



HAL
open science

GC-MS screening and evaluation of the anti-inflammatory and antioxidant activities of ethanolic leaves and stem barks extracts from *Dialium guineense* Willd, *Parkia biglobosa* (Jacq.) R. Br. ex Benth. and *Tamarindus indica* L

Senankpon Martial Gnansounou, Samy Iskandar, Lydia Abou, Maxime Robin, Carole Di Giorgio, Edwige Dahouenon, Philippe Piccerelle, Dominique Koko Sonhounhloue

► **To cite this version:**

Senankpon Martial Gnansounou, Samy Iskandar, Lydia Abou, Maxime Robin, Carole Di Giorgio, et al.. GC-MS screening and evaluation of the anti-inflammatory and antioxidant activities of ethanolic leaves and stem barks extracts from *Dialium guineense* Willd, *Parkia biglobosa* (Jacq.) R. Br. ex Benth. and *Tamarindus indica* L. *Journal of Pharmacognosy and Phytochemistry*, 2019, 8 (1), pp.295-301. <hal-01998405>

HAL Id: hal-01998405

<https://hal.science/hal-01998405v1>

Submitted on 4 Mar 2019

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization



E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(1): 295-301
Received: 21-11-2018
Accepted: 25-12-2018

Senankpon Martial Gnansounou

(1) Laboratoire d'Etude et de Recherche en Chimie Appliquée, Université d'Abomey Calavi, Abomey Calvi, Benin

(2) Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

(3) IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

Samy Iskandar

(1) Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

(2) IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

Lydia Abou

Laboratoire de Chimie Analytique, Qualitologie et Nutrition, UMR 910Génétique, UMR CNRS INRA/INSERM UMR-S 1263 C2VN Faculté de Pharmacie, 27 Blvd Jean Moulin, Marseille 13385, France

Maxime Robin

(1) Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

(2) IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

Carole DI Giorgio

IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

Edwige Dahouenon Ahoussi

Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

Philippe Piccerelle

(1) Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

(2) IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

Dominique Koko Sonhounhlope

Laboratoire d'Etude et de Recherche en Chimie Appliquée, Université d'Abomey Calavi, Abomey Calvi, Benin

Correspondence**Senankpon Martial Gnansounou**

(1) Laboratoire d'Etude et de Recherche en Chimie Appliquée, Université d'Abomey Calavi, Abomey Calvi, Benin

(2) Laboratoire de Pharmacie Galénique, Pharmaceuterie Industrielle, Biopharmacie et Cosmétologie, Faculté de Pharmacie, Aix Marseille Université, 27 Blvd Jean Moulin, Marseille 13385, France

(3) IMBE (Institut Méditerranéen de Biodiversité et d'Ecologie Marine et Continentale), UMR CNRS 7263, IRD 237, Faculté de Pharmacie, Aix Marseille Université, 27 Boulevard Jean Moulin, 13385 Marseille, France

GC-MS screening and evaluation of the anti-inflammatory and antioxidant activities of ethanolic leaves and stem barks extracts from *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) R. Br. ex Benth. and *Tamarindus indica* L

Senankpon Martial Gnansounou, Samy Iskandar, Lydia Abou, Maxime Robin, Carole DI Giorgio, Edwige Dahouenon Ahoussi, Philippe Piccerelle and Dominique Koko Sonhounhlope

Abstract

Inflammation has been shown to be greatly involved in the degenerative processes of human skin such as photo aging and atopy. Reduction of low-grade inflammatory reactions by topical products may be necessary in case of skin aggression, to obtain an optimal wound healing and restore the physiological balance of human skin. Present work evaluated the antioxidant, anti-inflammatory and phototoxic properties of ethanolic extracts from leaves and stem barks of *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) R. Br. Ex Benth. and *Tamarindus indica* L. The antioxidant power of the extracts, measured *in vitro* by the KRL method, showed that each gram of *D. guineense* bark extract, *P. biglobosa* leaves extract and *T. indica* bark and leaves extracts has an antioxidant capacity equivalent to 1585, 2092, 5071 and 2246 mg of Trolox respectively. Simultaneously with their actions on cell viability, the anti-inflammatory activity of the extracts was monitored measuring their effects on NO production by mouse macrophages submitted to LPS from *E. coli* to determine the anti-inflammatory ratio of each extract. The bark and leaves of *D. guineense* Willd., the leaves of *P. biglobosa* (Jacq.) R. Br. Ex Benth. and the bark of *T. indica* L. have anti-inflammatory ratios from 161 to 458.2, whereas Dexamethasone (positive control) has a ratio of 37.87. The *in vitro* 3T3 NRU test was used on mouse fibroblasts to determine the phototoxicity of the six extracts. Only *D. guineense* Willd. stem bark was photo-toxic with a photo-irritation factor greater than 5 (PIF = 8.39). Our study report some molecules such as lupeol, amaryn, sitosterol for the first time in the extracts and shows that the use of these plants in traditional medicine is justified.

Keywords: anti-inflammatory, antioxidant, GC-MS, ethanolic extracts, lupeol, sitosterol, stigmasterol

Introduction

Living beings have two major common enemies: aging and diseases. Although a natural process, aging can be accelerated by oxidative stress which seems closely related to inflammatory processes [1, 2]. Inflammation is the set of defense mechanisms by which the body recognize, destroy and eliminate all foreign substances. Inflammatory reaction sometimes exceeds its objectives leading to deleterious effects. It then needs to be managed. On the other hand, oxidative stress (due to increase of oxygen reactive species) is linked to many pathologies [3, 2, 1]. As implication of oxidative stress and inflammation in occurrence of several pathologies (diabetes, cancer, cardiovascular diseases) was highlighted [3-5] search for molecules with antioxidant and anti-inflammatory power now goes beyond the anti-aging or cosmetic framework. Therefore, during two past decades, scientific research focused on them worldwide. In Africa and many low and middle income countries, traditional knowledge based on medicinal plants is used to fight against oxidative stress and inflammation [6]. Resort to this form of medicine have been linked to cultural and economic reasons [7, 8]. However, we note a worldwide increase of medicinal plants use [9, 10] because of their renewable character and association with no side effects. It is then important to investigate the effectiveness of those medicines. This is the purpose of this study focused on three plants recurrent in Benin traditional medicine: *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) R. Br. Ex Benth., and *Tamarindus indica* L.. Valorization and safeguarding of those threatened plants in Benin [11, 12] and their recurrence in our ethnobotanical survey justify our choice on them. They belong to the Fabaceae family which is well know for its medicinal purposes [13-16].

Their aerial organs are widely used in the treatment of various infectious diseases in Benin. Because inflammatory reaction may occur in response to infections, natural compounds having both activities would be very interesting.

D. guineense is a dialioideae exclusively present in regions of tropical Africa and exceptionally in Sao Tome et Principe. It grows mainly in the wild as a shrub on land left fallow and in dry or dense forests, as well as forest galleries, from Senegal to southern Nigeria. In Benin, it is present from the coast to the southern region. Antibiotic properties are attributed to its roots, leaves and barks used alone or in combination with other plants in the treatment of malaria, coughs, bronchitis, diarrhea, palpitations, dysmenorrhea, ulcer, anemia, hemorrhoids [17, 18]. Concerning *P. biglobosa*, it is a Mimosoideae [19] present in tropical Africa between 3 ° and 15 ° North. It grows in tropical regions with high rainfall but also in arid environments. In Benin, it is an almost ubiquitous tree occupying the fifth position among the most used plants in traditional medicine [20]. Finally, *T. indica* is a detarioideae up to 25 meters tall [21] growing very well in semi-humid or arid regions [22]. With well known laxative and carminative effects, its organs are used in the treatment of a wide range of pathologies such as toothaches [23], bacterial infections, malaria [24].

Material and Methods

Leaves and bark of the plants were collected in three localities of Benin (Abomey-Calavi, Cotonou and Parakou). Samples were formally identified at the Benin National Herbarium under the voucher numbers AA 6727 / HNB, AA 6728 / HNB and AA 6729/ HNB for *Dialium guineense* Willd., *Parkia biglobosa* (Jacq.) R. Br. Ex Benth., and *Tamarindus indica* L. respectively.

RAW 264.7 macrophage assay has been used to monitor the inhibitory effects of the extracts on the low-grade inflammatory cascade (RAW 264.7, Sigma-Aldrich, N° P6110401, Lot. 09I006). Phototoxicity was assessed using Balb / c 3T3 mouse fibroblasts (3T3-L1) (ATCC, United States, ATCC® CL-173™, N° P6110401, Lot. 09I006). DMEM (Dulbecco's Minimum Essential Medium) with stable L-glutamine supplemented with Penicillin 100 IU/ml, streptomycin 100 µg/ml, and 10% of inactivated calf serum served as culture medium. It was freshly prepared with a pH of 7.2. Standardized horse blood was used for KRL test. All test materials were diluted in dimethyl sulfoxide (DMSO, Sigma-Aldrich).

Anti-inflammatory test and cell viability

This *in vitro* anti-inflammatory assay is based on the ability of macrophages to generate a strong inflammatory response when stimulated with antigens. Mouse immortalized macrophages (RAW 264.7 cell line) were stimulated by *E. coli* LPS and exposed to the extracts for 24 hours. At the end of the incubation period, NO production was evaluated indirectly by measuring the accumulation of nitrite/nitrate in the culture medium using a spectrophotometric method based on the Griess reaction. Negative and positive controls were DMSO and Dexamethasone (1, 5, 10, 50 and 100 µM) respectively. Cell mitochondrial respiration measurement allowed to assess cell viability. Inhibition of NO release and inhibition of cell viability were expressed as percentages as compared to the negative controls:

$$\text{Percentage of NO release (\%)} = 100 \times (\text{OD}_{\text{test well}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})$$

$$\text{Percentage of Cell viability (\%)} = 100 \times (\text{OD}_{\text{test well}} - \text{OD}_{\text{blank}}) / (\text{OD}_{\text{control}} - \text{OD}_{\text{blank}})$$

The concentrations of the test material causing respectively a 50% decrease of NO release (IC_{50-NO release}) and a 50% decrease of cell viability (IC_{50-cell viability}) were calculated using the software Table curve Version 2.0. The anti-inflammatory ratio corresponded to the ratio between the anti-inflammatory activity and the toxicity. It was expressed as follows:

$$\text{Anti-inflammatory ratio} = \text{IC}_{50\text{-cell viability}} / \text{IC}_{50\text{-NO release}}$$

Phototoxicity and cytotoxicity measurement

The *in vitro* 3T3 NRU phototoxicity test was used to evaluate phototoxicity of the six ethanolic extracts measuring relative reduction in viability of cells exposed to them in presence of light versus absence of light. The Photo-Irritation-Factor (PIF) was calculated with concentrations (obtained by the software Phototox Version 2.0.) of the test material causing a 50% release of the preloaded Neutral Red without irradiation (IC_{50 -Irr}) and with irradiation (IC_{50 +Irr}) as compared to the control culture using the following formula:

$$\text{PIF} = \text{IC}_{50 (-Irr)} / \text{IC}_{50 (+Irr)}$$

On the other hand, the degree of membrane damage (i.e. the increase of released Neutral Red) was measured by a fluorescence-luminescence reader Infinite M200 Pro (TECAN). The Optical Density (OD) of each well was read at 540 nm. The results obtained for wells treated with the test material were compared to those of untreated control wells (HBSS, 100% viability) and converted to percentage values. Neutral Red desorbed solution serves as blank.

The percentages of cell viability were calculated as:

$$\text{Viability (\%)} = \frac{\text{Mean OD of test wells} - \text{mean OD of blanks}}{\text{Mean OD of negative control} - \text{mean OD of blanks}}$$

Kit Radicaux Libres (KRL) test

Evaluation of the overall resistance of standardized horse blood submitted to oxygen reactive species attack was made in absence versus presence of our extracts. The overall resistance of the control blood to the radical attack in the presence or absence of our products was expressed by the time at which 50% of the blood cells were lysed (T1 / 2 in minutes). The antiradical efficacy of the products was then expressed as a percentage of overall potential for antiradical defense of the control blood (% T1 / 2 of the control blood). The results were standardized in Trolox equivalents (water soluble analogue of vitamin E) and in equivalents Gallic acid (phenolic acid).

Gas chromatography and mass spectrometry analysis

100µl of each filtered extract were dried with nitrogen, then 100µl of BSTFA were added and the whole is incubated at 70 °C for 20 min. 2µl were injected for GC-MS analysis. The analyses were carried with a GC-MS apparatus consisting of a chromatograph Shiwazu QP, a mass spectrometer GCMS-QP2010S equipped with an electronic impact ion source and a quadrupole analyzer type, a GC Solution acquisition software as well as banks (NIST, WILEY). The compounds were separated using a DB-1MS capillary column (30 m x 0.25 x mm internal diameter x 0.25 µm film thickness from JW Scientific) which temperature limits were between -60 °C and 350 °C. The mobile phase was helium. Pressure in the column

was 3psi and the flow rate was 1.59 mL /min. The injector temperature (Inlet) was 280 °C, interface temperature was 280 °C, the source and the quadrupole at 150 °C. Temperature gradient was programmed as follows: 1 min at 60 °C, then increasing from 100 to 260 °C at 4 ° / min, then 30 min at 260

°C. The total elution time was 82 min. We focus on compounds with high retention time (>60min) in this work.

Results and Discussion

Results of anti-inflammatory activities and phototoxicity are reported in Tables 1&2 below.

Table 1: Anti-inflammatory, cytotoxic activity and anti-inflammatory ratio of the extracts

	NO release IC ₅₀ (µM or µg/mL)	Cell viability IC ₅₀ (µM or µg/mL)	Anti-inflammatory ratio
<i>Dialium guineense</i> barks	0.15 ± 0.01	68.74	458.2
<i>Dialium guineense</i> leaves	0.22 ± 0.05	43.17 ± 8.2	196.2
<i>Parkia biglobosa</i> barks	77.78 ± 9.4	>100	>1.3
<i>Parkia biglobosa</i> leaves	0.62 ± 0.08	>100	>161
<i>Tamarindus indica</i> barks	0.28 ± 0.02	48.91 ± 10.1	174.6
<i>Tamarindus indica</i> leaves	>100	>100	-
Dexamethasone	4.31 ± 1.45 µM	163.22 ± 24.96 µM	37.87

Extracts of *Dialium guineense* (both leaf and stem), those of leaves of *Parkia biglobosa* and barks of *Tamarindus indica* exert higher anti-inflammatory activity than Dexamethasone, the positive control (Fig. 1). Barks of *Dialium guineense* extract showed the best anti-inflammatory activity (Table 1). Sitosterol (0.77%) and lupeol acetate (0.55%) are triterpenoids we found and reported for the first time in this extract. Those two molecules have been reported to have interesting anti-inflammatory activity^[25, 26]. In association with phenolic compounds contained in this extract^[27-29] they could explain our result and support the use of *Dialium*

guineense by traditional healers. This extract has a high photo-irritation factor (Table 2). Therefore, it appears suitable to investigate the three others as anti-inflammatory drugs for cutaneous use. The leaves of this plant for example have half of the anti-inflammatory activity compared to the barks but is not phototoxic. It has higher tenor of sitosterol (3.5%) and lupeol acetate (1.7%) which seems to play a key role in phototoxicity as they are also more abundant in leaves of *P. biglobosa* (2.02% and 4.41%) and barks of *T. indica* (0.80% and 1.53%) (Tables 7&8).

Table 2: Phototoxicity results

	Toxicity in non-irradiated cells IC ₅₀ (-irr) (µM)	Toxicity in irradiated cells IC ₅₀ (+irr) (µM)	PIF	Phototoxicity
<i>Dialium guineense</i> barks	75.31 ± 8.54	8.97 ± 1.54	8.39	phototoxic
<i>Dialium guineense</i> leaves	47.56 ± 9.64	36.87 ± 4.21	1.28	Not phototoxic
<i>Parkia biglobosa</i> barks	130.85 ± 9.21	202.78 ± 9.47	0.64	Not phototoxic
<i>Parkia biglobosa</i> leaves	123.61 ± 2.21	66.33 ± 1.34	1.86	Not phototoxic
<i>Tamarindus indica</i> barks	53.64 ± 8.88	49.94 ± 9.54	1.07	Not phototoxic
<i>Tamarindus indica</i> leaves	8.76 ± 9.47	>100	-	Not phototoxic
Chlorpromazine	46.33 ± 2.96	1.04 ± 0.25	44.54	phototoxic

-irr: not irradiated, +irr: irradiated

It clearly appears on Figure 1 that, despite its great anti-inflammatory activity, only *Dialium guineense* barks extract exerted phototoxicity in our study. Leaves extract of this plant have lower anti-inflammatory activity than barks but are not phototoxic. This can be due to the difference of abundance in

terpenoids like sitosterol (0.78% vs trace) and lupenone (0.42% vs trace). *Parkia biglobosa* leaves and *Tamarindus indica* barks extracts also aren't phototoxic and have higher amount of sitosterol, lupeol or lupenone than *Dialium guineense* barks extract.

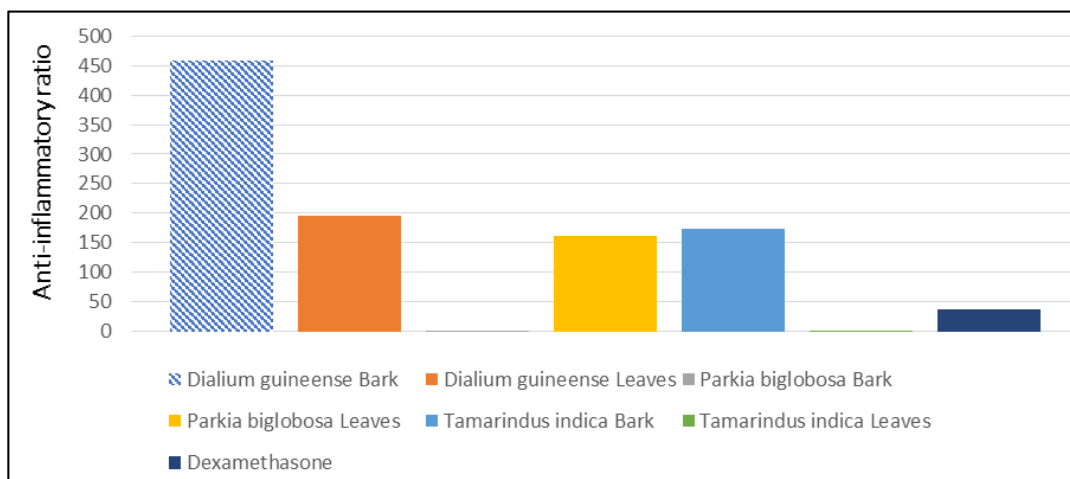
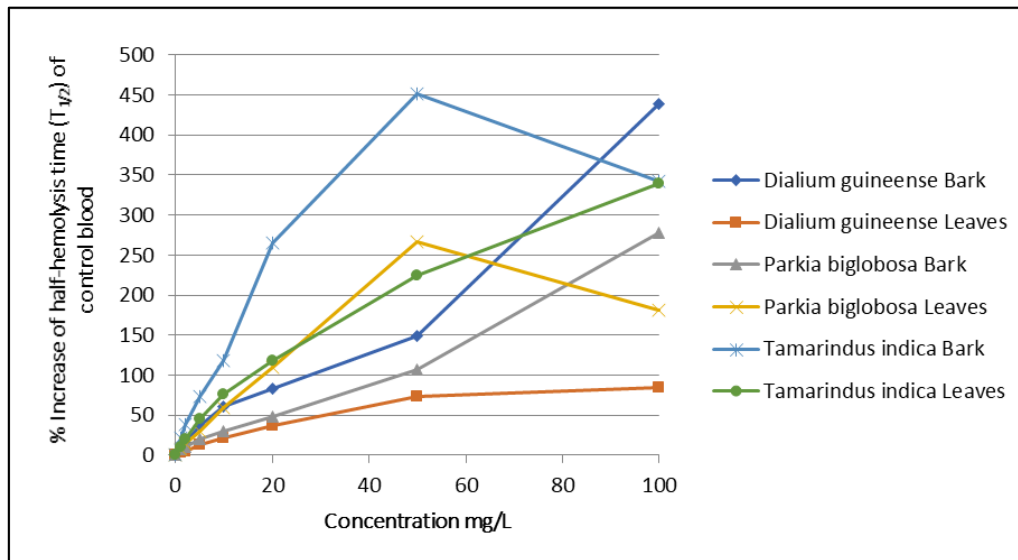


Fig 1: Anti-inflammatory activity and phototoxicity of ethanolic extracts

Table 3: antioxidant activity of the extracts

Concentrations (mg/L) *	% increase of half-hemolysis time ($T_{1/2}$) of control blood					
	<i>Dialium guineense</i>		<i>Parkia biglobosa</i>		<i>Tamarindus indica</i>	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	5.64	3.64	6.94	7.30	21.87	10.93
2	15.85	5.39	9.83	14.08	38.19	20.34
5	36.70	13.63	19.65	30.63	73.14	45.10
10	61.33	20.98	30.72	59.86	118.05	75.95
20	82.93	36.80	47.83	109.48	265.36	117.55
50	149.18	73.47	106.94	266.33	451.18	224.66
100	438.64	85.30	277.53	181.70	342.87	338.91

* Concentration in mg of extract per liter of reaction medium

**Fig 2:** Antioxidant activity of plant extracts

KRL test results indicate that all the tested plant extracts have a dose-dependent antiradical capacity (Fig. 2) and this antioxidant power is strongly increasing in a concentration range of 0 to 20 mg/L (Table 3). At 20 mg/L, leaves of *Parkia biglobosa*, leaves and barks of *Tamarindus indica* extracts

have remarkable results. They increased the resistance of the control blood to a radical attack up to 109.48, 265.36 and 117.55% respectively (Table 3). Also, those extracts were at least as effective as Trolox and gallic acid.

Table 4: Anti-radical power of extracts vs Trolox (Equivalent Trolox mg / g product)

Concentrations (mg/L) *	Antioxidant activity of plant extracts of Benin: Equivalents mg of Trolox/g of extract					
	<i>Dialium guineense</i>		<i>Parkia biglobosa</i>		<i>Tamarindus indica</i>	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	2157,55	1391,50	2654,27	2789,66	8360,74	4177,09
2	3028,74	1029,43	1879,17	2689,96	7297,49	3887,81
5	2805,47	1041,60	1502,12	2341,37	5591,19	3447,83
10	2344,19	802,04	1174,09	2287,81	4512,03	2902,86
20	1584,90	703,19	914,09	2092,13	5071,12	2246,38
50	1140,39	561,58	817,49	2035,83	3448,87	1717,37
100	1676,51	326,03	1060,73	694,48	1310,49	1295,33

Table 5: Anti-radical power of extracts vs Gallic acid (Equivalent Trolox mg / g product)

Concentrations (mg/L) *	Antioxidant activity of plant extracts of Benin: Equivalents mg of Gallic acid/g of extract					
	<i>Dialium guineense</i>		<i>Parkia biglobosa</i>		<i>Tamarindus indica</i>	
	Barks	leaves	Barks	leaves	Barks	leaves
0	0.00	0.00	0.00	0.00	0.00	0.00
1	1027,26	662,53	1263,76	1328,22	3980,74	1988,81
2	1442,05	490,14	894,72	1280,75	3474,50	1851,08
5	1335,75	495,93	715,19	1114,78	2662,09	1641,59
10	1116,12	381,87	559,01	1089,28	2148,28	1382,12
20	754,61	334,80	435,22	996,11	2414,48	1069,55
50	542,96	267,38	389,23	969,31	1642,08	817,68
100	798,22	155,23	505,04	330,66	623,96	616,74

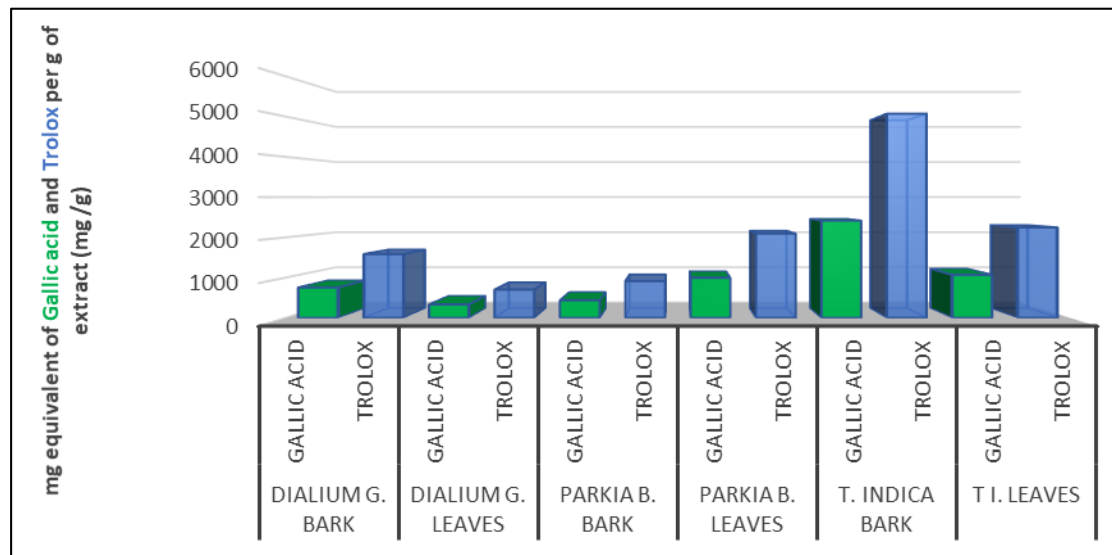


Fig 3: Comparison of the antioxidant power of the extracts with Gallic acid and Trolox at a concentration of 20 mg/L

As shown in Fig. 3, *Tamarindus indica*'s barks ethanolic extract is particularly interesting: it is five times more active than Trolox (Table 4) and 2.5 times more active than gallic acid (Table 5). Lupeol (0.83%), amyirin (0.65%) and lupeol acetate (1.53%) were detected in this extract by GC-MS analysis (Table 8). Those molecules were reported in the plant [30] and their free radical scavenging property is well known [31-33]. Leaves extract of this plant also showed high free radical scavenging activity [34, 35].

Chemical compounds identified by GC-MS in the extracts are summarized below. Identification was based on direct comparison and at least 80% matching of the retention times and mass spectral data with those in the National Institute of Standards and Technology (NIST) and WILLEY libraries. DPPH free radical scavenging test of the methanolic extract of *Dialium guineense* leaves showed a concentration-dependent antioxidant activity supposed to be due to the phenolics compounds [36]. Leaves ethanolic extracts of *Parkia biglobosa* showed an antioxidant activity of 109.48% according to the KRL test. It contains lupenone (2.59%), β -Amyrin (1.05%), Sitosterol (2.02%) and lupeol acetate (4.41) all known for biologic activities. Other studies reported similar antiradical activity of the barks according to Diphenyl Picryl Phenyl Hydrazine (DPPH) test: 87.68% for aqueous extract [37].

Table 6: Triterpenoids identified in *Dialium guineense* by GC-MS

RT	Compounds	Leaves	Stem-barks
61.774	Free Sitosterol	5.92 %	1.48 %
62.337-62.442	Stigmasterol TMS	0.78	trace
63.086	Lupenone	0.26	0.2
63.794-63.873	Lupeol	0.42	-
63.794-63.873	Lupeol	trace	trace
64.603-64.838	Sitosterol TMS	2.72	0.77
66.108-66.433	Lupeol acetate	1.74	0.51

RT: Retention time

Sitosterol and lupeol acetate are reported here for the first time in those extracts. Despite their low amount, we suppose they contribute to the anti-inflammatory role and the antioxidant activities of the extracts as reported by several papers [38, 25, 33].

Table 7: Triterpenoids identified in *Parkia Biglobosa* by GC-MS

RT	Compounds	Leaves	Stem-barks
60.665	Taraxerone	10.44%	7.31%
62.372-62.442	Stigmasterol TMS	-	0.66
62.883-63.086	Lupenone	0.37	0.72
62.883-63.086	Lupenone	2.59	1.14
63.840-63.873	Lupeol	trace	trace
64.457	beta-Amyrin TMS	1.05	-
64.636	beta Sitosterol	-	1.18
64.838	Sitosterol TMS	2.02	-
66.196-66.433	Lupeol acetate	4.41	3.61

Beta-Amyrin is present in *Parkia Biglobosa* leaves extract which is as active as gallic acid and two times more active than Trolox (Fig. 3). It is well known for its antioxidative and anti-inflammatory activities [39, 40].

Table 8: Triterpenoids identified in *Tamarindus indica* by GC-MS

RT	Compounds	Leaves	Stem-barks
62.25	Alpha-Amyrin TMS	4.04%	3.81%
62.316 & 62.385	Stigmasterol TMS	-	0.23
62.316 & 62.385	Stigmasterol TMS	trace	trace
62.869 & 63.023	Lupenone	1.75	trace
63.817	Lupeol	-	0.83
64.345	beta-Amyrin TMS	-	0.42
64.612	beta-Sitosterol	-	0.80
64.724	Sitosterol TMS	1.07	-
66.138 & 66.219	Lupeol acetate	1.22	1.53

Lupeol, beta amyirin and beta sitosterol could be involved in the good antioxidant and anti-inflammatory activity of *Tamarindus indica* barks extract. This extract is five times more active than Trolox and two times more active than gallic acid (Fig 3).

Conclusion

In the present study, ethanolic extracts of leaves and barks of *Dialium guineense*, *Parkia biglobosa* and *Tamarindus indica*, showed interesting antioxidant and anti-inflammatory properties. Lupeol acetate and Sitosterol were reported for the first time in *Dialium guineense* barks. Lupenone, amyirin, stigmasterol and taraxenone were also identified in the extracts and could explain their biological activity. The amounts of those compounds and their synergy also seem to play a key role in the biological activities. Except barks

extracts of *Dialium guineense* which is phototoxic, the other extracts can be investigate as cosmetic agents.

Abbreviations

DMSO: Dimethyl sulfoxide

DPPH: Diphenyl Picryl Phenyl Hydrazine

KRL: Kit Radicaux Libres

LPS: Lipopolysaccharide

PIF: Photo-Irritation-Factor

Declarations

- **Ethics approval and consent to participate:** Not applicable.
- **Consent to publish:** Not applicable
- **Availability of data and materials:** All data generated or analyzed during this study are included in this published article (However, raw data are available from the corresponding author on reasonable request).
- **Competing interests:** “The authors declare that they have no competing interests.”
- **Funding:** France and Benin governments
- Authors' Contributions

S.M.G. is corresponding author, PhD student working on the subject. He carried out most of the test and wrote this manuscript. S. A. performed KRL tests, figures and tables. L. A. make GC-MS analyzes. C.D.G. performed anti-inflammatory tests. M.R. make extractions and assisted L.A for GC-MS analyzes. E.D.A. is PhD supervisor and direct and correct this manuscript. P.P. is co- Supervisor of thesis. He also direct and correct the manuscript. D.C.S is member of thesis committee and he correct the manuscript.

All authors read and approved the final manuscript for submission. They have no conflict of interest to declare.

- **Acknowledgements:** Authors are grateful for Caroline ORNETO for her technical support and for Mr. for his english language support.

References

- Salzano S. *et al.* Linkage of inflammation and oxidative stress via release of glutathionylated peroxiredoxin-2, which acts as a danger signal,” *Proc. Natl. Acad. Sci.*, 2014; 111(33):12157-12162.
- Reuter S, Gupta SC, Chaturvedi MM, Aggarwal BB. “Oxidative stress, inflammation, and cancer: How are they linked?,” *Free Radic. Biol. Med.* 2010; 49(11):1603-1616
- Khansari N, Shakiba Y, Mahmoudi M. “Chronic Inflammation and Oxidative Stress as a Major Cause of Age- Related Diseases and Cancer,” *Recent Pat. Inflamm. Allergy Drug Discov.* 2009; 3(1):73-80.
- Eiró N, Vizoso FJ. Inflammation and cancer,” *World J. Gastrointest. Surg.* 2012; 4(3):62-72.
- Golbidi S, Badran M, Laher I. Antioxidant and anti-inflammatory effects of exercise in diabetic patients,” *Exp. Diabetes Res.*, 2012; 941868, 2012.
- Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN. “The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African,” *BMC Complement. Altern. Med.* 2015; 15:159
- Sato A. “Revealing the popularity of traditional medicine in light of multiple recourses and outcome measurements from a user’s perspective in Ghana, *Health Policy Plan.* 2012; 27(8):625-637.
- Oyebode O, Kandala NB, Chilton PJ, Lilford RJ. Use of traditional medicine in middle-income countries: a WHO-SAGE study,” *Health Policy Plan.* 2016; 31(8):984-991.
- Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health: an ethical analysis,” *Bull. World Health Organ.* 2008; 86(8):594-599.
- Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety, *Front. Pharmacol.* 2014; 4:177.
- Meregini AOA. Some endangered plants producing edible fruits and seeds in Southeastern Nigeria,” *Fruits*, 2005; 60(3):211-220.
- Lykke AM. Local perceptions of vegetation change and priorities for conservation of woody-savanna vegetation in Senegal,” *J Environ. Manage.* 2000; 59(2):107-120.
- Rosado-Vallado M, Brito-Loeza W, Mena-Rejón G, Quintero-Marmol E, Flores-Guido J. “Antimicrobial activity of Fabaceae species used in Yucatan traditional medicine,” *Fitoterapia.* 2000; 71(5):570-573.
- Koné W, Atindehou KK, Terreaux C, Hostettmann K, Traoré D, Dosso M. Traditional medicine in North Côte-d’Ivoire: screening of 50 medicinal plants for antibacterial activity, *J. Ethnopharmacol.* 2004; 93(1):43-49.
- Ma X, Zheng C, Hu C, Rahman K, Qin L. “The genus *Desmodium* (Fabaceae)-traditional uses in Chinese medicine, phytochemistry and pharmacology, *J. Ethnopharmacol.*, 2011; 138(2):314-332.
- Rahman AM, Parvin MIA. Study of medicinal uses on Fabaceae family at Rajshahi, Bangladesh,” *Res. Plant Sci.* 2014; 2(1):6-8.
- Odukoya OA, Houghton PJ, Adelusi A, Omogbai EKI, Sanderson L, Whitfield PJ. Molluscicidal triterpenoid glycosides of *Dialium guineense*, *J. Nat. Prod.* 1996; 59(6):632-634.
- Bero J, *et al.*, *In vitro* antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria,” *J. Ethnopharmacol.* 2009; 122(3):439-444.
- Hopkins HC. The taxonomy, reproductive biology and economic potential of *Parkia* (Leguminosae: Mimosoideae) in Africa and Madagascar,” *Bot. J. Linn. Soc.* 1983; 87(2):135-167.
- Eyog Matig O, Adjanohoun E, de Souza S, Sinsin B. Programme de Ressources Genétiques Forestières en Afrique au Sud du Sahara (*Programme saforgen*). Bioversity International, 2001.
- Tariq M, Chaudhary SS, Rahman K, Zaman HR, Shaikh I. *Tamarindus Indica: AN Overview*,” *J Biol. Sci. Opin.*, 2013; 1(2):128-131.
- Meher B, Dash DK, Roy A. A review on: Phytochemistry, pharmacology and traditional uses of *Tamarindus indica* L,” *World J Pharm Pharm. Sci.* 2014; 3(10):229-240.
- Tapsoba H, Deschamps JP. Use of medicinal plants for the treatment of oral diseases in Burkina Faso,” *J. Ethnopharmacol.* 2006; 104(1, 2):68-78.
- Doughari JH. Antimicrobial activity of *Tamarindus indica* Linn,” *Trop. J. Pharm. Res.* 2006; 5(2):597-603.
- Loizou S, Lekakis I, Chrousos GP, Moutsatsou P. “ β -Sitosterol exhibits anti-inflammatory activity in human aortic endothelial cells,” *Mol. Nutr. Food Res.* 2010; 54(4):551-558.
- Lucetti DL, *et al.*, “Anti-inflammatory effects and possible mechanism of action of lupeol acetate isolated

- from *Himatanthus drasticus* (Mart.) Plumel,” J. Inflamm. 2010; 7(1):60.
27. Orji. “Antibacterial Activities of Crude Leaf and Bark Extracts of ‘Icheku’ *Dialium guineense* on Bacterial Isolates from Bronchitis Patients,” IOSR J. Pharm. Biol. Sci., 2012; 1(3):21-25.
 28. Olajubu F, Akpan I, Ojo DA, Oluwalana S. Antimicrobial potential of *Dialium guineense* (Wild.) stem bark on some clinical isolates in Nigeria,” Int. J. Appl. Basic Med. Res. 2012; 2(1):58-62.
 29. Obonga W, Nnadi C, Agbo M, Kenechukwu F, Nwodo U. Distribution of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Apparently Healthy Population and its Susceptibility to Saponins from *Dialium guineense*,” Br. J. Pharm. Res. 2014; 4(18):2200-2209.
 30. Jain A, Bhadoriya S, Ganeshpurkar A, Narwaria J, Rai G. *Tamarindus indica*: Extent of explored potential,” Pharmacogn. Rev., 2011; 5(9):73.
 31. Santiago LA, Mayor ABR. Lupeol: An antioxidant triterpene in *Ficus pseudopalma* Blanco (Moraceae),” Asian Pac. J. Trop. Biomed. 2014; 4(2):109-118.
 32. Tchimine MK, Nwaehujor CO, Ezenwali M, Okoli CC, Iwu M. “Free Radical Scavenging Activity of Lupeol Isolated from the Methanol Leaf Extract of *Crateva adansonii* Oliv. (Capparidaceae),” 2016; 8(3):8
 33. Parvez MK, Alam P, Arbab AH, Al-Dosari MS, Alhowiriny TA, Alqasoumi SI. “Analysis of antioxidative and antiviral biomarkers β -amyrin, β -sitosterol, lupeol, ursolic acid in *Guiera senegalensis* leaves extract by validated HPTLC methods,” Saudi Pharm. J. 2018.
 34. Razali N, Abdul Aziz A, Lim CY, Mat Junit S. “Investigation into the effects of antioxidant-rich extract of *Tamarindus indica* leaf on antioxidant enzyme activities, oxidative stress and gene expression profiles in HepG2 cells,” Peer J, 2015; 3:e1292.
 35. Razali N, Mat Junit S, Ariffin A, Ramli NSF, Abdul Aziz A. Polyphenols from the extract and fraction of *T. indica* seeds protected HepG2 cells against oxidative stress,” BMC Complement. Altern. Med., 2015; 15:438.
 36. Ogu GI, Ezeadila J, Ehiobu JM. Antioxidant and antimicrobial activities of *Dialium guineense* (Willd) leaf extract,” Pharm Pharmacol Res. 2013; 1(1):1-7.
 37. Olabinri BM, Adetutu A, Olaleye MT, Oluwafunsho BO, Oyeniya OO. A study of the antioxidative potentials of acetone and aqueous extracts of *Parkia biglobosa* and *Tetracarpidium conophorum* stem barks *in vitro*,” Int. J. Med. Med. Sci. 2013; 5(8):368-373.
 38. Awad AB, Burr AT, Fink CS. Effect of resveratrol and β -sitosterol in combination on reactive oxygen species and prostaglandin release by PC-3 cells,” Prostaglandins Leukot. Essent. Fatty Acids, 2005; 72(3):219-226.
 39. Hernandez L, Palazon J, Navarro-Oca A. “The Pentacyclic Triterpenes α , β -amyrins: A Review of Sources and Biological Activities,” in Phytochemicals - A Global Perspective of Their Role in Nutrition and Health, V. Rao, Ed. In Tech, 2012.
 40. Krishnan K, Mathew LE, Vijayalakshmi NR, Helen A. “Anti-inflammatory potential of β -amyrin, a triterpenoid isolated from *Costus igneus*,” Inflammopharmacology, 2014; 22(6):373-385.