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Abstract

Our study explored the scientific rationale behind the traditional use of medicinal plants as a foundation for evidence-based strategies for the management of sickle cell disease (SCD) in developing countries. Firstly, we conducted a survey among people living at Kisangani city in DR Congo to identify the plants used alone or in combination for the SCD management considering the following criteria: used parts, preparation methods, administration routes and common combinations with other substances. This step allowed to select the most used plants which were harvested from two different soil types and prepared according to local practices. Secondly, different preparations were tested at laboratory focusing on the antioxidant and anti-inflammatory biological activities which are mainly observed with people suffering from SCD. Out of 384 surveyed people, 201 reported the use of 45 medicinal plants whereas the additives comprised sugar, caramel, ash and lemon juice. *Alchornea cordifolia* Müll. Arg and *Hibiscus tiliaceus* L. were selected for laboratory analyses. Their leaves were collected from both dry and marshy soils and prepared following local methods with additive. *A. cordifolia* showed significantly higher antioxidant (p = 0.001) and anti-inflammatory activity (p = 0.01) than *H. tiliaceus*. Soil type influenced activity in *H. tiliaceus*, favoring marshy soil. However, sugar and caramel significantly reduced bioactivity, ash and lemon juice enhanced antioxidant effects without significantly altering anti-inflammatory properties.

Keywords: medicinal plants; soil type; additives; antioxidant; myeloperoxidase; sickle cell disease

1. Introduction

Nowadays the implementation of traditional medicines is needing more evidenced, particularly when using new or unknown medicines due to unavailability, shortage and over chronical diseases such as the sickle cell disease (SCD). The latter is the most prevalent hemoglobinopathy worldwide and a major cause of morbidity and mortality [1]. The disease results from a single amino acid substitution in the β-globin chain, leading to hemoglobin polymerization, intravascular hemolysis

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and the release of free hemoglobin and heme. These events trigger oxidative stress and inflammation through myeloperoxidase (MPO) activation, reactive oxygen species (ROS) generation and neutrophil extracellular trap (NET) formation, contributing to vaso-occlusion and organ damage [2,3]. SCD is characterized by chronic anemia, vaso-occlusive crises, acute chest syndrome, stroke, renal impairment, and splenic dysfunction [4,5], that constitute a long-term family burden. The sickness affects millions of people worldwide, with approximately 300,000 new cases per year, predominantly in Sub-Saharan Africa, the Middle East, and India, although migration contributes to its global spread [6]. In Africa, around thousand children are born daily with SCD, and more than half die before five years due to infections and severe anemia [7]. In the Democratic Republic of the Congo (DRC), SCD prevalence is about 2% among newborns, putting the country in second of most affected African country after Nigeria [8] with nearly 50,000 annually affected births and high early mortality [9]. In Kisangani, the homozygous prevalence among newborns increased to 2.2% while for heterozygotes it remained 21% which is quite stable [10,11]. SCD's management is mainly symptomatic, relying on drug treatments such as hydroxyurea and validated phytomedicines (e.g., Niprisan®) to alleviate pain, anemia, and vaso-occlusive crises [12–14]. Although bone marrow transplantation is beneficial in developed countries, it remains inaccessible in settings such as Kisangani [15]. Consequently, SCD is often classified as a neglected tropical disease, leading many patients to depend on medicinal plants [16,17]. The reliance on traditional medicine in Kisangani, stems from the high cost and limited availability of modern medicines, making them inaccessible to people with SCD. This is a significant challenge given the chronic nature of the disease and the need for consistent medical follow-up, which is often beyond the financial means of the local population [18,19]. Several studies in DRC have identified plants commonly used in traditional medicine [20]. Only few have been subjected to scientific research to provide evidence of their therapeutic use [21–23], however focusing mainly on active compounds identification and biological activities assessment related to SCD. The importance of traditional practices was not clearly evidenced to the use of medicinal plants. Therefore, considering this gap in Kisangani, we conducted an ethnobotanical survey in that city (Figure 1) to document medicinal plants used in the management of SCD and their modalities of use. Based on these findings, the selected species were submitted to laboratory investigation to evaluate the scientific relevance in relation with the SCD health consequences.

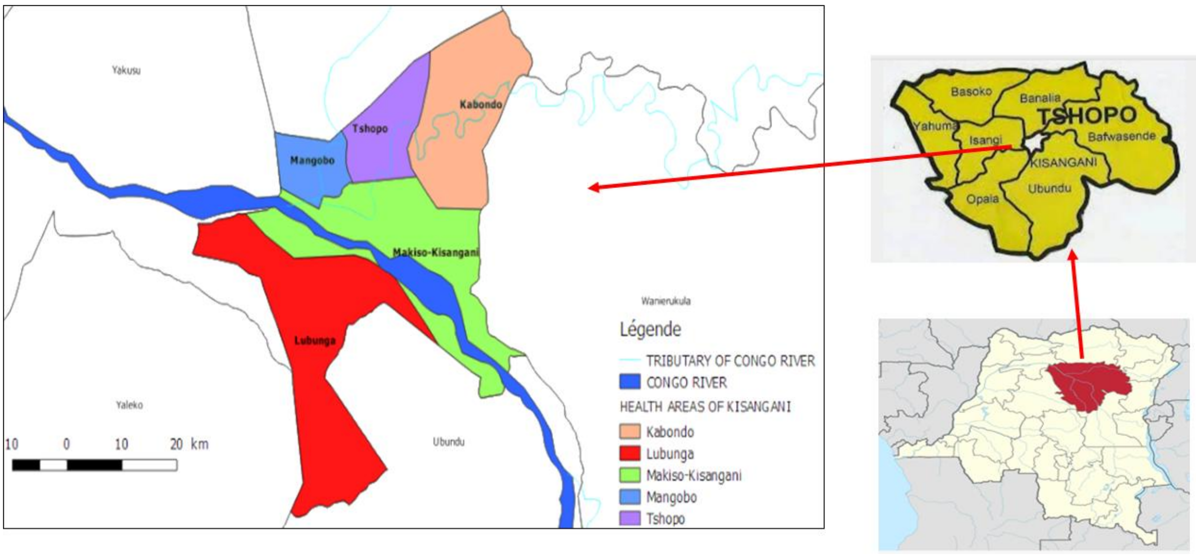

Figure 1. Map of Kisangani city crossed by the Congo river (0° 31′ 09″ north latitude and 25° 11′ 46″ east longitude, Google search, n.d.).

2. Results and Discussions

2.1. Ethnobotanical Survey Outcomes

2.1.1. Characteristics of Respondents

Out of 384 participants, 201 reported using medicinal plants for the management of SCD. Women (58.7%) represented more than half of the respondents which can be explained by their key role in caregiving and health management, particularly in children chronic diseases including SCD. Sociocultural perceptions in such disease often attribute responsibility to women. Another notable fact related to the survey is that most of the participants were educated, above 20-years, and filled free to claim being affected by SCD, either closely or indirectly, which explains why their responses may be reliable. In addition, their free participation along with the diversity of knowledge circumstances can testify that SCD acceptability and socio-cultural perception among people have evolved compared to 2007 where the patients were victim and rejected in the society [24]. The main knowledge circumstances were from friendship (39%), at the family (23%) and at school and university (15%). Media and churches were not much cited, whereas other participants were not clear with this point.

2.1.2. Plants Species Cited by Respondents and Way of Their Use

The respondents reported the use of 45 distinct plant species in Kisangani for the treatment of SCD. As shown in Table 1, 25 species were cited at least thrice by respondents meaning their usefulness. Seven species were mentioned once by two respondents and 13 species were mentioned by a single participant. The participants mentioned that some plants were discovered serendipitously, others by sharing knowledge with traditional healers, grandparents or friends. Even if they could not explain the biological mechanism of action of the plant recipes, the participants testified to have favorable results namely the loss of pain and the increase of hemoglobin. To ensure their identity, the plants were identified by their vernacular name and were given a deposit number from the Faculty of Science of Kisangani University along with the family. We also classified the plants according to use report (UR) which include the number of way or part a plant is used. For example, *Hibiscus tiliaceus* had 22 UR meaning that the 42 respondents have described 22 ways of using that plant, which is the highest UR value and confirms its importance and interest in SCD management. Concerning the used parts for all plants, no one could justify their choice. However, we noticed that leaves (fresh) were the most used as they are easily available. No one could also justify the requirements concerning the dry or fresh state of used parts, or the harvesting place, including the type of soil. It is worthy to remember that Kisangani city is located mainly in arid or swampy soils that can influence the recipes composition. Decoction was cited as the most used method preparation with a 108 UR (58.6%), followed by maceration (20.1%). The oral route was the most frequently cited for administration with a 154 UR (83.6%), since it is the easiest controllable route. However, the cutaneous and rectal routes, mentioned 10 and 3 times, respectively, caught our attention as they are little known routes of administration for such sickness as SCD. According to some believes, the cutaneous administration route seems appropriate because it alludes to a customary purification ceremony, given that SCD is considered as a curse. Thus, this use deserves further investigation to assess both their therapeutic value and safety, particularly in potentially vulnerable users such as children, pregnant women, SCD patients. Some respondents believe that plants grown in marshy soil would be more effective. This statement was confirmed by riverside communities namely "Genya" living in Congo river in Kisangani who testified employing water for spiritual deliverance and healing.

Table 1. Plants mentioned and use reports of each species cited.

Deposit number	Plant names	VN cited by participants	Family	Used parts	Preparation method	Administration route	Combination	FC	UR
MBA/01	*Acmella paniculata* (Wall. ex DC) R.K.Jansen	Kekemu	Asteraceae	WP, FL	Dec	Rectal, Oral	nd	2	2

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MBA/05	*Alchornea cordifolia* (Schumach.) Müll.Arg.	Mabanzi	Euphorbiaceae	FL, DLT	Inf, Dec, Mac	Oral	Ash, sugar, Caramel, canned tomato, Lemon juice	17	3
nd	*Amaranthus cruentus* L.	Muchicha	Amaranthaceae	FL	Cooking	Oral	Sugar, Limon Juice	3	3
nd	*Ananas comosus* (L.) Merr.	Anana	Bromeliaceae	Fruit	Mac, EJ, Dec	Oral	Canned tomato and Soft drink, Sugar, Eggs, Caramel	7	6
MBA/25	*Andasonia digitata* L.	Liguma	Malvaceae	FL, DF	Dec, Mac	Oral	Sugar, Sugar milk, Eggs and folic acid, Caramel, Milk and eggs, Eggs and sugar	8	6
MBA/35	*Anisopappus chinensis* Hook.& Arn.	Nzete ya makila	Asteraceae	FL, Flower	Mac, Dec	Oral, Cutaneous	Ash	5	4
MBA/28	*Annona reticulata* L.	Bizabibu, Libombi	Annonaceae	FL	Dec	Oral	Ash	1	1
nd	*Annonidium manii* (Oliv.)	Nzete ya bombi	Annonaceae	FL, Bark	Dec	Oral, Cutaneous	nd	4	2
MBA/6	*Bidens pilosa* L.	Police	Asteraceae	WP	Dec	Oral	nd	1	1
MBA/20	*Bridelia atroviridis* Müll.Arg.	Mgiangange	Phyllanthaceae	FL	Dec	Oral	nd	1	1
MBA/07	*Carica papaya* L.	Payipayi	Caricaceae	FL, DLT	Dec, Mac	Oral, Cutaneous	*C.citratus* root, Ash, Caramel, Peneapple juice, Sugar, Canned tomato, Soy flour, Caterpillar	14	13
MBA/23	*Catharanthus roseus* (L.) G. Don		Apocynaceae	Flower	Dec	Oral	Sugar	1	1
MBA/11	*Cocos nucifera* L.	Cocoti	Arecaceae	Juice, FL	Mixt, Dec	Oral	Soft drink, Sugar	3	3
MBA/29	*Coffea robusta* L. Linden	Kafé	Rubiaceae	FL, Seed	Dec, Mac, Grinding	Oral	*C. citratus leaves*, Sugar milk and Eggs, Caramel, Ash	4	5
MBA/22	*Cosmos sulphureus* Cav.	Maloti	Asteraceae	Flower, leaves, Root	Mac, EJ, Dec Grinding	Oral	Ash, Sugar	10	9
nd	*Cucurbita pepo* L.	Kasa ya maboke, djurubi	Cucurbitaceae	FL	EJ, Dec, Mac	Oral	Sugar, Ash, Caramel	6	6

MBA/21	*Cymbopogon citratus* (DC.)Stapf	Nyasi	Poaceae	FL, Root	Inf, Dec	Oral	*Bidens pilosa* and sugar, *Curcuma longa*	3	3
MBA/16	*Elaeis guineensis* Jacq.	Ngasi, Nzete ya lito	Arecaceae	Fruit	Mac	Rectal	nd	1	1
nd	*Fagara zanthoxyloides* (Lam.) B. Zepernick & Timler	-	Rutaceae	FL	Mac, Dec	Oral	Sorghum powder	3	2
nd	*Ficus mucuso* Welw. Ex Ficalho	Apendanyoka	Moraceae	FL	Dec	Oral	nd	1	1
MBA/13	*Harungana madagascariensis* Lam.ex Poir.	Botondolondo	Hypericaceae	FL	Dec	Oral	Sugar	2	2
MBA/33	*Hibiscus sabdariffa* L.	Ngai-ngai	Malvaceae	FL	Dec, Cooking	Oral	*C. citratus* leaves, Sugar	7	5
MBA/30	*Hibiscus tiliaceus* L.	Kasa ya makila	Malvaceae	FL	Dec, EJ, Mac, Inf	Oral, Cutaneous	Ash, Sugar; Soy flour, Corn flour , milk; Tomato juice, soft drink, lemon juice, Caterpillar, *C. citratus* root and leaves, Caramel,	42	22
MBA/26	*Ipomoea batata* (L.) Lam.	Matembela	Convolvulaceae	FL	Mac	Oral	Tomato juice, Eggs and Sugar milk	2	2
MBA/04	*Laportea canadensis* Wedd.	Ibenja ou Katolia	Urticaceae	WP	Dec	Rectal, Oral	nd	3	2
MBA/19	*Macaranga spinosa* Müll.Arg.	-	Euphorbiaceae	FL	Dec	Oral	nd	1	1
MBA/17	*Macaranga stipulosa* Müll. Arg.	-	Euphorbiaceae	FL	Dec	Oral	nd	1	1
MBA/34	*Mangifera indica* L.	Manga	Anacardiaceae	FL	Dec	Oral	Eggs	1	1
nd	*Manihot esculenta* Crantz	Sombe, Mwinja	Euphorbiaceae	FL	Dec, Inf, Mac, Cooking	Oral	Lemon Juice, Milk	8	7
MBA/10	*Morinda morindoides* (Baker) Milne-Redh.	Kongo bololo	Rubiaceae	FL	Inf	Oral	nd	1	2
MBA/08	*Moringa oleifera* Lam.	Moringa	Moringaceae	FL	Mac, Dec, Inf	Oral	Sugar	8	4

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MBA/24	*Musa paradisiaca* L.	Makemba	Musaceae	DLT	Dec	Cutaneous, Oral	Ash, Sugar	4	3
MBA/09	*Myrianthus arboreus* P. Beauv.	Bokomu	Moraceae	FL	Dec, Mixt	Cutaneous, Oral	Ash	4	3
nd	*Oryza sativa* L.	Loso	Poaceae	Seed	Grinding and calcined	Oral	Caramel, Sugar	4	4
MBA/12	*Passiflora edulis* Sims	Marakuja	Passifloraceae	Fruit, FL	EJ, Dec	Oral	Soft drink, Sugar	2	4
MBA/18	*Persea americana* Mill.	Avocati, Isandu igeboka	Lauraceae	FL, Fruit,Pit , Bark	Dec, Mac	Oral, Cutaneous	*C,citratus leaves*, Sugar milk; Tomato juice; Caramel; Ash; Soft drink; Eggs; Sugar; Baking powder;	21	17
MBA/14	*Ricinus communis* L.	Mbalika	Euphorbiaceae	FL	Heating	Cutaneous	nd	1	1
MBA/03	*Senna alata* (L.) Roxb.	Folele	Fabaceae	FL	Dec	Oral	Sugar	1	2
MBA/02	*Sida acuta* Burm.f.	Omongo, Uende ukamuita mama	Malvaceae	Stem, Root	Grinding and calcined, Dec	Oral	Vegetable salt and palm oil; Ash	2	2
nd	*Solanum betaceum* Cav.	Damudamu	Solanaceae	FL, Fruit	Dec, EJ	Oral	Sugar , Eggs	2	2
nd	*Solanum lycopersicum* L.	Tomate	Solanaceae	Fruit	Mixt	Oral	Soft drink	2	3
MBA/32	*Tectona grandis* L.f.	Tec	Verbenaceae	FL	Mac, Dec	Cutaneous	Tomato juice, Eggs, Soft drink, Sorghum flour, Caramel, Sugar	20	12
MBA/15	*Terminalia catapa* L.	Madamé	Combretaceae	FL	Dec	Cutaneous	Caramel	4	3
MBA/27	*Theobroma cacao* L.	Cacao	Malvaceae	FL, DLT	Dec	Oral	Ash, Sugar	4	3
MBA/31	*Zingiber officinal* Roscoe	Tangawisi	Zingiberaceae	Root	Inf	Oral	nd	1	3

Legend: **Dec**(Decoction); **DF**(Dried fruit); **DLT**(Dried leaves on the tree); **EJ**(Expression of juice); **FC** (Frequency of citation); **FL**(Fresh leaves); **Inf**(Infusion); **Mac**(Maceration); **nd** (not defined); **Soft drink** (Coca cola); **Sugar milk** (Nestlé); **VN** (vernacular name); **WP**(whole plant).

2.1.2. Frequency of Citation of Additives

Of the 184 URs, 111 involved a combination of plant extracts with other products (Figure 2). The most frequently associated products included sugar, ash, caramel, canned tomatoes, lemon juice and eggs. Some participants mentioned to use separately from herbal recipe some additives such as canned tomatoes, milk and eggs.

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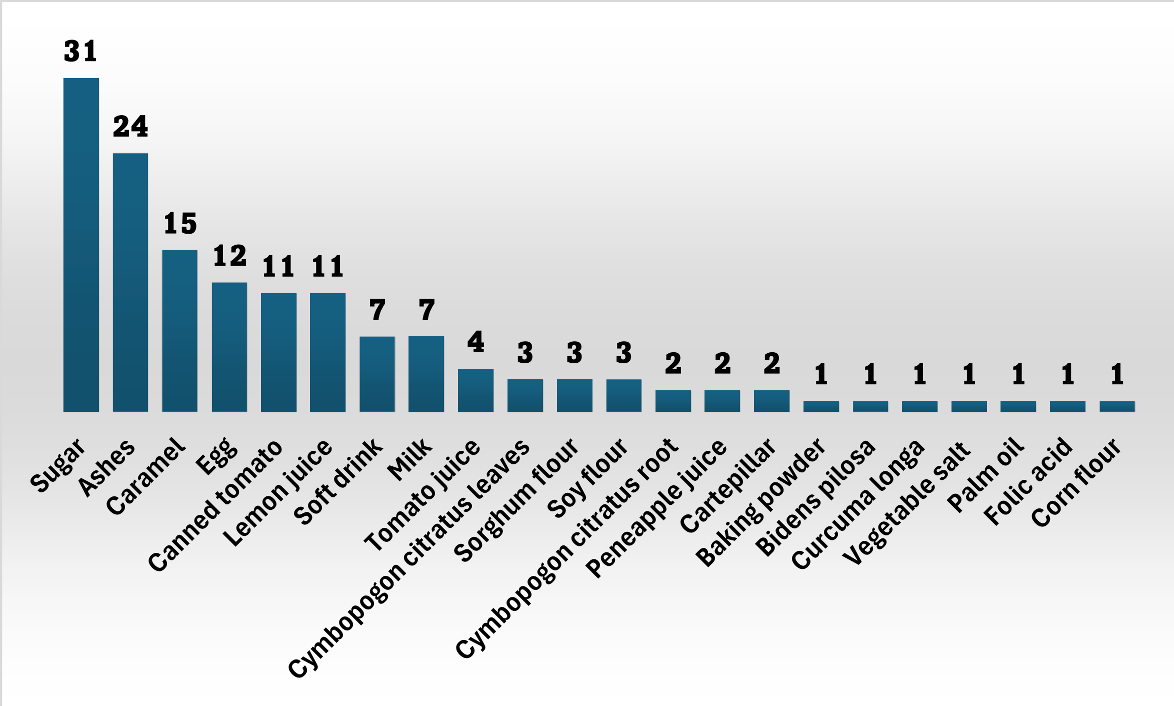

Figure 2. Frequency of citation of additives.

The following were added during the preparation of the herbal remedy: caramel, sugar, lemon juice and ash, whereas caterpillars, sorghum, maize and soya flour were consumed with the meal after ingestion of the herbal remedy. We found that the key difference was in the pre-treatment of certain additional products before their incorporation into the recipe. This is the case of sugar calcination or *C. citratus* roots. In some cases, for example topical application, the associated products are consumed orally after external administration of the principal product. Unfortunately, the participants were unable to explain the benefits of such combinations and administration way. Most of them limited themselves in describing the organoleptic changes occurred during the preparations after the addition of these products. Other participants claimed the tonic effects of these additives acting against anemia-related fatigue or as appetite stimulants. It is worthy to noticed that in most of African culture, ash is often associated to purification, transition and renewal, whereas fire denote destruction means that allows for regeneration. Its inclusion in remedies therefore symbolizes the transition from illness to healing. Lemon is often seen as a cleansing agent, even as a "hunter of evil spirits". Its acidity is perceived as a force capable of fighting disease or removing 'impurities' from the body [25–27]. Some informants believed that these additives are used not only for their antiseptic and preservative properties, but also for their ability to alter the taste of preparations, thus facilitating their administration. In a scientific point of view, the addition of lemon is beneficial because of the ascorbic acid presence which may contribute to Fe(III) reduction to Fe(II), facilitating its oral absorption and subsequently increasing hemoglobin levels [28].

2.2. Laboratory Validation

2.2.1. Plant Selection for Laboratory Assessment

We used the UR value for plant selection. Remind that a plant with a high UR is the one for which several variants were mentioned, either in terms of used parts, preparation methods or administration routes. In our survey, the top five species most frequently mentioned were *H. tiliaceus*, *P. americana*, *C. papaya*, *T. grandis* and *C. sulphureus* (see Table 1). However, we considered additional selection criteria such as the local species availability, the previous studies related to SCD, the soil type specificity mentioned by the respondents, the administration route and the type of additives associated with the preparations. Thus, we included only the plants for which the most cited additives (sugar, ash, caramel and lemon juice) were explicitly mentioned at the time of recipe preparation. The eggs were not included as they are not incorporated during preparation, but they are administered sometimes after the remedy has been taken. Among the above top five species, *P. americana* and *C. papaya* were excluded since they were not mentioned for both soil types. *T. grandis* was also excluded because it is not used orally, while *C. sulphureus* did not meet the criterion related to the use of the most reported additives. Only *H. tiliaceus* (HT) met all the selection criteria. To have a minimum representative species for evaluation, *A. cordifolia* (AC) was included to the selection group, although with only 3 UR, but all other selection criteria were met. AC is a shrub belonging to the Euphorbiaceae family, commonly found in tropical forests, especially along rivers. HT is a tree from the Malvaceae family, found in tropical regions of Africa, America and Asia, which is more drought tolerant and adaptable to different soil types.

2.2.2. Biological Activities Evaluation

The goal of these activities was to evidence the antioxidant and anti-inflammatory effects of the selected plants and the added value of the additives reported by respondents., knowing that the prominent role of oxidative stress in SCD yielding to several complications [29,30]. Anti-inflammatory activity was assessed using both the classical myeloperoxidase (MPO) assay and the SIEFED (Specific Immuno-Extraction Followed by Enzymatic Detection) method, given MPO role in SCD-related oxidative stress and inflammation. In addition to its peroxidase activity, MPO catalyzes chlorination reactions in the presence of chloride ions [31]. The classical MPO assay evaluates the direct inhibitory effect of plant extracts on enzyme activity in an open system, whereas the SIEFED assay selectively measures the activity of active, bound MPO, minimizing interference from other extract constituents [32,33]. HT plant was already subjected to chemical characterized in our previous study [34]. Antioxidant activity was assess using ABTS radical cation.

Antioxidant activity

As shown in Table 2, all AC extracts presented higher and significant antioxidant activity than their corresponding HT extracts, regardless the soil type with or without additives. This indicate that the antioxidant activity is species and concentrations dependent. For AC, we noticed that arid soil had globally high antioxidant activity than marshy soil. This figure was opposite to HT as marshy soils were more favorable. However, regarding AC extracts and additives, the use of lemon juice was found favorable since the antioxidant activity was higher compared to ashes, caramel and sugar additives. In the case of AC extracts, lemon juice and ashes were found favorable since the antioxidant activity was higher compared to sugar and caramel. These antioxidant activity patterns were confirmed with the ABTS tests. Globally, none of these additives improved significantly the antioxidant capacity of the extracts. This might be a possible interference or dilution effect of the phytochemical compounds. The antioxidant activity observed in the total extracts of the two plants supports the satisfaction expressed by the users through survey respondents, as a complementary strategy of SCD management. Furthermore, the antioxidant activity of AC and HT extracts has been confirmed in previous studies [34,35].

Table 2. IC_{50} of samples for antioxidant test.

Sample	**Antioxidant expressed as IC_{50} (in µg/mL) Mean ± Standard deviation (n=3)**
***Alchornea cordifolia* (Schumach.) Müll.Arg.**	
AC arid soil aqueous extract (AC AAe)	1.76 ± 0.17
AC AAe + caramel	nd
AC AAe+ Ash	12.46 ± 4.51
AC AAe + lemon juice	2.89 ± 0.26
AC AAe + sugar	nd

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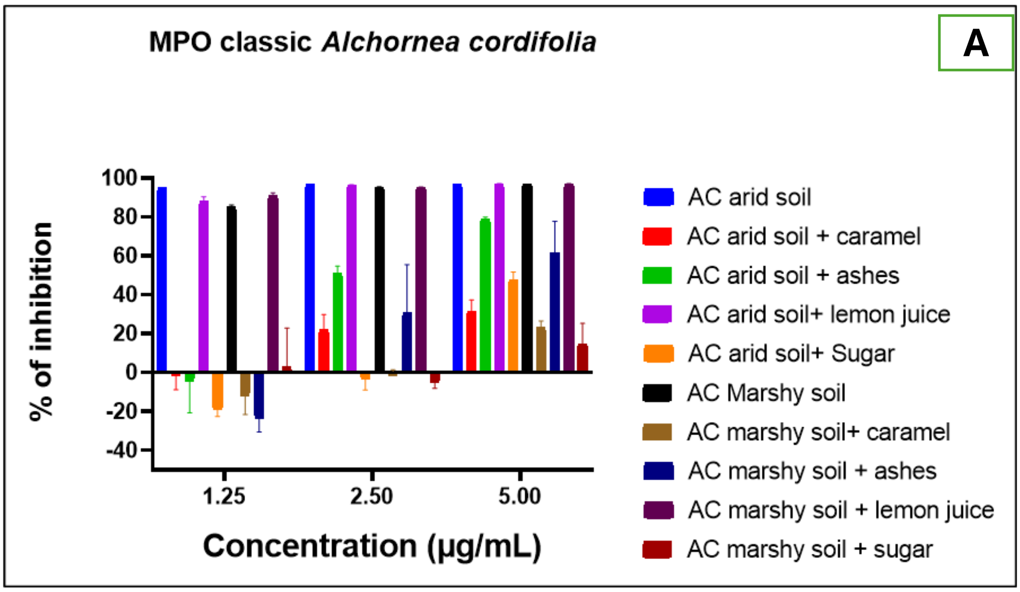
AC marsh soil aqueous extract (AC Mae)	3.11 ± 0.10
AC MAe + caramel	nd
AC MAe + Ash	36.04 ± 25.15
AC MAe + lemon juice	4.99 ± 3.59
AC MAe + sugar	nd
***Hibiscus tiliaceus* L.**	
HT arid soil aqueous extract (HT AAe)	18.71 ± 11.78
HT AAe + caramel	nd
HT AAe + Ash	nd
HT AAe + lemon juice	nd
HT AAe + sugar	nd
HT marsh soil aqueous extract (HT Mae)	4.85 ± 0. 86
HT MAe + caramel	nd
HT MAe + Ash	26.22 ± 10.02
HT MAe+ lemon juice	12.24 ± 3.51
HT MAe + sugar	nd

Legend: AAe: Arid soil aqueous extract; AC : *Alchornea cordifolia;* HT : *Hibiscus tiliaceus;* MAe: Marsh soil aqueous extract; nd: not defined.

Anti-inflammatory activity

In terms of inhibition of human MPO activity, the AC extract presented high activity compared to the HT extract. Among the tested additives, only the extract treated with lemon juice exhibited high activity, reaching approximately 90% for AC against 60% for HT at a concentration of 5 µg/mL. This level of inhibition is comparable to that of the untreated extract of AC, but remains lower than that observed with the untreated extract of HT. Furthermore, the HT extract from marshy soils showed greater inhibitory activity than that from arid soils. However, for AC, soil type did not appear to significantly influence this activity. A concentration-response dependent was observed with HT extracts, whereas AC extracts quickly reached an inhibition plateau at low concentrations, suggesting a saturation effect (Figures 3 and 4).

These results suggest that the extracts tested inhibit MPO activity by preventing the enzyme from interacting with its substrates, in particular hydrogen peroxide (H_2O_2) and nitrite ion (NO_2^-). This inhibition limits the formation of reactive oxygen species (ROS) responsible for oxidative stress and inflammatory lesions [36,37].



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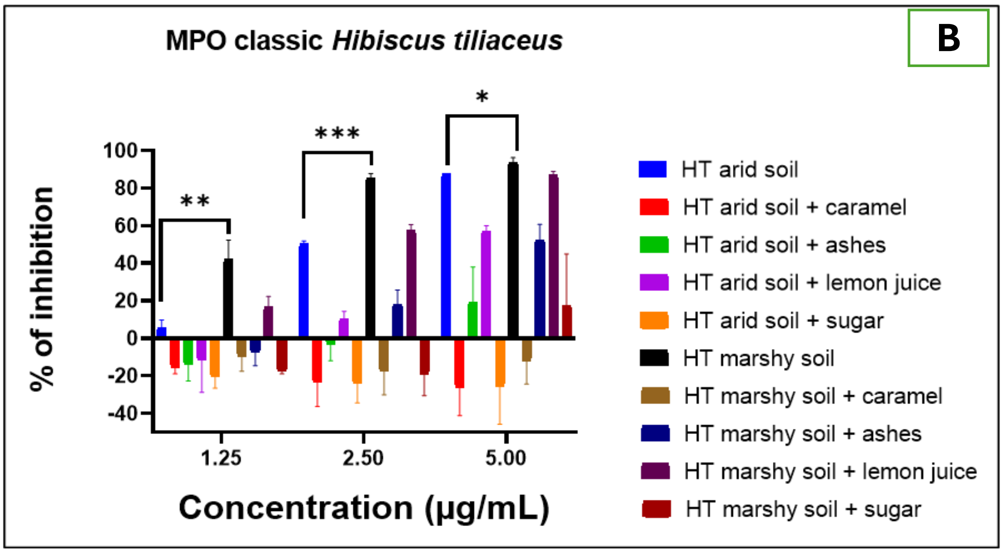
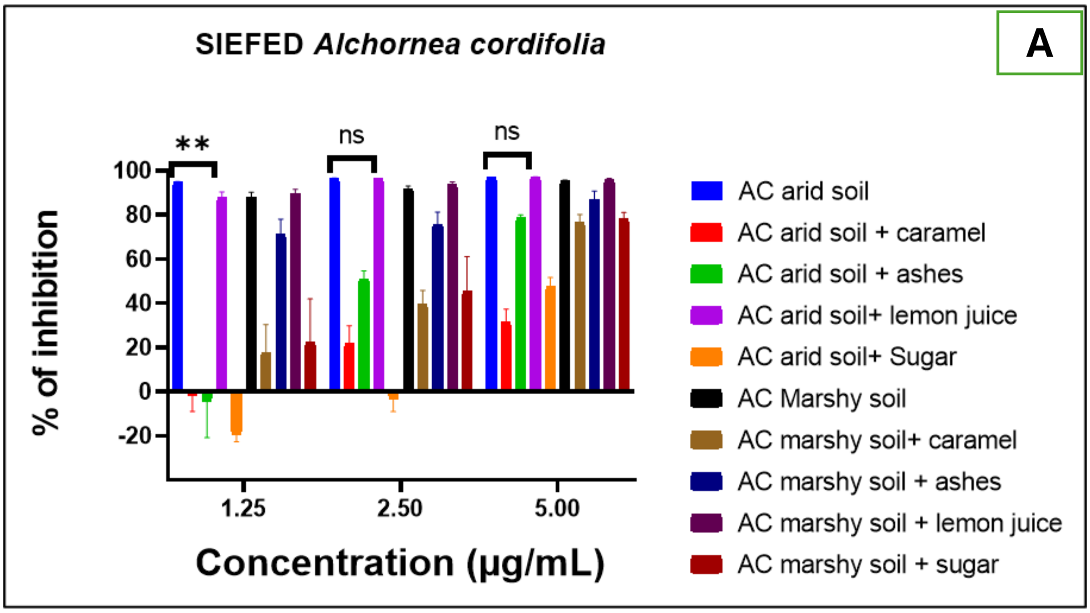


Figure 3. Anti-inflammatory profiles expressed as percentage of inhibition of classic MPO activity observed with Direct assay for *Alchornea cordifolia* (AC) (A) and for *Hibiscus tiliaceus* (HT) (B) in different soil types (arid or marshy) combined or not with additives.

Regarding myeloperoxidase (MPO) inhibition, this study demonstrated that among the four additives tested, the incorporation of ash or lemon juice did not significantly modify the inhibitory activity of extracts from either plant. Inhibition levels ranged from 90 to 100% at 5 µg/mL, with no significant difference compared to untreated extracts. Similarly, soil type exerted a negligible effect on MPO inhibition for both AC and HT. Unlike the classical MPO assay, which primarily measures inhibition through interference with reactive oxygen species (ROS) formation via electron or hydrogen transfer mechanisms, the SIEFED assay specifically assesses the spatial interaction between test molecules and the enzyme's active site. This approach, which focuses on the molecular arrangement, may explain some of the variations or unexpected results observed in inhibitory activity [32]. The anti-inflammatory activity observed for AC confirms the results reported in previous studies [35,37]. The same is true for HT, whose antioxidant and anti-inflammatory properties are well documented [38]. Several studies have also highlighted the immunomodulatory and thrombolytic effects of HT [39,40] and antibacterial effects of AC [41,42].



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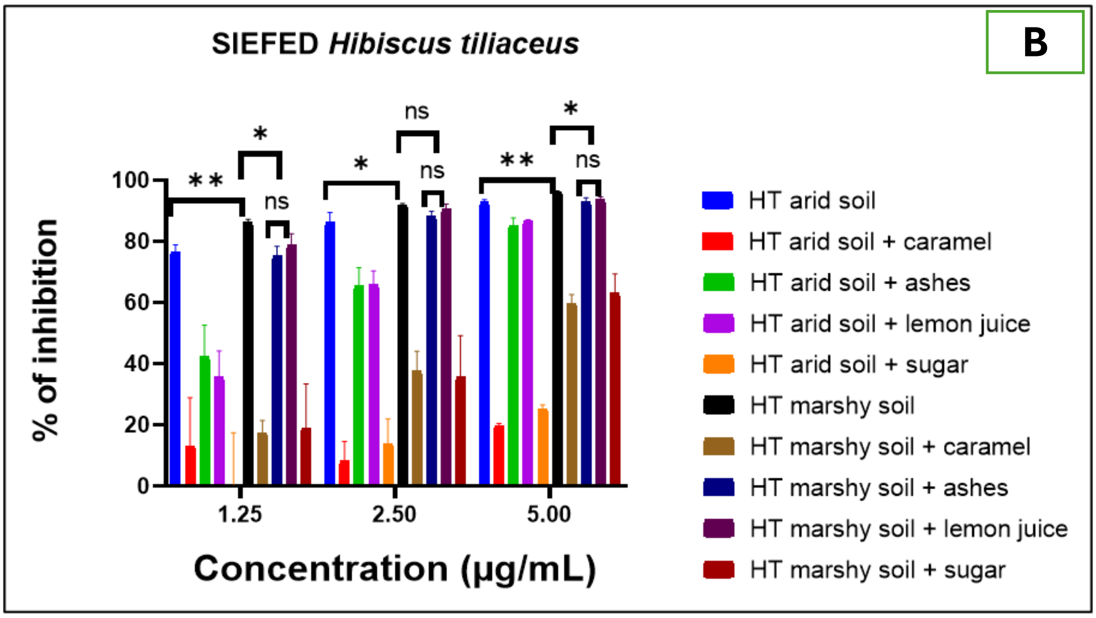

Figure 4. Anti-inflammatory profiles expressed as percentage of inhibition of MPO activity observed with Siefed for *Alchornea cordifolia* (AC) (A) and for *Hibiscus tiliaceus* (HT) (B) in different soil types (arid or marshy) combined or not with additives.

3. Materials and Methods

3.1. Materials

3.1.1. Survey Area and Period

The survey took place in the Kisangani city from June 2023 to December 2024. The city is in the north-east of the DRC, very close to the equatorial line and surrounded with a dense tropical rainforest zone. The climate is equatorial with a short, hot dry season, mainly in January and February, followed by a warm, oppressive and overcast rainy season that persist throughout most of the year.

3.1.2. Vegetable Materials and Reagents for Bioassays

3.1.2.1. Vegetable Materials

The plants material consisted of leaves from two selected plants species on basis of the ethnopharmacological survey. The leaves were harvested from marshy and arid soils. The leave extracts were prepared following the respondent's description.

3.1.2.2. Reagents

All the salts used to prepare the buffered solutions and methanol, were of an analytical grade from Merck VWR (Leuven, Belgium). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2 azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), Amplex Red, sodium nitrite, sodium persulfate, and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich (Steinheim, Germany). Bovine serum albumin (BSA) was obtained from Roche Diagnostics Gmbh (Mannheim, Germany), and human MPO was purchased from Calbio chem Millipore (Bellirica, Madison, WI, USA). The ash was obtained from charcoal of equatorial rainforest wood, the sugar from sugarcane (*Saccharum spp*), and lemon juice was obtained from yellow lemons of the species *Citrus limonia* (L.) Osbeck.

3.2. Methods

3.2.1. Ethnobotanical Survey

The survey team included eight pharmacy students and two assistants from the Department of Pharmacy, Faculty of Medicine and Equation (1) Pharmacy at the University of Kisangani. An anthropologist was also recruited to ensure full understanding of responses during the survey. The sample size of respondents (*n*) was estimated by mean of William Cochran formula described by Charan J. (Equation (1)) [43,44].

$$\boldsymbol{n} = \frac{\boldsymbol{z^2 \times p \times (1-p)}}{\boldsymbol{m^2}} \quad (1)$$

with *z* the confidence level (1.96 at 95%), *p* the estimated proportion of the population with the characteristic and *m* the tolerated margin of error equals to 0.05 when set at 5%.

Respondents were selected based on two criteria: being at least 18 years old and having stayed in Kisangani for 3 years minimum. The objectives of the survey were explained to the participants. Their voluntary, free and informed consent was obtained in accordance with ethical guidelines for research involving human subjects. Data were then collected using a semi-structured questionnaire focusing on knowledge and practices related to SCD management, particularly traditional medical approaches. Information gathered included plants names, the used parts, method preparation, administration routes, and specificity and individual perceptions of therapeutic efficacy, including plant combinations. The cited plants species were identified at the Faculty of Science, University of Kisangani, and taxonomically verified using the African Plant Database and World Flora Online.

3.2.2. Laboratory Assessment

3.2.2.1. Plant Selection and Collection

The plants species were sorted based on the survey data and using the following selection criteria : (*i*) the frequency of citations by respondents, (*ii*) the route of administration limited to oral use, (*iii*) the harvesting environment considering both arid and marshy soils for the same species, (*iv*) and the additives used during recipe preparation, focusing on the four most frequently reported additives associated with each plant. Practical considerations were added including the local availability and evidence from previous studies reporting a link between their medicinal properties and the management of SCD.

The part used of the selected plants were collected from two different soils (marshy and arid environments) and air-dried at room temperature in the laboratory of the Faculty of Medicine and Pharmacy, University of Kisangani. The dried plant material was subsequently ground into powder using a ZM 200 ultra centrifugal electric grinder (Resch, France). The resulting powders were packaged in hermetically sealed plastic vials and transported to the University of Liège for further analyses at the Pharmacognosy Laboratory (LPG) and the Oxygen Research and Development Centre (CORD).

3.2.2.2. Preparation of Plants Extracts

The selected and collected plants were treated with the most cited additives. The untreated raw extracts of each plant from arid soils were used as controls. The comparison was made with the plant extracts from marshy soils to find evidence to support the respondents' statements. For each selected plant, 25 g of powder was mixed to 500 mL of water. The mixture was boiled at 100°C for 15 minutes, then filtered through glass wool. The quantities of additives to be incorporated were determined on the basis of the proportions reported by the respondents, expressed in grams or milliliters per 100 mL of aqueous extract. In order to reproduce the traditional preparation method described by the respondents, 100 mL of hot aqueous extract was put into separate beakers. One specific additive was added to each of them. The resulting mixtures obtained were allowed to cool to room temperature, filtered through glass wool and then freeze-dried for 48 hours. The resulting dry extracts were transferred into glass vials and stored at 6°C until use in subsequent biological assays.

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3.2.2.3. Biological Activities Assays

Antioxidants activities

Antioxidant activity was assessed using the ABTS tests to provide a more comprehensive assessment of the antioxidant capacity of the different samples [45]. The ABTS test is based on the change of the blue-green color of the ABTS radical cation ($ABTS^{•+}$) solution into its colorless neutral form, as previously described by Re et al and modified by Widowati et al. [46,47]. To generate the $ABTS^{•+}$ radicals, an aqueous solution of sodium persulfate (2.45 mM) was mixed with ABTS (7 mM) and incubated overnight in the dark to obtain a dark colored solution. The stock solution of $ABTS^{•+}$was then diluted by adding pure methanol (100%) to give an absorbance of 0.70 (±0.02) at 734 nm at 25°C. Assays were performed in multiwell plates to obtain three replicates per condition which allow to minimize reagent consumption and save time. An aliquot of 2 µL of the tested extract was added to 198 µL of $ABTS^{•+}$. As a negative control, 2 µL of ultrapure water was added to 198 µL of $ABTS^{•+}$solution. To evaluate the absorbance of the different solutions at 734 nm, a microplate reader (Thermo Lab system, Vantaa, Finland) was used and the reducing capacity was determined according to the formula:

$$\%inhibition = (A\ control - A\ sample) * 100 / A\ control \qquad (2)$$

with A : Absorbance

Anti-inflammatory activities

Measurement of the peroxidase activity of MPO was performed by a classical enzymatic assay and by the SIEFED assay as described by Nyssen et al.[48]. The MPO solution was prepared with purified human MPO in dilution buffer (PBS 20 mM at pH 7.4 with 5 g/L BSA and 0.1% Tween-20). Solutions of each sample, at final concentrations ranging from 1.25 to 5.00 µg/mL, were incubated with human MPO at a final concentration of 5 mU/mL for 10 min before further use. The detection of MPO activity was performed by monitoring the enzyme-catalyzed oxidation of Amplex Red in the presence of H_2O_2 and nitrite in phosphate buffer at pH 7.4.

Classical assay of MPO activity

After incubation, mixtures containing 100 µL of each extract or vehicle (ultrapure water) and MPO were added to the wells of multiwell plate (transparent) and peroxidase activity was measured by adding 10 µL of sodium nitrite solution (4.5 mM, final concentration) and 100 µL of the reaction solution containing 10 µM H_2O_2 and 40 µM Amplex® Red (AR) in phosphate buffer (50 mM) at pH 7.4. The oxidation of AR to the fluorescent resorufin adduct (excitation = 544 nm; emission = 590 nm) was monitored for 30 min at 37 °C using a fluorescence plate reader (Fluoroskan Ascent, Fisher Scientific, Hampton, NH, USA).

SIEFED assay of MPO activity

Samples containing MPO and different concentrations of juglone were prepared and incubated as in the classical assay. An amount of 100 µL of each mixture (MPO alone or MPO+ extract) was then added to the wells of a SIEFED multiwell plate coated with rabbit polyclonal antibodies (3 g/mL) against human MPO and incubated for 2 h at 37 C in the dark. After washing the wells, the activity of the enzyme captured by the antibodies was measured by adding 10 µL of sodium nitrite solution (4.5 mM, final concentration) and 100 µL of a reaction solution containing 10 µM H_2O_2 and 40 µM Amplex® in phosphate buffer (50 mM) at pH 7.4. The oxidation of Amplex® Red to the fluorescent adduct resorufin (excitation = 544 nm; emission = 590 nm) was monitored for 30 min at 37°C using a fluorescence plate reader (Fluoroskan Ascent, Fisher Scientific). As for the direct MPO assay, a control assay, set as a relative value of MPO activity, was performed with purified MPO in the presence of PBS instead of the samples. In this SIEFED assay, MPO was bound to the wells by the antibodies and the test extract was discarded in the wash step before the enzymatic activity was measured. For both

MPO assays, the percentage inhibition was calculated using a similar formula as described above (See Equation(2)).

3.2.2.4. Statistical Analysis

For data treatment, we used statistical analysis with GraphPad Prism 8.0.1 software, developed by GraphPad. Two-way ANOVA multiple comparisons and Dunnett's post-tests were used to test for differences between treatment groups. Results were considered significant at p-values of less than 0.05, i.e. at the 95% confidence level.

4. Conclusions

The management of SCD in Kisangani remains a major public health challenge, leading many families with limited resources to rely on traditional medicine, particularly medicinal plants combined with additives. Ash, sugar, caramel and lemon juice were used. Laboratory evaluation of two frequently used species (*A. cordifolia* and *H. tiliaceus*) confirmed their antioxidant and anti-inflammatory activities, which may explain the positive perceptions reported by users. However, the addition of ash and lemon juice reduced antioxidant activity without markedly affecting anti-inflammatory effects, whereas sugar and caramel significantly impaired both activities. Although these additives may improve sweetness, compliance, or pharmacokinetic properties, their use cannot be recommended based on the present findings. Moreover, soil type influenced plant bioactivity in a species- and activity-dependent manner. These results underscore the need for community awareness initiatives leveraging churches, schools, and media to mitigate risks associated with unregulated additive use. Further research should broaden the range of biological and safety parameters to provide more comprehensive evidence-based guidance.

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