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Antioxidative, anti-inflammatory potentials and phytochemical profile of *Commiphora africana* (A. Rich.) Engl. (Burseraceae) and *Loeseneriella africana* (Willd.) (Celastraceae) stem leaves extracts

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ABSTRACT

Objective: To assess the antioxidant and anti-inflammatory activities as well as to determine the flavonoids and phenolic acids content of active fractions.

Methods: Two medicinal plant samples were extracted successively in Soxhlet apparatus with *n*-hexane, dichloromethane, acetonitrile, ethyl acetate, methanol and *n*-butanol. Five methods were used to evaluate the antioxidant activity. Anti-inflammatory activity was done through the inhibition of the cyclooxygenase enzymes (COX-1 and COX-2). Polyphenolic compounds were analyzed by using a spectrophotometrical and high performance liquid chromatography-mass spectrometry (HPLC-MS) methods.

Results: The data showed that the stem leaves extracts of *Commiphora africana* and *Loeseneriella africana* possessed significant *in vitro* antioxidant and anti-inflammatory activities. Polar extracts had radical scavenging effects and they reduced iron (III). The prostaglandin production was significantly stopped by acetonitrile and methanol extracts. These biological activities were supported by some bioactive compounds quantified by using the HPLC-MS. *p*-Coumaric acid, ferulic acid, isoquercitrin, quercitrin, quercetin, rutin, kaempferol and apigenin were the most metabolites quantified.

Conclusions: The present study may explain the effectiveness of plants in traditional medicine of Burkina Faso, singularly *Commiphora africana* and *Loeseneriella africana*. The next investigation was to sub-fractionate the methanol fraction in order to isolate new antioxidant and/or anti-inflammatory compounds.

1. Introduction

Previous ethnobotanical investigations have demonstrated the importance of traditional medicine in maintaining people health in Burkina Faso [1,2]. A large number of medicinal plants were well known and traditionally used for the treatment of malaria, wound, cancer, HIV, diarrhea, inflammation and cardiovascular diseases [1–4]. These data indicated the importance of ethnopharmacological knowledge in Burkina Faso. Moreover, there are few pharmacological and phytochemical studies for valorizing traditional medicine and phytotherapy according to Zizka *et al*. [1].

Commiphora africana (*C. africana*) and *Loeseneriella africana* (*L. africana*) were well-known medicinal plants used in the treatment of cancer, malaria, wound and inflammatory disease in Burkina Faso and other countries [2,5]. Previous biological investigations from Nigeria, Kenya and South Africa showed

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the antiradical scavenging, antimicrobial, anthelmintic, antiplasmodial and antitumoral activities of *C. africana* extracts [6–8]. These results were supported by *in vivo* studies such as antilipidaemic, hypoglycemic and anti-inflammatory properties [9–11]. As for *L. africana*, it was demonstrated that this plant possessed anti-malarial activities [5].

The phytochemical investigations focused on volatile compound from gum, resin and oil such as monoterpenes [12]. The preliminary phytochemical screening of *C. africana* extract revealed the presence of flavonoids, tannin, anthraquinone, triterpenoides, saponins and alkaloids [11]. A dihydroflavonol glucoside from *C. africana* that mediates DNA strand scission was identified [13]. In contrast, the phytochemical studies of *L. africana* were not yet done. This work fills a gap in pharmacology and phytochemical knowledge in Burkina Faso. Thereby, the interactions between the plant extracts and the antioxidant and the anti-inflammatory parameters were evaluated *in vitro*. This research was achieved by phytochemical investigations.

2. Materials and methods

2.1. Plants

Stem with leaves of *L. africana* (Celastraceae) and *C. africana* (Burseraceae) were collected from central region of Burkina Faso in September 2011. After identification and authentication, voucher specimens were deposited at the Herbarium of the University of Ouagadougou under the voucher specimen number mcompaore_5 and mcompaore_6 respectively.

2.2. Chemical material

Folin–Ciocalteu reagent, sodium phosphate mono- and dibasics, sodium tetraborate, potassium persulfate, aluminum trichloride, trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis(3-ethylbenzoline-6-sulphonate) (ABTS), gallic acid and trichloro acetic acid (TCA) were purchased from Sigma–Aldrich (Germany). Sodium carbonate, potassium hexacyanoferrate, ascorbic acid and ferric chloride were from Prolabo (France). Caftaric, gentisic, ferulic, sinapic, caffeic, chlorogenic and *p*-coumaric acids, and patuletin, luteolin, hyperoside, isoquercitrin, rutin, myricetin, fisetin, quercitrin, quercetin, kaempferol and apigenin were purchased from Roth (Germany) and Dalton (USA). All solvents used were high-performance liquid chromatography (HPLC) or analytic grades.

2.3. Apparatus and chromatographic conditions

An Agilent 1100 HPLC Series system (Agilent, USA) coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL) was used for chromatographic analysis. The full experimental description was showed in Compaoré *et al*. previous publication [14].

2.4. Extraction

Twenty-five grams of powder from each plant was successively fractioned with *n*-hexane, dichloromethane, acetonitrile, ethyl acetate, methanol and *n*-butanol in Soxhlet apparatus according to method of Dastmalchi *et al*. with slight modifications [15]. The organic solvents were removed in rotary evaporator system under reduced pressure. The mass of extractable compounds were indicated in Table 1.

2.5. Biological investigations

2.5.1. Inhibition of radical DPPH assay

Extracts capacity to inhibit DPPH radical was evaluated as described by Lamien-Meda *et al*. with some modifications [16]. In a 96 micro-well plate, 200 µL of DPPH$^{\bullet}$ (20 mg/L) and 100 µL of sample were incubated in dark for 10 min, following a reading at 517 nm (BioTek Instruments, USA). Quercetin was used to generate a standard curve ($y = -27.94x + 8.15$; $r^2 > 0.99$; $P < 0.000\,1$). The data expressed in mg equivalent of quercetin per gram was the average of 2 independent triplet tests.

Table 1
Antioxidant activities and total polyphenolic contents.

Plant	Extract	Yield* (mg/g)	Antioxidant activities					Phytochemical analysis	
			ABTS (mMET/g)	DPPH (mgQE/g)	FRAP (mgAAE/g)	I% LPO	I% DRB	TPC (mgGAE/g)	TFC (mgQE/g)
L. africana	HF	25.56	6.62 ± 1.30^{f}	7.49 ± 0.09	21.59 ± 4.65^{e}	Non active	65.33 ± 0.44^{b}	90.42 ± 6.53	13.70 ± 0.65
	DCMF	7.54	17.08 ± 1.34^{e}	56.10 ± 2.93	88.17 ± 3.81^{d}	Non active	66.53 ± 0.88^{b}	206.54 ± 9.47	17.58 ± 0.57
	ACNF	9.76	21.50 ± 0.73^{d}	63.33 ± 0.78^{ab}	118.67 ± 3.27^{c}	Non active	70.32 ± 0.84^{a}	246.45 ± 6.66^{c}	11.26 ± 0.36
	EAF	3.36	34.57 ± 2.27^{b}	63.51 ± 0.11^{ab}	271.52 ± 7.33^{a}	41.79 ± 2.24	69.65 ± 0.79^{a}	439.75 ± 3.33^{a}	8.47 ± 0.27
	MeOHF	185.24	26.08 ± 1.16^{c}	65.01 ± 0.33^{a}	186.59 ± 7.97	70.07 ± 0.36	66.72 ± 0.55^{b}	368.20 ± 11.53^{b}	3.84 ± 0.08
	BuOHF	2.20	21.59 ± 2.39^{d}	61.25 ± 0.13^{b}	133.95 ± 2.02^{bc}	28.71 ± 0.34	70.32 ± 0.52^{ac}	371.15 ± 4.61^{b}	30.21 ± 0.49
C. africana	HF	27.12	5.06 ± 0.48^{f}	Non active	15.03 ± 2.14^{e}	Non active	Non active	5.86 ± 0.64	Non detected
	DCMF	5.48	13.98 ± 0.72^{e}	32.99 ± 0.86	78.22 ± 3.55^{d}	64.57 ± 0.81	60.78 ± 0.48	150.51 ± 2.40	1.48 ± 0.06
	ACNF	15.40	40.75 ± 0.43^{a}	64.45 ± 0.05^{a}	435.77 ± 20.57	58.51 ± 1.68	83.79 ± 0.28	819.39 ± 10.56	0.21 ± 0.03
	EAF	15.40	37.60 ± 1.00^{ab}	64.16 ± 0.32^{a}	365.42 ± 8.99	65.25 ± 3.59	80.24 ± 0.33	719.45 ± 2.78	6.18 ± 0.13
	MeOHF	101.28	22.13 ± 1.19^{d}	61.13 ± 0.30^{b}	157.40 ± 4.67^{b}	78.57 ± 3.04	73.44 ± 0.17^{c}	260.73 ± 4.50^{c}	Non tested
	BuOHF	1.02	29.47 ± 0.42^{c}	62.50 ± 0.05^{ab}	264.25 ± 9.35^{a}	64.02 ± 1.19	77.06 ± 0.19^{a}	424.90 ± 12.55^{a}	Non tested

$n = 2 \times 3$ except asterisk data that were obtained by one procedure extraction. Data in each column were statistically different ($P < 0.05$) except data with same letters. TPC: Total phenolic content; TFC: Total flavonoid content; FRAP: Ferric reducing antioxidant power; I% LPO: Lipid peroxidation inhibition percentage; I% DRB: Deoxyribose degradation inhibition percentage; HF: *n*-Hexane fraction; DCMF: Dichloromethane fraction; ACNF: Acetonitrile fraction; EAF: Ethyl acetate fraction; MeOHF: Methanol fraction; BuOHF: *n*-Butanol fraction; mMET/g: Millimole equivalent of trolox/gram; mgEAA/g: mg ascorbic acid equivalents/gram; mgGAE/g: mg gallic acid equivalent/gram; mgQE/g: mg quercetin equivalent/gram.

2.5.2. Inhibition of radical ABTS assay

The method described by Compaoré *et al*. with some modifications was used to evaluate the sample $ABTS^{\bullet+}$ scavenging ability [14]. To 200 µL of diluted $ABTS^{\bullet+}$ solution, 50 µL of extract was added for 5 min dark incubation. The absorbance was read at 734 nm with microplate reader (BioTek Instruments, USA). Trolox was used for generating standard curve ($y = -72.38x + 54.57$; $r^2 > 0.99$; $P < 0.001$) and the result were expressed in mMET/g.

2.5.3. Reduction of iron III assay

The reducing power of extracts was determined according to Compaoré *et al*. method with some modifications [14]. In a tube, 0.5 mL of extract was mixed with 1.25 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 1.25 mL of 1% aqueous potassium hexacyanoferrate. This mixture was incubated for 30 min followed by an addition of TCA and centrifugated at 3 000 r/min for 10 min. In 96 micro-wells, the upper layer solution (125 µL) was mixed with 125 µL of H_2O and 25 µL of $FeCl_3$ fresh solution. Ascorbic acid was used to produce the calibration curve by reading the absorbencies at 700 nm ($y = 105.9x$; $r^2 > 0.99$; $P < 0.000\,1$). The iron (III) reducing activity of each sample was obtained from 2 independent triplet determinations and expressed in mgAAE/g of extract.

2.5.4. Lipid peroxidation inhibitory assay

The described method from Jaishree *et al*. in previous publication was used to evaluate the lipid peroxidation inhibition of samples [17]. The production of chromogen A from lecithin degradation was induced by $FeCl_3$, ascorbic acid, TCA and 2-thiobarbituric acid. The extract inhibitory powers (100 µg/mL) were obtained by reading the absorbance at 532 nm. The inhibition percentage was calculated by using a proper blank and negative control. All tests were achieved for 2 triplet independent assays.

2.5.5. Desoxyribose degradation inhibitory assay

The inhibition of desoxyribose degradation was assessed by using the experimental procedure developed by Perjési and Rozmer [18]. In the presence of Fe^{2+}-ethylene diamine tetraacetic acid and H_2O_2 (10 mmol/L), the desoxyribose was degraded to methane dicarboxylic aldehyde according to a Fenton reaction. A combination of methane dicarboxylic aldehyde and 2-thiobarbituric acid produced a color indicator reaction at 532 nm. The extract was used at 100 µg/mL of final concentration. All experiments were accomplished in triplicate for 2 independent tests. Hydroxyl radical scavenging effect was expressed as the percentage of inhibition of 2-deoxyribose oxidation.

2.5.6. Cyclooxygenase (COX) inhibition assay

Inhibition assay of COX-1 and COX-2 activities was performed by using a commercial available colorimetric COX (ovine) inhibitor screening assay kit (Cayman Chemical Company, USA). All the inhibitors added to the reaction system were dissolved in appropriate solvent and prepared just before using. In this assay, the COX activity was measured by using N,N,N,′N′-tetramethyl-*p*-phenylenediamine (TMPD) as a co-substrate with arachidonic acid. TMPD oxidation was monitored spectrophotometrically with a 96-well plate reader at 590 nm (BioTek instruments, USA). Different extracts were used at 100 µg/mL final concentration in total volume of 220 µL.

2.6. Phytochemical investigations

2.6.1. TPC determination

TPC was evaluated by using Folin–Ciocalteu colorimetric assay described by Compaoré *et al*. with minor modifications [14]. Twenty-five microliters of sample was added to 125 µL of Folin–Ciocalteu reagent (0.2 mol/L) and incubated during 5 min following by adding 100 µL of sodium carbonate (75 g/L). After 1 h of dark incubation, the absorbance was recorded at 760 nm with microplate reader. Gallic acid was used to produce the standard curve ($y = 201x - 21.22$, $r^2 > 0.99$, $P < 0.000\,1$) and the results were done in mgGAE/g of extract.

2.6.2. TFC determination

TFC was determined according to previous method described by Compaoré *et al*. with some modifications [14]. One hundred microliters of sample and 100 µL of $AlCl_3$ (2%) were mixed in 96 micro-well plate and incubated for 10 min. The absorbance was measured at 415 nm with microplate reader. The standard curve was generated with variable concentration of quercetin at 415 nm ($y = 39.8x - 3.5$; $r^2 = 0.99$, $P < 0.000\,1$) and the results were done as mgQE/g of sample.

2.6.3. Identification and quantitative determinations of polyphenols

To assess the quantities of polyphenolic compounds in the bioactive fractions, the previous experimental processing was used according to Compaoré *et al*. [14]. Seven phenolic acids and 11 flavonoid compounds were used as internal standard HPLC analysis. The mass spectrometry was used for compound identification and the UV trace was used for the identified compound quantification.

2.7. Data analysis

All data were analyzed by using Excel for calculating means and standard deviation. One-way ANOVA of GraphPad software was used for measuring the statistical difference and obtaining the graph and correlation.

3. Results

3.1. Antioxidant activities

The antioxidative activity of the plant extracts was evaluated by 5 methods and the results were shown in Table 1. ABTS radical scavenging activities were decreasing from (40.75 ± 0.43) to (5.06 ± 0.48) mMET/g. In contrast, all fractions were presented similar DPPH radical scavenging effect (around 60 mgQE/g) except HF and DCMF. Interestingly, all fractions were showing powerful iron (III) reduction ability which increased from (15.03 ± 2.14) to (435.77 ± 20.57) mgAAE/g. Concerning the lipid peroxidation inhibition effect, all the fractions (100 µg/mL final concentration) of *C. africana* were active except HF. Meanwhile, only BuOHF, MeOHF and EAF from *L. africana* were able to inhibit lipid peroxidation. MeOHFs from *C. africana* and *L. africana* were presenting the

best inhibition percentage with (78.57 ± 3.04)% and (70.07 ± 0.36)% respectively. All active fractions presented an IC_{50} inferior to 100 µg/mL (final concentration) in desoxyribose degradation inhibition, with ACNF from *C. africana* inhibiting (83.79 ± 0.28)%. According to the total antioxidant activity, the best fractions were EAF, MeOHF and BuOHF from *L. africana* and ACNF, EAF and BuOHF from *C. africana*. In general, *C. africana* fractions showed better antioxidant activity than *L. africana* fractions.

3.2. Anti-inflammatory effect

The inhibition percentage of prostaglandins biosynthesis was presented in Figure 1. According to our data, COX-1 inhibition was decreased from (67.36 ± 0.29)% to (10.16 ± 2.71)% and COX-2 inhibition from (64.24 ± 1.16)% to (12.74 ± 1.90)%. In considering the type of extract, it was shown that COX inhibitors were in polar extract. Certainly, the different extracts contained some bioactive metabolites.

3.3. Phenolic and flavonoid contents

The yield data in Table 1 showed the existence of extractive compounds with extensive polarity. Particularly, plants contained some variable phenolic and flavonoid metabolites. The TPC was ranging from (819.39 ± 10.56) to (5.86 ± 0.64) mgGAE/g and the highest flavonoid content was (30.21 ± 0.49) mgQE/g. Remarkably, all the extracts from *C. africana* were very poor in flavonoid content contrary to the fractions from *L. africana*. For example, flavonoid content represented around 8% of phenolics from BuOHF from *L. africana*.

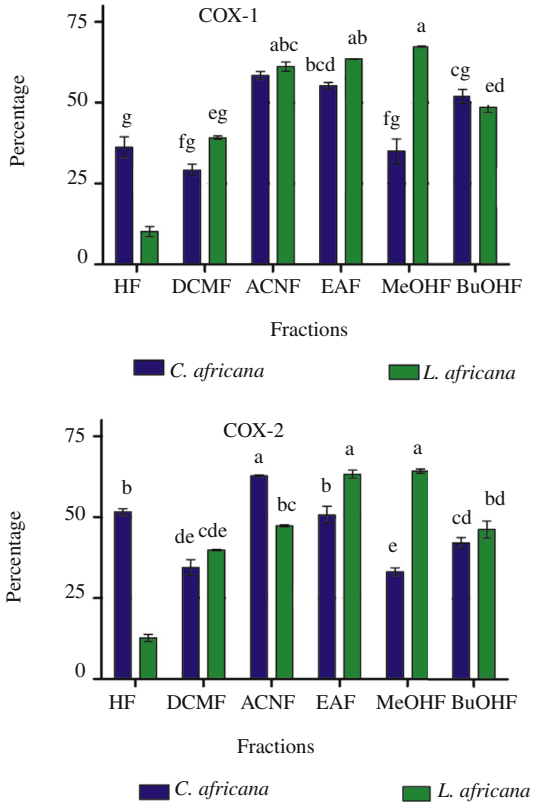

Figure 1. Inhibition percentage of COX-1 and COX-2.
Data with same letters were not statistically significant. $n = 2 \times 3$.

3.4. Phenolic compositions analysis

Polar fractions of plants were analyzed by high performance liquid chromatography-mass spectrometry (HPLC-MS) but only data from EAF, ACNF (*L. africana*) and BuOHF (*C. africana*) analysis were shown in Tables 2 and 3. The two fractions from *L. africana* presented the similar chromatographic profile with isoquercitrin, rutin, quercetin, quercitrin and *p*-coumaric acid that were quantified. EAF from *C. africana* was distinguished to EAF from *L. africana* by the presence of kaempferol and apigenin. The profile of BuOHF from *C. africana* was characterized by the presence of ferulic acid, *p*-coumaric acid [931.36 µg equivalent/gram (µgE/g)], rutin, isoquercitrin, quercetin and quercitrin (126.83 µgE/g). Gentisic and ferulic acids were detected in ACNF from *L. africana*, gentisic and chlorogenic acids in EAF and gentisic and caffeic acids in BuOHF from *C. africana*. In contrast, caftaric and sinapic acids, hyperoside, myricetin, fisetin, patuletin and luteolin were not identified in the fractions of plants. According to the chromatogram profiles (data not shown), five unknown major compounds were notified at 8.5, 9.4, 12.2, 22.3 and 28 min (retention time) in EAF and 3 compounds at 22–25 min in BuOHF from *C. africana*. As in *C. africana* fractions, *L. africana* fraction showed five major compounds at 18.7, 21.1, 21.9, 23.4 and 24.9 min in EAF and seven compounds from 21.5 to 33 min in ACNF. The unknown compounds with the retention superior to 20 min could be flavonoids according to their high retention time and UV absorbance. While the HPLC analysis data of MeOH, BuOHF from *L. africana* and ACNF from *C. africana* were not presented in the present study. According to their biological and phytochemical profiling, they will be used in the anti-COX compound isolation. The metabolites contributed to antioxidant and anti-inflammatory effects significantly (Table 4). There were a significant correlation between phenolic contents and antioxidant activity and a good specific contribution of flavonoids that was

Table 2
Identified and quantified compounds (µgE/g) in *L. africana* by HPLC-MS analysis.

Compounds	EAF	ACNF	Total quantity
p-Coumaric acid	22.83	34.10	56.93
Isoquercitrin	19.80	15.95	35.75
Rutin	12.55	08.85	21.40
Quercitrin	20.90	34.95	55.85
Quercetin	04.35	04.93	09.28

Data were obtained by one procedure analysis.

Table 3
Identified and quantified compounds (µgE/g) in *C. africana* by HPLC-MS analysis.

Compounds	EAF	BuOHF	Total quantity
p-Coumaric acid	–	951.45	951.45
Ferulic acid	–	29.10	29.10
Rutin	51.50	88.25	139.75
Isoquercitrin	15.40	70.40	85.80
Quercitrin	22.52	126.83	149.35
Quercetin	26.25	15.35	41.60
Kaempferol	14.30	–	14.30
Apigenin	13.38	–	13.38

Data were obtained by one procedure analysis.

Table 4
Positive correlation matrix.

	TPC	ABTS	FRAP	COX-1	COX-2	DPPH	I% LPO	I% DRB
TPC	1							
ABTS	0.95	1						
FRAP	0.98	0.96	1					
COX-1	0.63	0.75	0.64	1				
COX-2	0.59	0.63	0.58	0.87	1			
DPPH	0.70	0.82	0.68	0.75	0.50	1		
I% LPO	0.55	0.60	0.60	0.32*	0.27*	0.51	1	
I% DRB	0.66	0.68	0.61	0.31*	0.06*	0.77	0.47	1

Asterisk data mean an unsignificant correlation.

varying from 0.3 to 1.5. Similarly, phenolic compounds contributed to COX inhibition significantly ($r = 0.60$, $P < 0.05$).

4. Discussion

According to World Health Organization statistical studies, people in Burkina Faso have a very low economic level and the population used the plants for health care appropriately [19]. These facts lead scientists from Burkina Faso to focus their interest in improving traditional medicine. The purpose of this investigation was to assess the antioxidative and anti-inflammatory effects as well as to determine the phenolics composition of active extracts of medicinal plants. Our finding has demonstrated the interesting antioxidant activity of EAF, MeOHF and BuOHF from the 2 plants as well as the enzymes inhibitory effect of ACNF, EAF and MeOHF. According to Fawole *et al*. [20] and Vogl *et al*. [21], MeOHF, EAF, ACNF from *L. africana* and ACNF from *C. africana* which presented a hight COX-1 inhibition at 100 µg/mL could contain some potential inhibitors in a first level, and MeOHF and EAF from *L. africana* and ACNF from *C. africana* in a second level. According to previous study, *C. africana* stem leaves extracts showed the anti-DPPH$^{\bullet}$ and anti-ABTS$^{\bullet+}$ effects and didn't present any 5-lipoxygenase inhibitory effect, an inflammatory enzyme [6].

These antioxidant and anti-inflammatory activities can be explained by the presence of metabolites notably, including the total phenolics and flavonoids found in different extracts and the identified compounds. The significant correlation of phenolics to the biological activity conformed with previous data that indicated the important contribution of phenolics and flavonoids [14,16,22]. Thereby, the anti-DPPH$^{\bullet}$ and anti-ABTS$^{\bullet+}$ activities of quercitrin, isoquercitrin, rutin and *p*-coumaric acid have been demonstrated [23–25]. Additionally, rutin, isoquercitrin, quercetin, apigenin and kaempferol, ferulic acid and caffeic acid were good lipid peroxidation and desoxyribose degradation inhibitors [23,24,26,27]. In the same way, *p*-coumaric acid, rutin, isoquercitrin, quercetin and kaempferol have been shown to possess anti-COX activity [28–31]. Interestingly, the significant correlation between antiradical scavenging and COX inhibition in the first way and between the Fe^{3+} reduction and COX inhibition in the second way suggested a mechanism of fractions inhibitory actions (Table 4). For instance, plant extract by reducing iron in COX heme could jam enzyme activity. Mechanistically, these data could be explained the *in vivo* anti-inflammatory effect and the DNA scission regulation of extracts from *C. africana* and its dihydroflavonol [11,13,32]. The antioxidant and anti-inflammatory properties of these plants could justify its importance in the fight against oxidative stress diseases and its corollaries.

The presented study could explain the effectiveness of plants in traditional medicine in Burkina Faso, singularly *C. africana* and *L. africana*. In biological activities, the plant extracts presented some potential antioxidant and anti-inflammatory effects. The biological effects were explained by the content of bioactive compounds revealed by HPLC-MS analysis. Thereby, isoquercitrin, rutin, quercitrin, quercetin and *p*-coumaric acid, gentisic and ferulic acids were found to be the bioactive compounds in *L. africana* and *C. africana* extracts. Additionally, caffeic and chlorogenic acids, kaempferol and apigenin were detected in *C. africana* extract. The near future is to isolate new bioactive compounds form these plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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