1 and COX-2, providing evidence that flavonoids elicit anti-inflammatory activity. Furthermore, other studies have shown that the hydroethanolic extracts of *X. americana*, *T. macroptera* and *S. senegalensis* contain high levels of polyphenols and flavonoids (Ballo et al., 2021), suggesting that the present characterized inhibitory effects of the selected plant extracts on COX-1 and COX-2 are therefore probably due to the presence of secondary metabolites such as flavonoids and polyphenols in general, contained in significant levels in these extracts.

Among the present investigated plant extracts, 3 extracts appeared to be of significant interest in terms of therapeutic perspective for inflammatory conditions. Nevertheless, subject to thorough investigation, these products represent interesting potential alternatives for anti-inflammatory/analgesic medicines with less adverse effects.

Inhibition of protein denaturation

The effect of the extracts on the inhibition of protein denaturation is shown in Table 1. All extracts at all dose levels (100, 200 and 500 µg/ml) showed significant inhibition of protein denaturation. At 100 µg/ml, the reference drug diclofenac (51.32 ± 1.22%) was more effective ($p < 0.0001$) compared to all extracts. At such concentration, aqueous extract of *C. tinctorium* (42.07 ± 1.16%) and hydroethanolic extract of *X. americana* (41.33 ± 0.96%) were the most effective extracts against protein denaturation, when compared to the other extracts, and their effect was similar to that of diclofenac ($p>0.05$). The hydroethanolic extract of *X. americana* was the most effective (66.24 ± 0.55%) at 200 µg/ml and had no significant difference with diclofenac (67.34 ± 0.93%), all other extracts were very less active. At the highest concentration (500 µg/ml), the hydroethanolic extracts of *C. tinctorium, and X. americana* showed the highest activity against protein denaturation with a percent inhibition of 86.61 ± 1.22 and 84.5 ± 0.56% respectively and their effect was similar to that of the reference drug diclofenac (83.57 ± 1.61; $p<0.001$). No significant difference was observed between these two extracts ($p>0.05$) (Table 1). Moreover, the results are confirmed by the inhibitory activity of *X. americana* (77.51 ± 2.25%) against protein denaturation demonstrated by other authors (Shettar et al., 2015). Moreover, a plant of the same family as *Terminalia macroptera* Guill. & Perr., *Terminalia glaucescens* Planch. ex Benth, its anti-inflammatory activity has been proved by inhibition of protein denaturation (Das et al., 2020). The pathophysiology of the autoimmune disease rheumatoid arthritis involves protein denaturation and thus the production of autoantigens *in vivo*. Denaturation probably leads to an alteration of electrostatic, hydrogen, hydrophobic and disulphide properties (Deshpande et al., 2009; Ghosh et al., 2015). BSA denaturation was strongly inhibited by hydroethanolic extract of *C. tinctorium* and *X. americana* suggesting that both extracts could prevent protein denaturation in rheumatoid arthritis and thus be a potential anti-arthritic agent.

In vitro antioxidant activity

Antioxidants are substances that protect cells from the

Table 1. Effects of selected plant extracts on protein denaturation.

Plant	100 µg/ml		200 µg/ml		500 µg/ml	
	AE	HE	AE	HE	AE	HE
C. tinctorium	42.07 ± 1.16	23.33 ± 0.61***	51.48 ± 0.32***	56.39 ± 0.35***	77.49 ± 1.15***	86.61 ± 1.22
X. americana	19.47 ± 1.53***	41.33 ± 0.96	39.15 ± 0.61***	66.24 ± 0.55	73.83 ± 0.61***	84.5 ± 0.56
V. paradoxa	7.51 ± 0.61***	3.25 ± 1.22***	32.66 ± 0.7***	12.58 ± 1.53***	43.0 ±0.7***	54.56 ± 0.93***
T. macroptera	23.33 ± 0.61***	24.34 ± 0.93***	37.53 ± 1.26***	36.92 ± 0.93***	51.12 ± 0.7***	67.55 ± 0.7***
S. senegalensis	16.63 ± 0.61***	30.22 ± 0.93***	25.15 ± 0.61***	35.3 ± 0.35***	46.13 ± 0.31***	66.12 ± 0.7***
Diclofenac	51.32 ± 1.22		67.34 ± 0.93		83.57± 1.61	

AE: Aqueous extract; HE: hydroethanolic extract. The effects on protein denaturation were expressed as mean ± SD. Differences were considered statistically significant when p <0.05 (*), very significant when p <0.001 (**) and highly significant when p <0.0001 (***) compared all extracts to Diclofenac.
Source: GraphPad Prism 5.03, Ballo et al., 2022

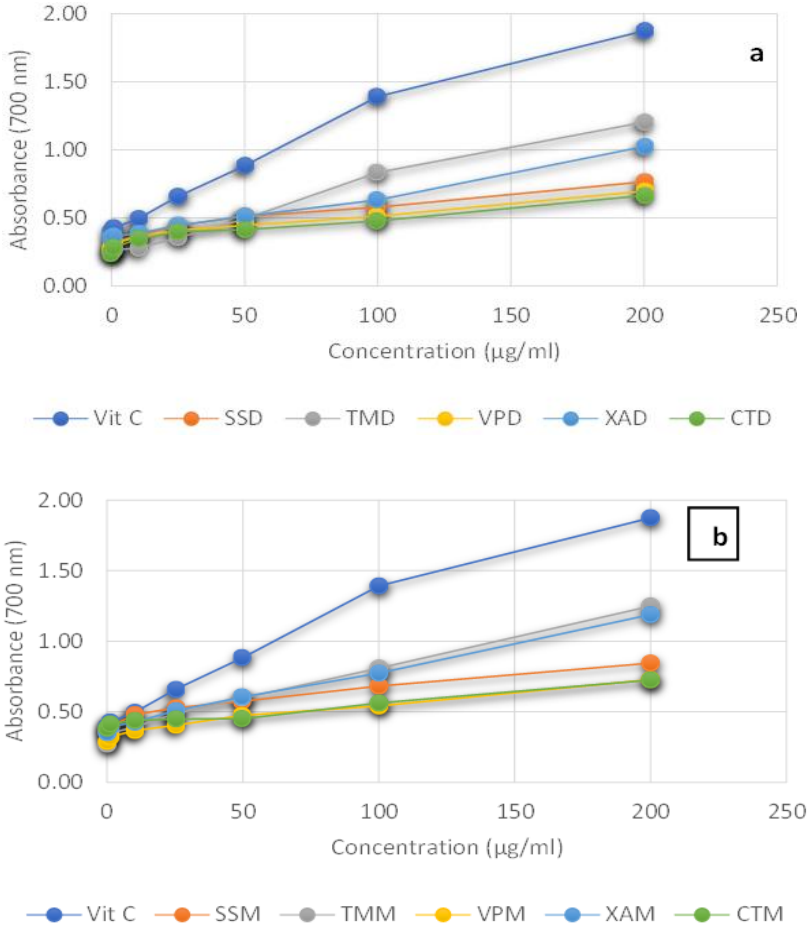

Figure 3. The reducing power of aqueous (a) and hydroethanolic (b) extracts.
Source: Microsoft Excel 2016, Ballo et al., 2022

harmful effects of reactive oxygen species. Recently, natural antioxidants have been sought after for their potential to prevent disease (Shettar et al., 2015). In the present work, two methods were used to evaluate the antioxidant capacity of aqueous and hydroethanolic extracts.

Table 2. R^2 and IC_{50} coefficient of determination (µg/ml) of extracts on reducing power activity and H_2O_2 scavenging activity.

Plant	FRAP				H_2O_2 scavenging	
	AE		ME		AE	ME
	R^2	IC_{50}	R^2	IC_{50}	IC_{50}	IC_{50}
C. tinctorium	0.931	105.05	0.978	62.13	39.31 ± 3.60***	44.52 ± 1.59***
X. americana	0.992	44.85	0.996	29.00	42.97 ± 1.61	53.54 ± 1.34**
V. paradoxa	0.959	92.00	0.967	83.25	72.12 ± 2.57***	77.37 ± 2.16***
T. macroptera	0.987	50.55	0.99	35.46	40.54 ± 1.60***	48.7 ± 1.91***
S. senegalensis	0.924	69.57	0.944	35.61	54.13 ± 2.73**	62.46 ± 1.79***
Vit C	R^2 = 0.997		IC_{50} = 8.42		21.76 ± 1.64	

AE: Aqueous extract; HE: Hydroethanolic extract. Values were expressed as mean ± SD. The differences were considered statistically significant when p < 0.05 (*), very significant when p < 0.00l (**) and highly significant when p < 0.000l (***).
Source: GraphPad Prism 5.03, Ballo et al., 2022

Ferric reducing antioxidant power (FRAP)

In this study, the results show that the reductive power of extracts and vitamin C increased with concentration in a very strongly linear manner (Figure 3a and b). The most important effects were observed with vitamin C (R^2 = 0.997; IC_{50} = 8.42 µg/ml), hydroethanolic extracts of *X. americana* (R^2 = 0.996; IC_{50} = 29 µg/ml), *T. macropteria* (R^2 = 0.990; IC_{50} = 35.46 µg/ml). In addition, the hydroethanolic extract of *X. americana* was more than twice higher than hydroethanolic extracts and three times higher than aqueous extracts of *C. tinctorium* and *V. paradoxa* (Table 2). Previous studies confirm the results. Thus, a higher activity of the aqueous extract of *Terminalia macroptera* and *X. americana* have been reported (Shettar et al., 2015; Sobeh et al., 2017; Sombie et al., 2018). In this study, the hydroethanolic extract (IC_{50} = 35.61 µg/ml) *S. senegalensis* showed good antioxidant activity and the aqueous extract (IC_{50} = 69.57 µg/ml) had moderate activity. Belemlilga et al. (2019) obtained a contrary result in their study, but the DCM fraction of the hydroethanolic extract presented the highest reducing power with a concentration of 88.88 mmol TE/g. Studies have shown that the polyphenolic compounds in the extracts may be good electron and hydrogen atom donors, and thus able to stop radical chain reactions by converting free radicals into more stable products (Amarowicz et al., 2004; Shettar et al., 2015).

Hydrogen peroxide scavenging activity

Hydrogen peroxide is naturally produced in living organisms, it is not very reactive, but it causes cytotoxicity by the synthesis of hydroxyl radicals in the cell system. Therefore, H_2O_2 scavenging can contribute to the preservation of human health (Upadhyaya et al., 2007; Jayakumar et al., 2016). The H_2O_2 scavenging capacity of the extracts is presented in Table 2. The IC_{50} value of H_2O_2 scavenging activity for extracts ranged from 39.31 ± 3.6 to 77.37 ± 2.16 µg/ml. However, the H_2O_2 scavenging effect of the best extracts and the standard decreased in the following order: vitamin C > aqueous extract of *C. tinctorium* > aqueous extract of *T. macropteria* > aqueous extract of *X. americana* (Table 2). Kiessoun et al. (2018) in his study on the polyphenol rich fraction of *Ximenia americana* roots had a higher IC50 = 130 µg/ml compared to our results.

Conclusion

The study clearly demonstrates that hydroethanolic extracts of *X. americana, T. macroptera* and *C. tinctorium* are potent inhibitors of cyclooxygenase-2 with low IC50 values. At the concentration of 500 µg/ml, hydroethanolic extracts of *C. tinctorium* and *X. americana* inhibited BSA denaturation more than 80%. They also have high antioxidant activity. Our data suggest that these extracts have significant anti-inflammatory activity. COX-1 and COX-2 inhibition, protein denaturation and antioxidant activity were performed to provide scientific data for in *in vivo* studies.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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