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Original Research Article

In vitro antisickling, antimicrobial and antioxidant potentials of extracts of *Sorghum bicolor* (L) Moench seeds and *Mangifera indica* (L) Anacardiaceae leaves and their formulations

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Abstract

Purpose: Seeds of *Sorghum bicolor* (SB) and leaves of *Mangifera indica* (MI) are used in ethnomedicine in the management of sickle cell disorder (SCD). The present study involves phytochemical analysis of the methanolic extracts of the plant parts and evaluation of their antibacterial, antioxidant and antisickling potentials as well as syrups containing the extracts.
Methods: Phytochemical analysis of the methanolic extracts were evaluated using standard methods while the functional groups were elucidated using Fourier transform infrared spectroscopy (FT-IR). Antioxidant properties were determined by comparing their radical scavenging activity against gallic acid and ascorbic acid while antibacterial properties of the plant extracts were evaluated against *Salmonella typhi, Pseudomonas aeruginosa, Escherichia coli and Staphylococus auerus.* The syrups containing the extracts were evaluated and compared to Ciklavit^{R}.
Results: Phytochemical screening and FI-TR spectra revealed the presence of bioactive molecules including flavonoids and phenols. The two extracts had comparable antioxidant properties however, only *Mangifera indica* extract had antibacterial activity against tested organisms. Both extracts including their syrup formulation had antisickling activity with the syrup containing 500 mg SB and 250 mg MI having superior activity.
Conclusion: *Sorghum bicolor* seeds and *Mangifera indica* leaves extracts and their formulations might play a great role in the management of SCD.

Keywords: Antisickling; antioxidant; antimicrobial; *Mangifera indica; Sorghum bicolor*

Indexing: Index Copernicus, African Index Medicus

Introduction

Sickle-cell disorder (SCD) has been described as an autosomal recessive genetic blood disorder characterized by red blood cells that assume an abnormal, rigid and sickle shape [1]. SCD is as a result of substitution of the glutamate by valine at the sixth position of the β chain of the normal hemoglobin. Patients with SCD have numerous acute and chronic medical problems and it has been described as one of the diseases afflicting the population living mostly in sub-Saharan Africa, South America and Asia. More than 75 % of SCD occurs in sub-Saharan Africa, where scarce health resources, inadequate awareness among health care providers and the general public contribute to shocking rates of early mortality [2]. There is no specific drugs yet available for the treatment of the genetic hereditary disease, however the first line clinical management of SCD includes the use of hydroxyurea, antimalarial prophylaxis, amino acids supplementation, folic acid, antibiotics, blood transfusions and bone marrow transplantation. The first line clinical management are expensive and have risk factors [3]. Continued researches for the antisickling properties of medicinal plants have been very promising and the use of phytomedicines has proven to reduce crisis and reverse sickling [4].

Nutritional deficiencies, elevated oxidative stress and inflammation have been linked to the vicious cycle in sickle cell disease [3]. Mandese *et al.,* [5] observed maximum nutrient deficiencies in intake of calcium, iron, vitamin B1, and vitamin C, while carbohydrates, lipids were moderately insufficient in patients with sickle cell anaemia. The main advantage of the use of plants in SCD management is the benefit of maximizing all the essential ingredients contained in such plant materials. Apart from antisickling activity, some of these plants also have antioxidant; anti-inflammatory, antimicrobial and immune system boosting potentials [6]. Different antisickling herbs being used in local treatment of SCD in West Africa had been reported [7] and some current herbal formulations marketed for the management of SCD include Nicosan®, Ciklavit®, Dioscovite®, and Jobelyn®.

Sorghum *(Sorghum bicolor* (L.) Moench) is the world's fifth largest most important cereal grain, after wheat, maize, rice and barley and Africa's one of the main subsistence food crop [8]. It contains important phytochemical groups such as phenolic compounds, flavonoids, plant sterols, policosanols, tannins, anthocyanin, and saponins [9]. The antisickling activity of *Sorghum bicolor* against SCD had been identified and attributed largely to the presence of anthocyanins [10, 11]. Anthocyanins are reported to have vasoprotective, anti-inflammatory properties, anti-cancer, and chemoprotective properties [10].

Mango (*Mangifera indica* (L) Anacardiaceae) is a large evergreen tree and the phytochemical contents in *Mangifera indica* leaves include phenolic acids, flavonoids, triterpenoids, tannins, steroids, and glycosides [12]. Mangiferin, a normal metabolite found in *Mangifera indica* is a polyphenolic antioxidant and a glucosyl xanthone [13]. It has strong antioxidant, anti-lipid peroxidation, and immune-stimulating effect on both cellular and hormonal immunity [14]. The xanthone mangiferin compound from the leaves is called Homomangiferin, *Mangifera indica* possess powerful antioxidant activity because of its high total phenols and total flavonoids content [14], hence may be useful in the effective management of SCA.

Recent survey revealed the use of combination of *Sorghum bicolor* (SB) and *Mangifera indica* (MI) by some Nigerian traditional practitioners for the management of SCD. The present study was aimed at investigating the antisickling, antioxidant, and antimicrobial properties of *Sorghum bicolor* and *Mangifera indica*, as well as evaluation of liquid dosage forms formulated with the mixture of the two plant parts.

Methods

Collection of plant materials and microorganisms

The leaves of *Mangifera indica* and the seeds of *Sorghum bicolor* were collected on 30th of June 2016 from the University of Lagos, Akoka, Nigeria botanical garden and Boundary market, Ajegunle, Lagos, Nigeria respectively. They were identified and authenticated at Forestry Research Institute of Nigeria, Ibadan, Nigeria and voucher specimen numbers 110696 (*Mangifera indica*) and 110695 (*Sorghum bicolor*) were assigned and samples deposited in the herbarium. The micro-organisms (*Salmonella typhi, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa*) were clinical isolates obtained from Pharmaceutical Microbiology laboratory of the Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Lagos, Nigeria.

Collection of blood samples

Blood samples used in the evaluation of the antisickling activity were obtained from patients with confirmed sickle cell status not in crisis and aged between 18 and 30 years. The collected samples placed in sodium ethylene-diaminetetracetic acid (EDTA) tubes were stored at 4 □ for the experiment. A written informed consent was signed by the participating patients. The research procedures employed in the study received the approval of College of Medicine University of Lagos, Nigeria Health Research Ethics Committee with approval number CM/HREC/11/16/076.

Extraction of the extracts

Air-dried leaves of *Mangifera indica* were weighed and transferred into a glass jar. It was pulverized and macerated with 80 % methanol for 24 h with interval agitations. The mixture was then filtered with a mesh of 200 µm, reduced to 50 ml, and transferred into a beaker to evaporate completely in a water bath at 40 □. The extracts obtained were transferred into a

sample bottle. The procedure was repeated thrice to ensure complete extraction of the plant material. The same method was employed for the extraction of the seeds of *Sorghum bicolor*. The extracts obtained were stored in the refrigerator and used for the study.

Phytochemical screening

Qualitative phytochemical screening of extracts of *Mangifera indica* and *Sorghum bicolor* were carried out for the presence of alkaloid, steroid, tannin, saponin, phytosterol, and glycoside using established protocols [15]. Quantitative phytochemical screening were carried out for total phenol content using method reported by Devi *et al.* [16] while total flavonoid content and total proanthocyanidin content using procedures reported by Ojewunmi *et al.* [17].

Proximate content analysis

Proximate content analysis was carried out on the samples to determine the moisture, ash, crude fiber, fat, protein and carbohydrate content using the procedures detailed by Association of Official Analytical Chemist (AOAC) [18].

The mineral content of the samples were determined by using the method described by AOAC [18]. The mineral elements; iron, magnesium and calcium were determined using Thermo Scientific iCE 3300 AAS Atomic Absorption Spectrometer.

FT-IR Study

Approximately 5 mg of perfectly milled and dried powder of the extracts were placed on the sample chamber of FT-IR optical lens (Bruker, South Africa). The spectra were scanned from 500 cm^{-1} - 4000 cm^{-1} under dry air at room temperature.

Antioxidant activity

The antiradical activity of the *Mangifera indica* and *Sorghum bicolor* extracts were evaluated based on the scavenging effect of the stable DPPH free radical [17]. An aliquot of 0.5 ml of the extract in ethanol (95 %) at different concentrations (25 µg ml^{-1}, 50 µg ml^{-1}, 75 µg ml^{-1}, 100 µg ml^{-1}) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 min the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) were read at 517 nm. The scavenging effect was calculated using Equation 1:

$$\% \text{ Inhibition} = (Ao-A1)/Ao\times100 \quad \ldots\ldots\ldots\ldots (1)$$

Where Ao is the absorption of the blank sample and A1 is the absorption of the extract

Antimicrobial activity

The antibacterial screening was carried out using the agar diffusion method as described by Doughari and Manzari [19]. The test bacteria include; *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* were first inoculated into tubes of nutrient broth separately and incubated at 37 ˚C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standard and inoculated (0.2 ml each) onto Mueller Hinton agar plates (MHA, Oxoid) (diameter: 15 cm). A sterile cork borer was used to make seven wells (6 mm diameter) for different concentrations of the extract on each of the plates containing cultures of the different test organisms. The extracts were separately dissolved in sterile glycerol at concentrations of 50, 100, 200, and 250 mg ml^{-1}. The extracts (0.5 ml) were then introduced into the wells using sterile Pasteur pipettes. Only 0.5 ml of sterile glycerol was introduced in another well to serve as negative control. Wells containing the standard antimicrobial drug (Levofloxacin tablet 500 mg) at a concentration of 0.5 mg ml^{-1} were used as positive control. The culture plates were allowed to stand on the working bench for 30 minutes and were then incubated at 37 ˚C for 24 h. After 24 h antibacterial activity was determined by measurement of diameter zones of inhibition (mm) against the test organisms, around each of the extracts and the antibiotics.

Formulation and physical evaluations of herbal syrups

Four different formulations (F1, F2, F3 and F4) were prepared (Table 1). Active solution used in the formulation was prepared by dispersing the required quantity of hydroxyethylcellulose in 30ml of purified water and allowed to swell at room temperature (26 ± 2 ^{o}C). Thereafter, the sodium benzoate was added to the solution. The solution was heated at 80 □ under stirring for 1 h

then cooled to 25 ˚C; 0.0025 g of sorbic acid was then incorporated to obtain the active solution.

A mixture of 0.015 ml peppermint flavour and 3g propylene glycol (flavour solution) were prepared separately.

The required quantity of sorbitol 70 % solution was incorporated, 15 g glycerol was added also to the mixing vessel under stirring, and container was rinsed with 5 ml purified water. The resultant mixture was poured into the mixing vessel containing the active solution under stirring.

The required quantities of each of the herbal extracts were mixed in the vessel and then the solution was cooled to 35 ˚C. The solutions of prepared peppermint flavour and propylene glycol were added to the mixing vessel under stirring, the container was rinsed with 1 ml purified water and added to mixing vessel under stirring the volume of syrup was made to 100 ml with purified water and stirring was continued for 15 min to get syrup. The pH was adjusted with ortho-phosphoric acid.

The physical appearance (colour, odour and taste), pH and viscosity of the herbal formulations were evaluated using methods reported in a previous study [20]. Stability testing of the herbal syrup was carried out by keeping the samples at accelerated temperature conditions. Nine portions of the final syrup were taken in amber colored glass bottles and kept at accelerated temperature at 4 ˚C, 25 ˚C, and in a stability chamber (60 ˚C/75 % RH). The samples were tested for their physical appearance, pH, viscosity, turbidity, microbiological quality, and homogeneity at different intervals (day 1, day 7, and day 21, 3rd month and 6th month).

In vitro antisickling activity

Antisickling tests were carried out as outlined by Pauline *et al.,* [1] with some modifications. The antisickling effects were investigated by incubating 0.2 ml of the extracts of *Sorghum bicolor* (250 mg, 500 mg) and 0.2 ml of Hemoglobin S (HbSS) blood. A drop of the mixture was placed on the slides (i.e. different slide was used for each test sample), covered with a cover slip, rimmed with Vaseline^{R}, the slide was kept in a damp chamber and incubated for 24 h. The procedure was repeated for *Mangifera indica* (250mg and 500 mg) leaves extract, formulated herbal syrup containing both extracts at varied concentrations (F1, F2, and F3), Ciklavit® (positive control), and (normal saline) negative control. All tests were done in triplicates. After incubation of the samples, the number of sickled cells remaining in all the samples and control were counted under a ×40 magnification binocular light microscope (Olympus cx21fs2 Tokyo Japan).

Statistical Analysis

Mean comparison with the standard was evaluated using one-way analysis of variance (ANOVA). Significant differences ($p < 0.05$) of mean values were determined by Tukey test. OriginPro 2016 (64-bit) software (OriginLab Corporation Northampton, MA 01060 USA) was used for statistical analysis.

Table 1: Composition of the seeds of *Sorghum bicolor* and leaves of *Mangifera indica* extracts syrup formulations.

Ingredient	**Syrup Formulations**			
	F1 (%w/v)	**F2 (%w/v)**	**F3 (%w/v)**	**F4 (%w/v)**
Sorghum bicolor extract	2.500	5.000	2.500	5.000
Mangifera indica extract	2.500	2.500	5.000	5.000
Hydroxylethyl cellulose	0.200	0.200	0.200	0.200
70% sorbitol	35.000	35.000	35.000	35.000
Glycerol	15.000	15.000	15.000	15.000
Propylene glycol	3.000	3.000	3.000	3.000
Peppermint flavour	0.020	0.015	0.015	0.015
Sodium benzoate	0.002	0.002	0.002	0.0022
Sorbic acid	0.025	0.025	0.025	0.025
Purified water to	100.000	100.000	100.000	100.000

Results

Phytochemical screening

Phytochemical screening of methanol leaves extract of MI revealed the presence of saponins, tannins, glycosides, phenols, steroids, reducing sugars and cardiac glycosides while that of the seeds extract of SB revealed the presence of saponins, tannins, reducing sugars, alkaloids, phenols, phytosterols, and anthocyanins. The total phenol content of the extracts measured as mg gallic acid/g dry weight, showed that MI extract had a higher phenolic concentration (22.02 ± 0.06 mg g^{-1}) than SB (14.76 ± 2.31 mg g^{-1}), and gallic acid (14.58 ± 9.55 mg g^{-1}). The total flavonoid content of the extracts measured as mg quercetin/g dry weight, showed that SB had higher flavonoid content (19.08 ± 5.74 mg g^{-1}) than MI (12.28 ± 0.09 mg g^{-1}), and standard (11.67 ±7.64 mg g^{-1}). The total proanthocyanidin content results showed that SB seed extract had 93.34 ± 0.81µg g^{-1}, while MI was 86.99 ± 0.45µg g^{-1}.

Proximate content analysis

The results for the proximate content analysis for the SB seeds and MI leaves extracts presented in Table 2 showed that there was no significant difference between the moisture content of the SB seed and MI leaves ($P<0.05$). SB seeds had higher lipid, crude fibre and protein content compared to MI but lower ash value and carbohydrate content. The data for the mineral content of SB seeds and MI leaves also presented in Table 2 revealed that there was significant difference between SB and MI for K, Na, Mg, Mn, Fe, Zn, and Cu content analysis but no difference was observed for Ca. Generally, SB had a higher mineral content except for Mn.

FT-IR Spectral analysis

The FT-IR spectra (Figure 1) of SB and MI extracts revealed the presence of multiple functional groups. The spectral data of the two extracts confirmed the presence of functional groups such as hydroxyl, ester group and aldehyde group among others. Prominent IR absorption frequencies are indicated in the two spectra in Figure 1.

Antioxidant activity

The results for the DPPH analysis (Table 3) showed that gallic acid had the maximum inhibition at a concentration of 100 µg ml^{-1} followed by ascorbic acid, SB and MI respectively. The results obtained also showed that the inhibition was concentration dependent and there was a statistically significant difference amongst the samples used and between the test samples and standards.

Table 2: Proximate and Mineral contents of *Sorghum bicolor* seeds and *Mangifera indica* leaves

Dietary nutrient	Composition SB	Composition MI
Moisture (%)	5.03±0.00	4.67±0.00
Ash (%)	1.98±0.00	4.35±0.00
Lipid (%)	2.01±0.00	1.52 ± 0.03
Crude fiber (%)	19.00±0.00	4.01±0.00
Protein (%)	9.82 ± 0.00.	5.18 ± 0.00
Carbohydrate (%)	40.67 ± 0.05	59.25 ± 0.00
Potassium (mg g^{-1})	212.57 ± 5.80	212.35 ± 5.84
Sodium (mg g^{-1})	100.01 ± 0.00	110.81 ± 5.82
Calcium (mg g^{-1})	1154.01 ± 5.80	1155.53 ± 14.45
Magnesium (mg g^{-1})	80.52 ± 5.81	73.97 ± 5.82
Iron (mg g^{-1})	337.15±5.83	41.21±5.81
Manganese (mg g^{-1})	16.21 ± 2.82	36.92 ± 3.80
Zinc (mg g^{-1})	10.18 ± 1.80	8.44 ± 1.81
Copper (mg g^{-1})	1.72 ± 0.00	1.30 ± 0.08

SB is Sorghum bicolor seeds extract and MI is Mangifera indica leaves extract

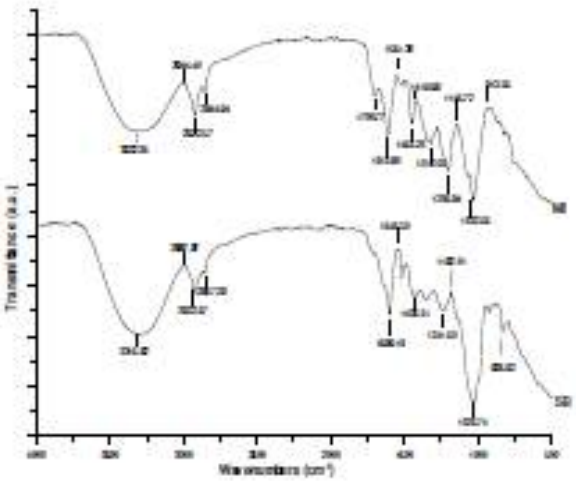

Figure 1: Normalized FT-IR spectra of *Sorghum bicolor* and *Mangifera indica* extracts highlighting some major bands/peaks (from top; MI is *Mangifera indica* and SB is *Sorghum bicolor*). **Some major bands on MI spectrum: - 3322.35 cm^{-1} -H-bonded O-H stretching, 2922.57 cm^{-1} - –C-H stretching, 1700.77 cm^{-1} – aldehyde/ketone –C=C- stretching, 1182.01 cm^{-1} - -C-C(O) –C acetate/ester stretching *Some major bands on SB spectrum: - 3315.82 cm^{-1} - H-bonded O-H stretching, 2922.57 cm^{-1} - –C-H stretching, 1145.72 - -C-C(O) –C acetate/ester stretching*

Table 3: DPPH radical scavenging activity

Sample	DPPH concentration			
	20 µg ml^{-1}	40 µg ml^{-1}	80 µg ml-^{1}	100 µg ml^{-1}
Sorghum bicolor	32.42 ± 0.81	38.30 ± 0.64	59.84 ± 0.22	72.60 ±0.54
Mangifera indica	28.41 ± 0.00	38.28 ± 0.32	51.62 ± 0.63	68.53 ± 0.41
Ascorbic acid	42.29 ± 0.00	51.33 ± 0.00	63.74 ± 0.00	71.32 ±0.00
Gallic acid	46.31 ± 0.70	61.29 ± 0.00	74.53±0.02	83.76 ±0.00

Table 4: Antimicrobial properties of *Mangifera indica* and Levofloxacin

Assay organism	*Mangifera indica* Zones of inhibition (mm) at the stated concentration (µg ml^{-1})				Levofloxacin Zones of inhibition (mm) at the stated concentration (µg ml^{-1})			
	400	200	100	50	80	40	20	10
E. coli	37.47± 0.06	23.51± 0.03	20.07± 0.03	16.5± 0.05	29.67±0.29	28.50± 0.08	27.50± 0.05	25.50± 0.13
P. aeruginosa	21.47 ±0.76	17.06± 0.26	15.03± 0.14	10.50± 0.06	21.45 ±0.14	28.50± 0.18	24.00± 0.07	20.00± 0.11
S. aureus	26.23 ±0.46	16.00± 0.12	126.02± 0.10	13.50± 0.04	27.47 ±0.06	24.50± 0.15	21.00± 0.09	19.00± 0.13
S. typhi	17.33 ±0.06	15.58± 0.03	15.50± 0.12	13.50± 1.03	28.47 ±0.26	25.50± 0.36	19.50± 0.18	11.00± 0.07

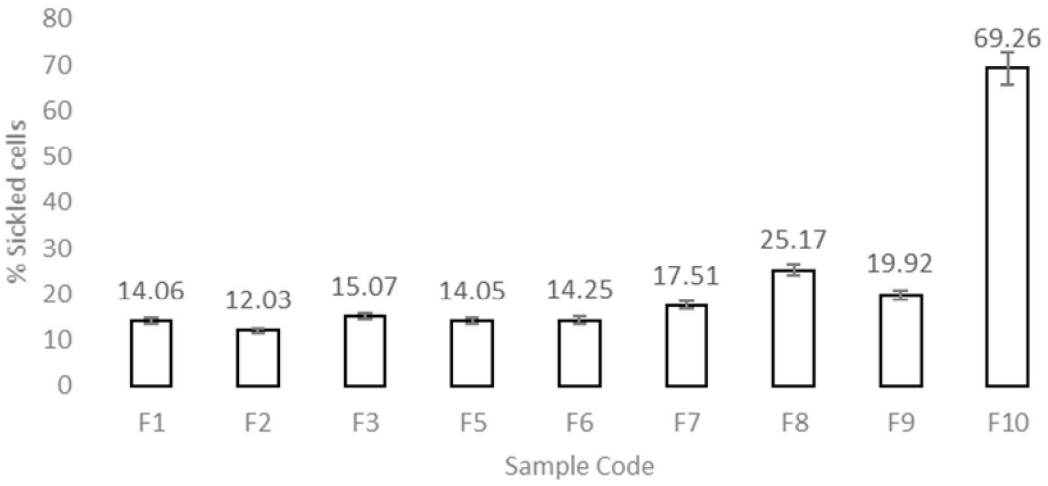

Figure 2: Percentage sickled cells left after antisickling activity testing
F1- syrup containing SB 250mg and MI 250mg, F2 - syrup containing SB 500mg and MI 250mg, F3-syrup containing SB 250mg and MI 500mg, F5- 500 mg SB extract only, F6- 500 mg MI extract only, F7-250mg SB extract only, F8-250mg MI extract only, F9- Ciklavit® and F10- negative control

Antibacterial activity

Sorghum bicolor produced no zone of inhibition at tested concentrations, *Mangifera indica* and Levofloxacin showed concentration-dependent inhibition for the different organisms as depicted in Table 4.

Formulation and evaluation of herbal syrups

The formulated syrups were clear liquid, viscous, orange color, sweet taste and minty smell with pH ranged from 4.2 to 4.8. These parameters remained unchanged throughout the testing period and conditions (60 ºC/75 %RH).

In vitro antisickling activity

The result of antisickling activity of the extracts and their formulations is presented in Figure 2. The result suggests that apart from the negative control (F10), F8 had the least antisickling activity and F2 had the best antisickling property amongst the samples.

Discussion

Phytochemical screening

The phytochemical screening of plants extracts provides essential information regarding the

chemical constituents of the extracts and hence insights on their pharmacological activities. The presence of important phytochemical groups (saponin, tannin, phenol, anthocyanin, steroid, phytosterol, and flavonoid) of the methanol extracts of the seed of *Sorghum bicolor* and *Mangifera indica* shows that they might possess good antioxidant, antimicrobial properties and hence might be useful in the management of SCD. Several reports have indicated lower levels of flavonoids in SCD patients [21]. Flavonoid quercetin has been shown to provide protection against hemoglobin oxidation and other cellular modifications promoted by peroxides [21]. Kumar and Pandey [22] reported that the functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions. Saponins consist of aglycones (either steroid C_{27} or triterpene C_{30}) attached to one or more sugar side chains, Srivastava *et al.* [23] reported the aglycone part of saponin is responsible for its good antioxidant properties. The total phenolic acid obtained from the SB extract did not correlate with that obtained from similar studies [16, 24]. The reason for the variations in the bioactive molecules of plant materials such as phenolics have been attributed to the differences in the structure and chemical compositions of plant parts [1]. Tannins can affect the organoleptic properties of the plant especially its taste and astringency [25] and they account for about 19% of total dietary antioxidant capacity compared to other phytochemical groups [26].

Proximate analysis

Moisture content in plant extracts can affect their microbiological, physical, and chemical stabilities when formulated as dosage forms. The extracts of SB seeds and MI leaves had low moisture contents (Table 2) making them good products to be formulated into dosage forms. Ash, the inorganic residue remaining after the water and organic matter has been removed by heating, provides a measure of the total minerals in the product [27]. The total ash content obtained for MI leaves extract in this study was higher than that of the SB seeds. The crude fibre of SB seeds was higher than that of MI leaves and even higher than that reported in a similar study [28]. The protein content (9.8 %) of SB was lower than 11.3% obtained by Stefoska-Needham *et al*. [28] in a similar study. Similarly, the protein content (5.18 %) of MI leaves was lower than that obtained (15.8 %) by Fafiolu *et al.*, [29]. The carbohydrate content of SB seed (40.67 %) was also lower than that obtained by Stefoska-Needham *et al*., [28] 72%. These variations have been attributed to several factors reported in a previous study [1]. Manadese *et al.* [5] reported an inverse relationship between hemoglobin F levels in SCD patients and intake of carbohydrates, lipids and protein, hence the two plant extracts might be useful in the management of SCD.

Comparatively, with exception of copper content that was low, the two extracts had reasonable amount of potassium, sodium, calcium, magnesium, iron, manganese and zinc (Table 2) and therefore could be good sources of these mineral. The presence of calcium in large quantities might be very helpful in osteoporosis and high iron content might be useful in anaemia that is common in SCD patients. Low levels of total magnesium in sickle cell erythrocytes have been associated with increased sickling due to propensity for red cell dehydration and increased HbS polymerization and during crisis magnesium, zinc, and potassium present in sufficient quantities may help in management of pain [30] Increased plasma copper levels in individuals with HbSS has also been reported [30], hence SB seeds and MI leaves extracts and their formulations might been useful in the management of patients with SCD.

FT-IR analysis

FT-IR can be employed in characterization and identification of compounds /functional groups present in an unknown mixture of plants extract [31]. Functional groups influence the biological activity of bioorganic materials to a large extent. In this study, the two extracts showed the presence of different phytochemicals. The result of the FT-IR analysis is consistent with findings from phytochemical screening since it has been established that OH functionality is an integral part of phenolic groups like flavonoids and tannins [32]. The FTIR studies indicate that the two extracts contain various bioactive molecules as depicted by their functional groups like ester, alcoholic, among others, hence confirming that the plant extracts possess bioactive molecules [32]. A more detailed description of the FT-IR spectrum of plant materials has been reported [32, 33].

Antioxidant assay

DPPH is scavenged by antioxidants through the

donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance [34]. It is believed that the higher the antioxidant property of an antisickling agent, the higher its possible antisickling effect due to a reduction of oxidative stress that contributes to sickle cell crisis and increase membrane protection of the cells, since SCD patients undergo oxidative stress at the onset of crises [1]. In the present work, both extracts showed good antioxidant activity comparable to the reference standards. This result is predictable due to their respective phenolics, alkaloids and flavonoids contents. The observed high antioxidant capacity of the extracts supports the significance of SB seeds and MI leaves as promising natural sources of antioxidants and hence would be useful in the management of SCD.

Antibacterial assay

The antibacterial activity of the plant extracts can be due to the presence of various phytochemicals, which are known to be synthesized by plants in response to microbial infection [35]. Evaluation of the antimicrobial activity of plants used in management of SCD is important because infections are recurrent pathologies of SCD that slowdown blood circulation. Osteomyelitis, meningitis and septicemia are common infections in SCD patients and *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli* are mainly the causal micro-organisms [36]. In our present work, only the MI extract showed a good antibacterial activity against the four tested organisms, hence preparations containing the MI leaves extract would be useful for SCD patients. The difference in antibacterial activity of both extracts might be attributed to the variations in the bioactive molecules of plant materials especially the flavonoid and proanthocyanidin contents.

Physical properties of the formulated syrups

The syrup formulated did not show any change at the different storage conditions. At 4˚C, no crystallization was observed; this may be due to the presence of sorbitol. Sorbitol acts as a crystallization modifier or inhibitor, it also prevents a reduction in syrup viscosity. Anti-microbial properties and antioxidant properties of the syrup may have impacted positively on the stability of the formulation.

In vitro antisickling activity

The best antisickling activity observed in F2 (syrup containing 500 mg SB and 250 mg MI) might be attributed to the synergism of phytochemical groups present in both plant extracts such as phenol, flavonoid, steroid, glycoside, and anthraquinone. The higher antisickling activity of SB extract than MI extract might be attributed to the high flavonoid content of SB and its good radical of scavenging activity. The extracts used in the study are both potent antioxidants, antioxidants were found to be potent inhibitors of sickle cell hemoglobin polymerization [1], and this was observed in both extracts but majorly in SB extract and syrup formulation containing a higher concentration of SB. Studies by Mpiana *et al.,* [10] reported that the high antisickling activity observed in SB extract was due to its high content of phenolic compound anthocyanin. The antisickling property of MI extract might be due to its anthraquinone content, it has been shown to improve Fe^{2+}/Fe^{3+} ratio, hence facilitating the conversion of met-hemoglobin to hemoglobin increasing the oxygen affinity of sickle cell hemoglobin and change its pathophysiology [37].

Conclusion

The study revealed that the methanolic extracts of *Mangifera indica* leaves and *Sorghum bicolor* seeds demonstrated significant antioxidant, antimicrobial and antisickling activities and syrups containing the mixtures of the two extracts would be useful in management of sickle cell disorder. Further study is required to isolate and identify the bioactive molecules responsible for these activities from the crude extracts and also evaluate the *in vivo* bioactivities of the isolated compounds.

Conflict of Interest

No conflict of interest associated with this work

Contribution of Authors

We declare that this study was carried out by the four authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. CAU and CII conceived and designed the study, and contributed to the manuscript write-up. CPA and NHI supervised data

collection, analysed the data and drafted the manuscript. All authors approved the manuscript for publication.

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