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ANTISICKLING ACTIVITY-GUIDED FRACTIONATION OF SOME PLANTS USED FOR THE MANAGEMENT OF SICKLE CELL DISEASE IN SOUTHWEST NIGERIA

J. M. Agbedahunsi*, C. A. Elusiyan, M. C. Cyril-Olutayo, F. B. Adewoyin, A. O. Oriola, O. A. Agbaje and T. A. Ajayi

Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria

**Corresponding author: jagbedah@oauife.edu.ng ; +2348034093594*

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ABSTRACT

The study evaluated the antisickling activities of some plants used in the Southwestern Nigeria ethno-medicine for the management of sickle cell disease (SCD), with a view to determining the most active plant extract and fraction. A semi-structured questionnaire was administered to two hundred and ten (210) respondents, which comprised traditional medical practitioners, herb sellers and community elders. Aqueous extracts of the plants with high fidelity levels alongside the positive controls (vanillic acid and *p*-hydroxybenzoic acid) were tested *in vitro* against the sickled erythrocytes, using the inhibitory and reversal assay methods. The active extracts were solvent-partitioned into petroleum spirit, ethyl acetate and aqueous fractions, and subsequently bio-assayed. A total of 166 plants were mentioned as being used for the management of SCD within the zone, among which 27 plants were selected. Six of the selected plant extracts demonstrated remarkable antisickling activities. The aqueous extracts of *Moringa oleifera* leaf (**MOL**), *Alchornia laxiflora* leaf (**ALL**), *Kigelia africana* fruit (**KAF**), *Olax subscorpioidea* leaf (**OSL**), *Pyrenacantha staudtii* leaf (**PSL**) and *Parquentina nigrescense* leaf (**PNL**) demonstrated 88.0, 86.2, 76.2, 75.8, 54.4 and 52.2% inhibitory activities respectively, while the reversal activities were 76.9, 65.3, 75.5, 66.6, 52.4 and 66.0% respectively. The ethyl acetate fraction of **MOL** and **KAF** (4 mg/mL) demonstrated 94.6 and 87.2% inhibitory activities, and 94.7 and 81.4% reversal activities respectively. The mode of activity of **KAF** was by membrane stabilisation with 81.1% activity. Silica gel column purification, followed by preparative thin-layer chromatography of the ethyl acetate **KAF** fraction afforded 5 bands, $\mathbf{M_1}$ – $\mathbf{M_5}$. At 4 mg/mL, band $\mathbf{M_2}$ demonstrated the highest activity with 78.9% inhibitory and 70.8% reversal, and also showed strong free radical scavenging property. The findings validated the ethnomedicinal use of the six listed plants, especially *M. oleifera* leaves and *K. africa*na fruits for the management of SCD and identified the ethyl acetate fractions of both plants to contain the putative compounds.

INTRODUCTION

Sickle cell disease (SCD) is an inherited genetic disorder in human, caused by mutation in the haemoglobin beta gene (HbB), thereby resulting in a single amino acid substitution of glutamic acid for valine at position six of the beta globin chain. This substitution leads to the production of haemoglobin S (HbS) gene, an abnormal form of haemoglobin A gene [1]. This abnormality often manifest as chronic anaemia, painful episodes, enlarged spleen, persistent infections, pulmonary hypertension and organ damage [2]

SCD is a non-communicable genetically autosomal recessive blood disorder, inherited from two parents with defective genes either as carriers or sickling [3]. It is a major public health concern in many parts of the world. Over 100 million people, accounting for about 5% of the world population live with the disease with about 300,000 children born annually [4]. Up to 70% of children born with the disease are from sub-Sahara African countries including Nigeria [5]. Nigeria has the highest occurrence of the disorder and sickle cell carrier in the world with about 2.5% and 24 % respectively, and an annual infant mortality of about 100,000 [6].

Various measures have been employed to prevent and manage the crisis associated with SCD. These include chemotherapy, blood transfusion, and most recently bone marrow transplantation which is expensive and therefore cannot be afforded by many people living in developing countries [7]

In developed countries, most SCD patients live up to forty years before death due to early newborn screening for diagnosis, penicillin prophylaxis and other chemotherapeutic treatments. Unfortunately, most patients living in the rural areas die before the age of five in most developing countries where early diagnosis and treatment are unavailable to most of the population, making early death inevitable [8].

Hydroxycarbamide (hydroxyurea), clotrimazole and erythropoietin are the synthetic drugs used for the management of SCD. They are moderately toxic when administered over a long period of time, and are also associated with side effects such as iron overload, teratogenic and mutagenic assertions on patients [9,10]. This has necessitated the search for more efficacious and safe antisickling agents from nature.

The role of medicinal plants in the management of SCD cannot be overemphasized. Some plants with established antisickling properties include: *Carica papaya* [11-13]*; Fagara zanthoxyloides* roots [14]; *Terminalia catapa* leaves [15]; *Cajanus cajan* seeds [16]; *Calliandra portoricensis* roots [17]; *Cnidoscolus aconitifolius* leaves [18] amongst others.

In the Nigerian pharmaceutical industry, Niprisan® and Ciklavit® are nutraceuticals used for the management of sickle cell anaemia (SCA). They have been developed from combined extract of *Sorghum bicolor* leaf sheath, *Eugenia caryophyllus* fruit, *Piper guineense* seed and *Pterocarpus osun* stem, and an extract of *C. cajan* respectively [19, 20]. Many more plants still need to be exploited for effective and safe treatment of the disease. Therefore,

it is expedient to exploit the indigenous knowledge of plants used in traditional medicine for the management of SCD by evaluating their antisickling activities and determining their active fractions; hence, this report.

RESULTS AND DISCUSSION

Ethnomedicinal Survey

The result (**Table 1**) showed a total 210 respondents across the three states, majority of them between the ages of 41 – 70 years. About 85% of the respondents were literate and were knowledgeable about sickle cell disease. Also, 84% of the respondents engaged in herbal medicine practice and acquired the knowledge by inheritance and apprenticeship. This was in consonance with the report of Mahwasane *et al*. [21] that majority of respondents in the Limpopo Province of South Africa were adults, female, who were knowledgeable in the traditional use of plants in the management many diseases.

One hundred and sixty-six (166) plants belonging to 111 genera and 65 families were mentioned as being used for the management of SCD across the study area (**Table 2**). Result (**Figure 1**) also showed the top five families of plants with high frequency of occurrence as Fabaceae (13), Rutaceae (10), Leguminosae (7), Euphorbiaceae (6) and Annonaceae (6). The frequently mentioned plants by the respondents were *Piper guineensis* with 3.88% fidelity level, *Citrus aurantifolia* (3.55%), *Carica papaya* (3.35%), *Khaya grandifoliola* (3.17%), *K. senegalensi*s (3.17%), *Morinda lucida* (2.82%),*Uvaria chamae* (2.65%), *Petivera alliaceae* (2.47%) and *Sorghum bicolor* (2.12%). Leaf was the mostly mentioned plant part with 37% frequency, followed by root with 26% usage **(Figure 2)**. Majority of the respondents indicated that their remedies were prepared as decoction, while quite a few of them mentioned maceration as a method of preparation.

The study sought knowledge from part of Southwest Nigerian traditional medicine (TM) and used ethnomedicinal survey as a tool. This was based on the fact that TM remains the sum total of the knowledge, skills and practices of the theories, beliefs and experiences indigenous to different cultures that are used for the maintenance and improvement of health, as well as for the prevention, diagnosis and treatment of illnesses [22]. It is in the interest of the World Health Organization (WHO) that documentation on the use of medicinal plants by native peoples from different part of the world are made for further scientific investigation.

Among the one hundred and sixty-six (166) plants mentioned, forty-three (43) have not been validated for their antisickling activities; hence, twenty-five (25) of them were readily available and so were evaluated.

Table 1: Demographic features of respondents on the plants used as remedy against SCD in Part of Southwest Nigeria

Features	Categories	Frequency	Percentage (%)
Gender	Male	91	43.33
	Female	119	56.67
Age	18-30	9	4.29
	31-40	22	10.48
	41-50	48	22.86
	51-60	55	26.19
	61-70	49	23.33
	Above 70	27	12.86
Occupation	Traditional medical practitioners	71	33.81
	Herb sellers	109	51.90
	Others	30	14.29
Level of Education	Illiterate	35	16.67
	Primary	36	17.14
	Secondary	84	40.00
	Tertiary	55	26.19
Acquisition of Knowledge	Inheritance	101	48.10
	Apprenticeship	77	36.67
	Others	32	15.24

Footnote: *Percentage = (Frequency/Total)*100*

Table 2: Medicinal Plants used in Part of Southwest Nigeria Ethnomedicine for the management of Sickle Cell Disease

S/N	Botanical name	Family name	Vernacular name	Part used	Frequency	Fidelity Level FL (%)
1.	*Carica papaya* L.	Caricaceae	Ibepe	Leaves, unripe fruits	19	3.35
2.	*Zanthozylum zanthoxyloides* Linn.	Rutaceae	Orin ata	Bark, roots	2	0.35
3.	*Cajanus cajan* (L.) Millsp	Fabaceae	Otili	Seeds	6	1.06
4.	*Harungana madagascarensis* Lam (Ex. Poir)	Hypericaceae	Amuje	Bark	3	0.53
4.	*Glycine max* (L.) Merr	Fabaceae	Ewa soya	Seeds	3	0.53
5.	*Anthocleista vogelii Linn*	Loganiaceae	Sapo	Roots, leaves, Bark	8	1.41
6.	*Citrus aurantifolia* (Christm) Swingle	Rutaceae	Osan wewe	Leaves, Fruit juice	20	3.53
7.	*Gossypium barbadensis* Linn	Malvaceae	Owu	Leaves	1	0.18
8.	*Khaya grandifoliola* C. DC	Meliaceae	Oganwo	Bark, Roots	18	3.17
9.	*Khaya senegalensis* (Descr) A. Juss	Meliaceae	Oganwo	Bark, Roots	18	3.17
10.	*Jatropha curcas* Linn	Euphorbiaceae	Botuje/ Lapalapa funfun	Roots	5	0.88
11.	*Carpolobia lutea* G. Don	Polygalaceae	Osunsun	Roots	2	0.35
12.	*Olax subscorpiodea* Oliv	Olacaceae	Ifan/ifon	Roots	6	1.06
13.	*Anarcardium Occidentale* Linn	Anarcardiaceae	Kaaju	Bark, Roots	4	0.71
14.	*Calliandra haematocephala* Linn	Leguminosae/ Mimosoideae	Tude	Leaves, Roots	4	0.71
15.	*Allium sativum* Linn	Liliaceae	Ayu	Bulbs	4	0.71
16.	*Lannea egregia* Engl& K.klause	Anacardaceae	Ekudan	Bark	1	0.18
17.	*Theobroma cacao* L.	Malvaceae	Koko	Bark	2	0.35
18.	*Petivera alliaccae* L.	Phytolacaceae	Awogba, oju saju	Roots, leaves	14	2.47
19.	*Chassalia kolly* (Schum.) Hepper	Rubiaceae	Isepe-agbe	Roots, leaves	1	0.18
20.	*Morinda lucida* L.	Rubiaceae	Oruwo	Roots, Bark, Leaves	16	2.82
21.	*Euphorbia neriifolia* Linn	Euphobiaceae	Soro	Leaves, Roots	3	0.53
22.	*Microdermis puberula* Hook f. ex Planch	Pandaceae	Apata	Roots	2	0.35
23.	*Lecaniodiscus cupaniodes* Planks Exbth	Sapindaceae	Aka	Roots	1	0.18
24.	*Garcinia kola* Heckel	Guttiferae	Orogbo	Fruits	8	1.41
25.	*Kigelia africana* (Lam) Benth	Bignoniaceae	Pandoro	Leaves, Fruits, Bark	7	1.23
26.	*Mangifera indica* L.	Anacardaceae	Mangoro	Bark, Leaves	7	1.23
27.	*Melissa officinalis* L.	Lamiaceae	Jogbo	Leaves	1	0.18
28.	*Ocimum gratissimum* L.	Lamiaceae	Efirin	Leaves	3	0.53
29.	*Ageratum conyzoides* L.	Compositae	Imi-esu	Leaves	2	0.35
30.	*Cynomentra vogeli* Hook.f	Leguminosae	Ata	Roots	1	0.18
31.	*Alafia barteri* Oliv		Agbari etu	Roots, Leaves	4	0.71
32.	*Clausena anisata* (Willd.) Hook K.f.exbenth	Rutaceae	Agbasa	Bark, Roots	4	0.71
33.	*Clitoria ternatea* L.	Fabaceae	Irere	Fruits	1	0.18
34.	*Adansonia digitata* Linn	Bombaraceae	Ose	Roots	1	0.18
35.	*Ocimum basilicum* Linn	Lamiaceae	Efirin wewe	Leaves	2	0.35
36.	*Citrus sinensis* L.	Rutaceae	Osan	Fruit juice	2	0.35
37.	*Citrus tangerine* Tanaka	Rutaceae	Tangerine	Fruit juice	2	0.35
38	*Capsicum frutescens* Linn	Zingiberaceae	Ata ijosi	Seeds	3	0.53
39	*Piper guineese* schum & Thorn	Piperaceae	Iyere	Fruits	22	3.88
40	*Curcuma longa* L.	Zingeberaceae	Ata ile pupa	Root, Fruits	10	1.76
41	*Picralima nitida* Staph	Apocynaceae	Abere	Fruits	3	0.53
42	*Sorghum bicolor* (L.) Moench	Poaceae	Poporo okababa	Bark, Seeds	12	2.12
43	*Alstonia boonei* De wild	Apocynaceae	Ahun	Bark, Roots	4	0.71
44	*Citrus aurantium L.*	Rutaceae	Osan jaganyin	Fruit juice, Roots	4	0.71

S/N	Botanical name	Family name	Vernacular name	Part used	Frequency	Fidelity Level FL (%)
45	*Securidaca longipedunculata* Frer	Polyganaceae	Ipeta	Roots, Leaves	6	1.06
46	*Plumbago zeylanica* (L.) Herbs	Plumbaginaceae	Egbo Inabiri	Roots	5	0.88
47	*Pennisetum purpureum* Schumach	Poaceae	Asun	Roots	3	0.53
48	*Viscum album* Linn	Santalaceae	Afomo	Leaves	2	0.35
49	*Bambusa vulgaris* (Schrad. Ex wendel)	Poaceae	Oparun	Leaves	4	0.71
50	*Cleistopholis patens* Benth	Annonaceae	Pako/ apako	Roots	5	0.88
51	*Vernonia amgdalina* Delile	Asteraceae	Ewuro	Leaves	2	0.35
52	*Parquetina nigrescens* (Afzel) Bullock	Periplocaceae	Ogbo/gbo	Bark, Roots	7	1.23
53	*Axonopus compressus* Engl&Diels	Combretaceae	Idi	Bark	1	0.18
54	*Dioscorea domentorum* (Kunth) Pax	Dioscoreaceae	Esuru pupa	Tuber	1	0.18
55	*Uvaria chamae* P.Beau	Annonaceae	Eruju	Roots	15	2.65
56	*Paullinia pinnata* Linn	Sapindaceae	Kakasenla	Roots	1	0.18
57	*Anogeissus leiocarpa* (DC) Guill&Perr	Combretaceae	Ayi	Bark	1	0.18
58	*Cocos nucifera* Linn	Palmae (Araceae)	Agbon	Fruit juice	1	0.18
59	*Uvaria afzelii* SC.Elliot	Annonaceae	Gbogbonise	Roots	3	0.53
60	*Azadirachta indica* A.Juss	Meliaceae	Dongoyaro	Roots, Bark	2	0.35
61	*Chasmanthera dependens* Hocst	Menispermaceae	Egbo ato	Roots	6	1.06
62	*Aristolochia repens* Vahl	Aristolochiaceae	Akogun	Roots	1	0.18
63	*Ficus asperifolia* Miq	Moraceae	Ipin	Leaves	2	0.35
64	*Eugenia aromaticum* Linn	Myrtaceae	Kanafuru	Clove	3	0.53
65	*Caesalpinae bonduc*	Caesalpiniaceae	Seeyo	Roots	2	0.35
66	*Vernonia amygdalina* L.	Asteraceae	Ewuro	Roots, Leaves	6	1.06
67	*Heliotropium indicum Linn.*	Boraginaceae	Olojogbura	Aerial part	1	0.18
68	*Solenostemon monostachyus* (P. Beauv.)	Asclepiadaceae	Olojogbodu	Leaves	1	0.18
69	*Ritchiea capparoide* (Andr.)	Capparidaceae	Ologbokiyan	Leaves	2	0.35
70	*Criestis ferruginea*	Cyatheaceae	Esan abo (ekuro)	Fruits	1	0.18
71	*Cycnium camporum*	Scrophulariaceae	Arojoku	Leaves	1	0.18
72	*Butyrospermum paradoxum*	Sapotaceae	Emi	Bark	2	0.35
73	*Abrus precatorius*	Leguminosae	Oju Ologbo	Seeds	1	0.18
74	*Biophytum petersianum*	Oxalidaceae	Patanmo	Leaves	1	0.18
75	*Plumbago zeylanica*	Plumbaginaceae	Inabiri	Roots	3	0.53
76	*Eucalyptus* spp.	Myrtaceae	Ukaliptu	Roots	2	0.35
77	*Telfaria occidentalis*	Cucurbitaceae	Igbale, Ewe ugu	Leaves	3	0.53
78	*Pterocarpus osun*	Papilionaceae	Osun	Stems	1	0.18
79	*Milicia excels*	Moraceae	Iroko	Leaves	6	1.06
80	*Aloe vera*	Aloaceae	Alo vera	leaves	1	0.18
81	*Lagenaria siceraria*	Cucurbitaceae	Igba	Bark	1	0.18
82	*Cassytha filiformis*	Lauraceae	Omo onigelegele	Leaves	1	0.18
83	*Paspalim conjugatium*	Poaceae	Awo Iki	Bark	1	0.18
84	*Ananas comosus*	Bromeliaceae	Opon Oyinbo	Fruits	1	0.18
85	*Erythrophleum suaveoleus*	Caesalpiniaceae	Obo	Bark	1	0.18
86	*Cassia alata*	Caesalpiniaceae	Asunwon/ Kasia	Leaves, Roots, Bark	7	1.23
87	*Chrysophyllum albidum*	Sapotaceae	Agbalumo	Stems	1	0.18
88	*Peperomia pellucid*	Piperaceae	Rinrin	Leaves	2	0.35
89	*Bryophyllum pinnatum*	Crassulaceae	Odundun	Leaves	3	0.53
90	*Brachyestegia eurycoma*	Asteraceae	Akoo	Stems	1	0.18
91	*Rauvolfia vomitoria*	Apocynaceae	Asofeyeje	Leaves, Roots	4	0.71
92	*Adenopus breviflorus* Benth	Cucurbitaceae	Tagiri	Fruits	2	0.35
93	*Cynometra megalophylla* Harms.	Leguminosae	Aka	Roots	1	0.18

S/N	Botanical name	Family name	Vernacular name	Part used	Frequency	Fidelity Level FL (%)
94	*Acacia ataxacantha* DC.	Fabaceae	Ewon agogo	Leaves	1	0.18
95	*Allium ascalonicum* L.	Liliaceae	Alubosa onisu	Bulbs	2	0.35
96	*Dioclea reflexa* Hook.f.	Leguminosae	Arin pupa	Seeds	1	0.18
97	*Chenopodium ambrosioides* L.	Chenopodiaceae	Arun pale	Leaves, Roots	2	0.35
98	*Motandra guineensis* (Thonn.) A.DC.	Apocynaceae	Agbaboje	Roots	1	0.18
99	*Amaranthus hybridus* L.	Amaranthaceae	Atetedaye	Leaves	1	0.18
100	*Musa sapentum var paradisiaca* L.	Musaceae	Ogede agbagba	Unripe fruits	1	0.18
101	*Indigofera nummulariifola*	Fabaceae	Rekureku	Leaves	1	0.18
102	*Baphia nitida* Lodd.	Leguminosae	Iyere osun	Leaves	1	0.18
103	*Anthocleista djalonensis* A Chev.	Loganiaceae	Sapo	Bark	1	0.18
104	*Nicotiana tabacum* L.	Solanaceae	Taba	Leaves	2	0.35
105	*Colocynthis citrullus* L.	Cucurbitaceae	Bara	Leaves	2	0.35
106	*Grewia pubescens* P. Beauv.	Tiliaceae	Oora igbo	Roots	1	0.18
107	*Calliandra portoricensis* (Jacq.) Benth.	Mimosoideae	Tude	Leaves, Roots	1	0.18
108	*Lophira alata* Banks ex Gaertn.	Ochnaceae	Epo eki	Bark	2	0.35
109	*Mimosa pudica* L.	Mimosoideae	Ewe patanmo	Leaves	1	0.18
110	*Aeglopsis chevsheri*	Rutaceae	Igo oke/ewe oke	Roots	1	0.18
111	*Costus afer* Ker–Gawl.	Costaceae	Ireke omode	leaf sheaths	1	0.18
112	*Piptadeniastrum africanum*	Leguminosae	Epo agboyin	Bark	2	0.35
113	*Citrus medica* L. *var. acida* Brandis	Rutaceae	Osan ganyinganyin	Leaves and Roots	3	0.53
114	*Aframomum melegueta* (Roscoe) K. Schum	Zingiberaceae	Ata ire	Fruits	7	1.23
115	*Cremaspora triflora* (Thonn.) K. Schum.	Rubiaceae	Ina	Leaves	1	0.18
116	*Musa sapientum* L.	Musaceae	Ogede wewe	Suckers	1	0.18
117	*Cassia podocarpa* Guill. et Perr.	Caesalpiniaceae	Asunwon	Leaves	1	0.18
118	*Phyllanthus amarus* Schum. & Thonn.	Euphorbiaceae	Eyin olobe	Leaves	1	0.18
119	*Xylopia aethiopica* (Dunal) A. Rich.	Annonaceae	Eru	Fruits	3	0.53
120	*Nauclea latifolia* Smith	Rubiaceae	Egbo egbesi	Roots	6	1.06
121	*Cochlospermum tinctorium* A. Rich.	Cochlospermaceae	Feeru	Leaves	1	0.18
122	*Enantia chlorantha* Oliv.	Annonaceae	Oso pupa	Stems	13	2.29
123	*Momordica charantia* L.	Cucurbitaceae	Ejinrin	Leaves	2	0.35
124	*Strophanthus hispidus* DC.	Apocynaceae	Egbo sagere	Roots	11	1.94
125	*Alchornea laxiflora* (Benth.) Pax and Hoffman	Euphorbiaceae	Epepe	Roots and Bark	1	0.18
126	*Cynometra mannii* Oliv.	Leguminosae	Aka	Fruits	1	0.18
127	*Argemone Mexicana* L.	Papaveraceae	Mafowokanmi	Leaves	1	0.18
128	*Desmodium gangeticum* DC.	Fabaceae	Ema	Leaves	1	0.18
129	*Acanthospermum hispidum* DC.	Asteraceae	Dagunro	Leaves	1	0.18
130	*Talinum portulacifolium* (Forssk.) Asch. ex Schweinf	Portulacaceae	Egbure	Whole plant	1	0.18
131	*Ficus capensis* Thunb.	Moraceae	Opoto	Leaves	1	0.18
132	*Tetrapleura tetraptera* (Schumach. And Thonn) Taub	Fabaceae	Aridan	Fruits	1	0.18
133	*Achillea millefolium L.*	Asteraceae	Aka	Fruits	7	1.23
134	*Aerva lanata* (Linn.) Juss. Ex Schult.	Amaranthaceae	Efun Eleelo	Leaves	5	0.88
135	*Allium cepa* L.	Amaryllidaceae	Alubosa were	Bulbs	1	0.18
136	*Jatropha gossypiifolia* L.	Euphorbiaceae	Lapalapa pupa	Fruits, Leaves	4	0.71
137	*Corchorus olitorious* L.	Malvaceae	Ewedu	Leaves	3	0.53
138	*Solanum microcarpum* Vahl.	Solanaceae	Igba	Fruits	2	0.35

S/N	Botanical name	Family name	Vernacular name	Part used	Frequency	Fidelity Level FL (%)
139	*Uvariodendron angustifolium* Engl.	Annonaceae	Ewe igbere	Leaves	8	1.41
140	*Pergularia daemia* Forssk. Chiov.	Asclepiadaceae	Ewe teji	Leaves	2	0.35
141	*Chrysophyllum cainito* L.	Sapotaceae	Igi onisan	Stems	2	0.35
142	*Dalbergia lactea* Vatke	Fabaceae	Ewe ojiji	Leaves	4	0.71
143	*Caesalpinia pulcherrima* L.	Fabaceae	Ewe omode	Leaves	7	1.23
144	*Nauclea diderrichii* (De Wild.) Merr.	Rubiaceae	Ewe opepe	Leaves	4	0.71
145	*Secamone afzelii* (Schult.) K. Schum	Asclepiadaceae	Ewe ailu	Leaves	1	0.18
146	*Osteopermum ecklonis* (DC.) Norl.	Asteraceae	Ewe akila	Leaves	3	0.53
147	*Acacia nilotica* (L.) Wild ex. Del.	Fabaceae (Mimosoideae)	Ewe kasia	Leaves	2	0.35
148	*Entadrophragma angolense* (Welw.) C DC.	Meliaceae	Eepo igi Ijebo	Bark	1	0.18
149	*Lanchocarpus laxiflorus* Guill. & Perr.	Fabaceae	Ewe apapa	Leaves	2	0.35
150	*Amaranthus caudatus* L.	Amaranthaceae	Panukuro	Roots	1	0.18
151	*Alternanthera sessilis* (L.) R.Br. ex DC	Amaranthaceae	Ewe rekureku	Leaves	2	0.35
152	*Anchomanes difformis* (Blume) Engl.	Araceae	Epo ogiri sako	Bark	1	0.18
153	*Calotropis procera* (Aiton) W.T Aiton	Asclepiadaceae	Bomubomu	Leaves, Fruits	3	0.53
154	*Cissampelos owariensis* P Beauv.	Menispermaceae	Ewe jenjoko	Leaves	1	0.18
155	*Citrus japonica* Thunb.	Rutaceae	Egbo osan nla	Roots	1	0.18
156	*Ehretia cymosa* (Thonn.)	Boraginaceae	Ewe ijan – oke	Leaves	1	0.18
157	*Gladiolus psittacinus* Hook	Iridaceae	Eso baka	Fruits	3	0.53
158	*Ficus exasperata* Vahl	Moraceae	Ewe eepin	Leaves	1	0.18
159	*Mallotus oppositifolius* (Geiseler) Mull.Arg.	Euphorbiaceae	Ewe orokoro	Leaves	1	0.18
160	*Microdesmis puberula* Hook. f. ex Planch	Pandaceae	Egbo esunsun	Roots	1	0.18
161	*Newbouldia laevis* (P.Beauv) Seem. ex Bureau	Bignoniaceae	Ewe akoko	Leaves	2	0.35
162	*Okoubaka aubrevillei* Pellegr. & Normand	Santalaceae	Epo igi nla	Bark	4	0.71
163	*Pyrenacantha staudtii*	Icacinaceae	Alukorogbaja	Leaves, Bark	7	1.23
164	*Senecio abyssinicus* Sch. Bip.	Compositae	Ewe amunimuye	Leaves	1	0.18
165	*Sida rhombifolia* L.	Malvaceae	Egbo iseketu	Roots	1	0.18
166	*Treculia africana* Decne.	Moraceae	Egbo ifon	Roots	1	0.18

Footnote: N (total number of mention) = 567, F = frequency, Fidelity Level, FL (%) = F/N*100

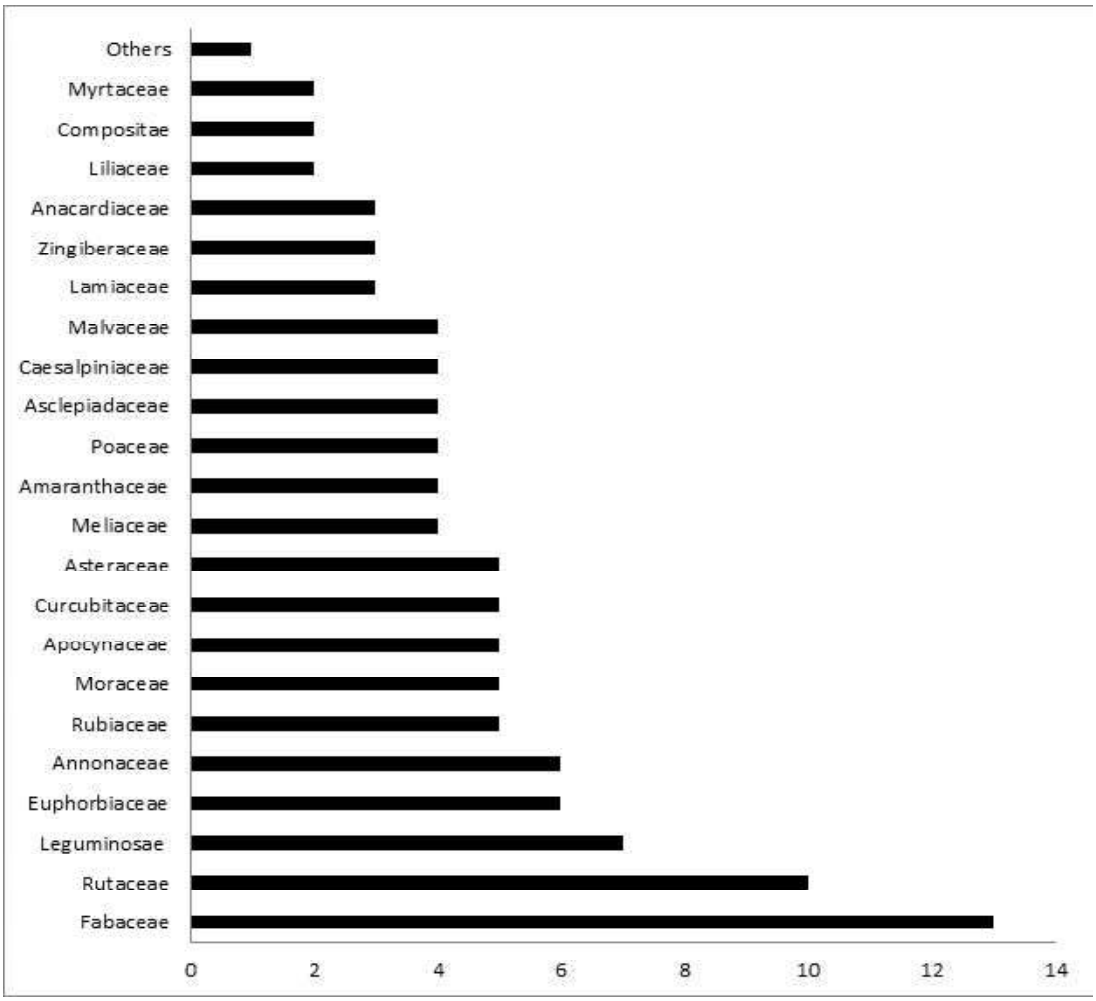


Figure 1: Frequency distribution of Southwestern Nigeria SCD plants across the families

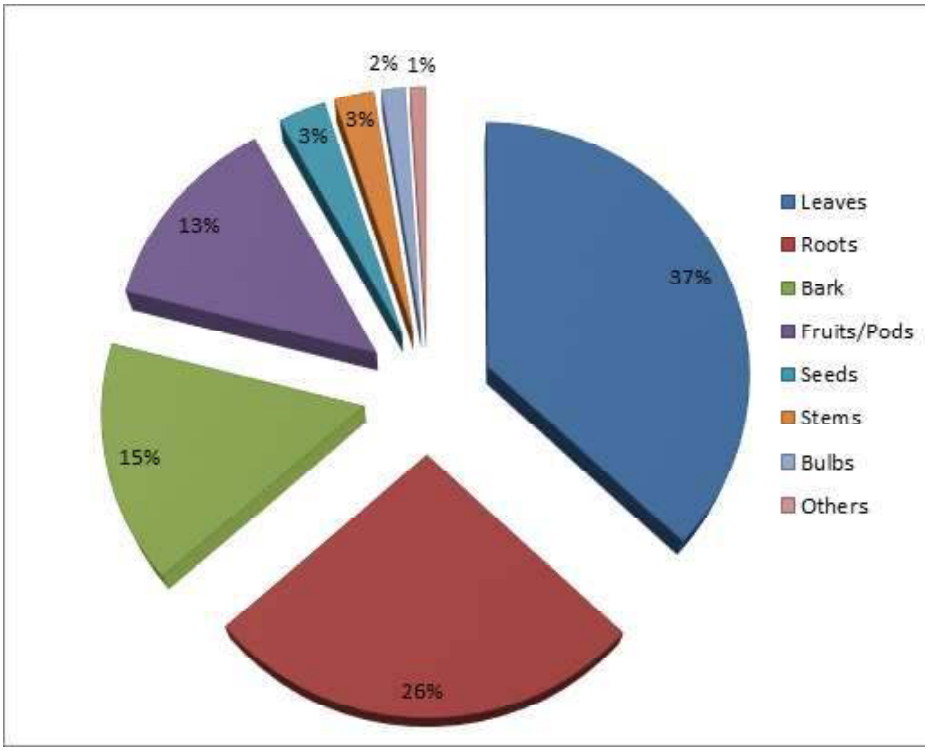


Figure 2: Frequency distribution of plant parts used for the management of SCD in the Southwestern part of Nigeria

Evaluation of Antisickling Activities of the Twenty-seven Selected Plants

The result of antisickling activities of aqueous extracts obtained from twenty-seven selected Nigerian medicinal plants, presented in **Table 3** showed that six of the extracts demonstrated >50 % activities; hence, justified their further investigation. The plants include: *Moringa oleifera* aerial part (**MOL**)*, Alchornia laxiflora* leaf (**ALL**), *Kigelia africana* fruit **(KAF**), *Olax subscorpiodea* leaf (**OSL**), *Pyrenacantha staudtii* leaf (**PSL**) and *Parquentina nigrescense* leaf (**PNL**).

In the inhibitory model, **MOL** (4.0 mg/mL) demonstrated 88.09% activity, which was the highest among the plant extract, followed by **KAF** which exhibited 76.16% inhibitory activity. However, **MOL** was significantly lower in activity (P<0.05) when compared with vanillic acid (VA), the positive control (93.31%). In the reversal model, **MOL**, **KAF** and **ALL** exhibited comparable activities, and they demonstrated the best activities among the extracts. However, each one of them demonstrated a significantly (P<0.05) lower activity compared with the positive control, *p*-hydroxybenzoic acid (PHBA), which gave 86.51% reversal activity.

The result of antisickling activities of the partition fractions from the six candidate plants (**Figures 4 and 5**) showed that purification enhanced both the inhibitory and reversal activities of *M. oleifera* and *K. africana.* A critical evaluation of the inhibitory antisickling activities showed that the aqueous fraction of **MOL** demonstrated 94.63% inhibitory activity, which was comparable (P>0.05) in activity with its ethyl acetate fraction and vanillic acid (**VA**) at 94.33% and 92.67% activities respectively. This was closely followed by the ethyl acetate fraction of **KAF** with 87.20% activity. In the reversal model, the ethyl acetate (94.70%) and petroleum spirit (94.04%) fractions of **MOL** were comparable (P>0.05) in their activities. This was also followed by the ethyl acetate fraction of **KAF** with 81.40% activity. On the whole, the ethyl acetate fractions of *M. oleifera* and *K. africana* were the best among the fractions based on their remarkable inhibitory and reversal antisickling activities. The marked antisickling activity of *M. oleifera* leaf has been attributed to its membrane stabilizing effect [23].

Therefore, our report on the membrane stabilizing activity of the aqueous extract and the ethyl acetate fraction of *K. africana* fruit when incorporated into sickled red blood cells showed that purification of the extract led to a significant increase in the activity of the plant. The ethyl acetate fraction exhibited the best activity among the samples across all concentrations. It is noteworthy that the ethyl acetate fraction (55.88%) demonstrated a significantly (P<0.05) two-fold higher percentage membrane stabilisation than Ibuprofen (26.49%), the positive control at a minimum test concentration of 0.375 mg/mL. At 1.50 mg/mL, the ethyl acetate fraction (81.09%) was also significantly higher in percentage membrane stabilisation than Ibuprofen (53.81%).

Based on the demonstrated membrane stabilising activity of **KAF**, it can be deduced that the plant gave a better protection against lysis and also stabilized the red blood cell better than Ibuprofen. This mechanism of action confers on both *M. oleifera* and *K. africana* the ability to bind with the human erythrocytes' membranes and subsequent alteration of the surface charges of the cells, thereby preventing physical interaction with aggregating agents or promotes dispersal by mutual repulsion of like charges involved in the haemolysis of red blood cells [24].

Several reports have supported the fact the membranes of human erythrocytes HbAA, HbAS and HbSS blood types have varying stability as determined from the mean corpuscular fragility [25,26].

The ethyl acetate fraction of **KAF** afforded a relatively pure band $\mathbf{M_2}$ upon its separation on a Silica gel preparative thin-layer chromatography. Band $\mathbf{M_2}$ gave an immediate reaction by its bleaching effect against the purple DPPH radical solution (**Plate 1**); thus, suggesting it to be a phenolic compound with strong free radical scavenging property.

Therefore, plants extracts and fractions capable of positively affecting the red cell membrane and with demonstrable free radical scavenging property such as *M. oleifera* and *K. africana* would be very useful in sickle cell disease management.

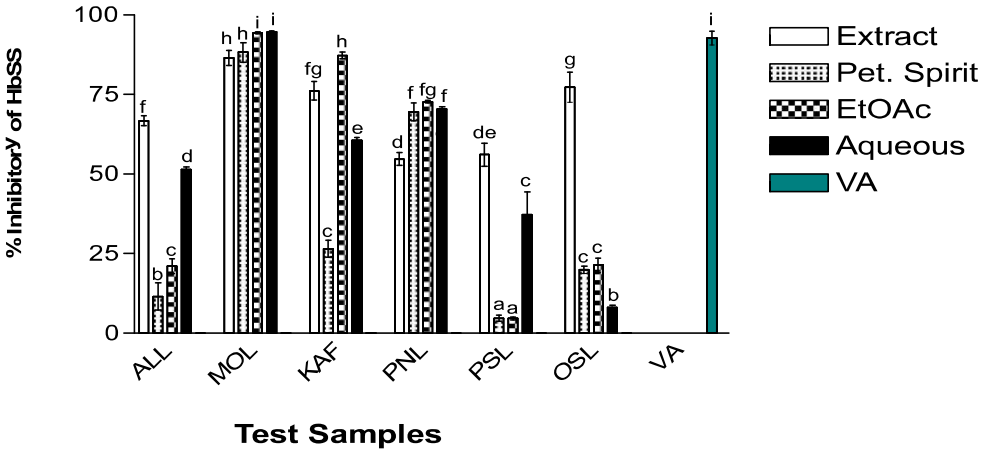
CONCLUSION

The study showed that the respondents were elderly between the ages 41-70 years, and they were knowledgeable in the management of sickle cell disease. The study identified one hundred and sixty-six plants belonging to 111 genera and 65 families, used for the management of SCD in part of Ondo, Osun and Oyo states, Southwest Nigeria. Fabaceae represented the most abundant family of plants, and the leaves were the most frequently used plant parts. Aqueous extracts of *M. oleifera* leaf, *A. laxiflora* leaf, *K. africana* fruit, *O. subscorpoideae* leaf, *Parquetina nigrescence* leaf and *Pyrenacantha staudtii* leaf showed remarkable antisickling activity; hence, validates their ethnomedicinal use for the management of SCD. The ethyl acetate fraction of *M. oleifera* and *K. africana* exhibited remarkable antisickling activities, partly due to their membrane stabilising and free radical scavenging properties.

Table 3: Antisickling activity of selected medicinal plants used for the management SCD in Southwest Nigeria

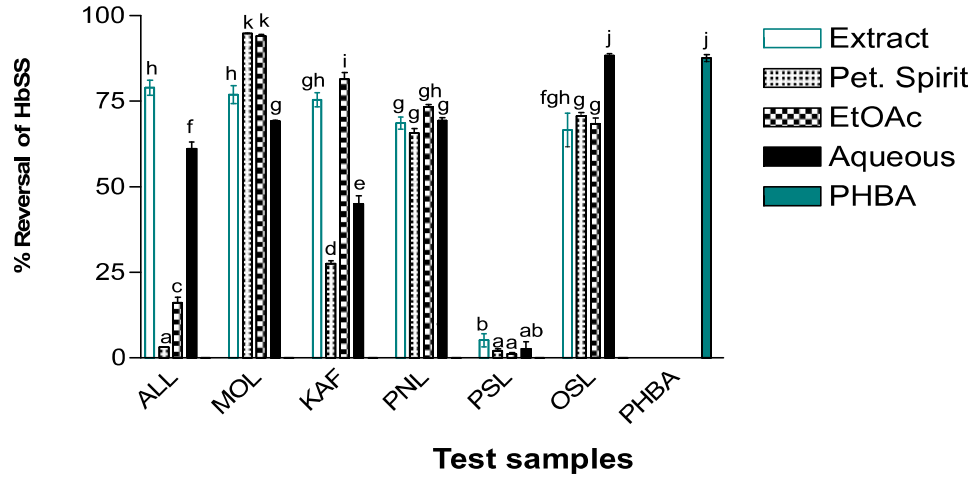
S/N	Aqueous Extract (4mg/mL)	% Inhibition	% Reversal	Ranking
1	*Ageratum conyzoides* Leaf	37.53 ± 0.98	38.63 ± 2.57	12th
2	*Alchornea laxiflora* Leaf	69.45 ± 2.52***	78.20 ± 1.86***	4th
3	*Anacardium occidentale* Stem	59.65 ± 1.75***	23.20 ± 3.03	10th
4	*Anthocleisa vogelli*	47.48 ± 2.39	2.49 ± 1.77	15th
5	*Bambusa vulgaris* Root	11.39 ± 2.41	11.06 ± 3.62	24th
6	*Baphia nitida* Leaf	16.08 ± 2.81	17.40 ± 3.11	22nd
7	*Bryophyllum pinnatum* whole plant	36.86 ± 10.68	2.86 ± 2.43	20th
8	*Caesalpinia pucherrima* Leaf	12.32 ± 0.10	7.41 ± 0.08	25th
9	*Cassia sieberiana* Seed	55.59 ± 13.04	36.15 ± 3.29	8th
10	*Chasmanthera dependens* Root	15.27 ± 2.14	8.11 ± 2.69	23rd
11	*Cleistopholis patens* Root	65.20 ± 0.67***	4.59 ± 1.14	9th
12	*Harungana madagascariensis* Leaf	27.10 ± 11.79	15.59 ± 5.47	19th
13	*Kalanchoe crenata* Aerial part	43.54 ± 8.96	1.27 ± 1.91	15th
14	*Kigelia africana* Fruit	76.16 ± 2.84***	75.47 ± 2.01***	3rd
15	*Lecanodiscus cupanioides* Leaf	41.93 ± 6.12	8.11 ± 2.33	17th
16	*Mangifera indica* Stem	30.45 ± 4.40	34.55 ± 2.58	14th
17	*Mimosa pudica* whole plant	44.60 ± 1.68	9.29 ± 3.01	17th
18	*Morinda lucida* Leaf	25.59 ± 12.72	28.92 ± 9.12	19th
19	*Moringa oleifera* Leaf	88.09 ± 3.32***	76.90 ± 2.58***	2nd
20	*Olax subcorpiodea* Leaf	75.81 ± 2.52***	66.63 ± 4.91***	5th
21	*Parquetina nigrescence* Leaf	52.24 ± 2.86	66.04 ± 3.17***	6th
22	*Pyrenacantha staudtii* Leaf	54.40 ± 3.04	52.40 ± 1.88	7th
23	*Senna alata* Leaf	16.15 ± 2.88	26.35 ± 2.18	21st
24	*Spondias mombin* Root	38.89 ± 7.25	29.31 ± 8.10	12th
25	*Tetrapleura tetraptera* Fruit	36.10 ± 5.64	51.50 ± 2.79	11th
26	*Theobroma cacao* Pod	35.50 ± 1.72	42.01 ± 3.08	14th
27	*Vernonia amygdalina* Leaf	34.01 ± 2.53	42.45 ± 1.98	14th
	Negative control (Blank)	2.14 ± 0.35	3.53 ± 0.74	29th
	Positive control	93.31 ± 0.36***	86.51 ± 2.52***	1st

Footnote: n=3, Data are expressed as mean±SEM, Positive control = vanillic acid (inhibitory), p-hydroxybenzoic acid (reversal). Samples were compared using One-way ANOVA followed by Bonferroni post-hoc test. Values in asterisks (***) are significantly (P<0.05) higher compared with that of A. vogelli (inhibitory) and T. tetraptera (reversal).



Footnote: n=3, Data are expressed as mean±SEM, Positive control = vanillic acid (inhibitory), p-hydroxybenzoic acid (reversal). Samples were compared using One-way ANOVA followed by Bonferroni post-hoc test. Values in asterisks (***) are significantly (P<0.05) higher compared with that of *A. vogelli* (inhibitory) and *T. tetraptera* (reversal).

Figure 4: Antisickling activity (Inhibitory) of selected plant fractions



Footnote: Data are represented as mean ± SEM in bars, Data analysis was based on One-way ANOVA, followed by Student's Newman-keul post-hoc test, where bars with different alphabets were considered significant (P<0.05) and those with same alphabets were comparable (P>0.05); n=3, **ALL**: Alchornea laxiflora leaf, **MOL**: Moringa oleifera leaf, **PNL**: Parquetina nigrescense leaf, **PSL**: Pyrenacantha staudtii leaf, **OSL**: Olax subscorpoideae leaf, **PHBA**: p-hydroxybenzoic acid.

Figure 5: Antisickling (Reversal) activity of candidate plant fractions

Table 4: Percentage membrane stabilisation of *K. africana* fruit

	% Membrane Stabilisation			
Test Sample	**0.375 mg/mL**	**0.750 mg/mL**	**1.125 mg/mL**	**1.500 mg/mL**
KAF aqueous extract	46.57±2.75^{b}	54.73±1.98^{b}	65.18±1.36^{b}	75.26±2.53^{b}
KAF ethyl acetate fraction	55.88±2.31^{c}	67.88±2.52^{c}	76.08±1.72^{c}	81.09±5.57^{b}
Ibuprofen (Positive control)	26.49±2.68^{a}	28.64±3.16^{a}	45.27±0.59^{a}	53.81±2.39^{a}

Key: KAF - *Kigelia africana* fruit, n=3, data expressed as % mean±SEM, data were analysed using One-way ANOVA followed by Student-Newman-Keul's post-hoc test, where values with different alphabets in superscript were considered significant in activity at P<0.05, while those with same alphabets in superscript were comparable (P>0.05) in activity.

Table 5: Antisickling activity of EtOAc column bulked fractions of *K. africana* Fruit

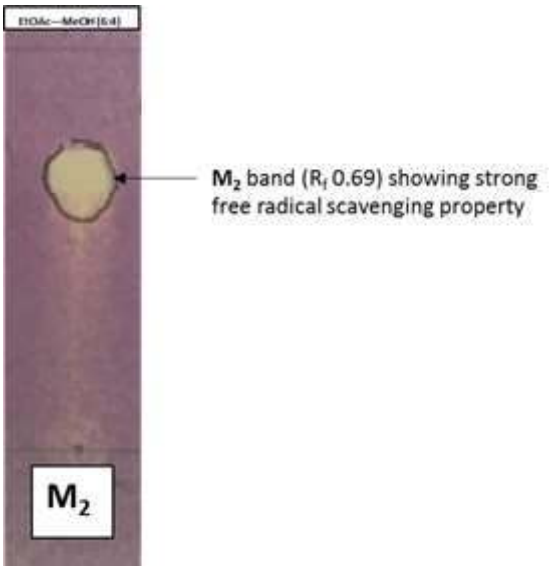
Bulked Column Fraction (4mg/mL)	% Inhibition	% Reversal
PHBA	81.47 ± 2.68	80.23 ± 3.57
A	10.23 ± 0.33	09.85 ± 1.75
B	15.50 ± 0.46	12.55 ± 0.56
C	12.55 ± 1.52	15.30 ± 2.32
D	25.26 ± 3.25	20.10 ± 1.54
E	10.24 ± 0.33	15.20 ± 2.35
F	8.78 ± 0.26	12.25 ± 1.35
G	30.65 ± 0.37	22.62 ± 1.52
H	38.22 ± 0.25	21.15 ± 0.59
I	35.42 ± 2.36	20.10 ± 0.63
J	27.58 ± 1.56	19.40 ± 0.75
K	15.63 ± 0.46	20.10 ± 0.65
L	47.24 ± 0.26	40.22 ± 0.87
M	79.80 ± 2.55***	76.56 ± 4.35***
N	63.66 ± 0.21	60.22 ± 4.50
O	69.23 ± 0.65	59.45 ± 0.27
P	65.56 ± 0.85	52.70 ± 0.55
Q	39.25 ± 0.80	35.86 ± 0.33
R	25.53 ± 0.65	20.20 ± 0.58
S	58.24 ± 0.76	50.54 ± 2.64
T	40.95 ± 3.12	35.25 ± 3.59
U	32.82 ± 2.66	30.15 ± 4.25
V	29.64 ± 0.95	22.54 ± 2.36
W	15.23 ± 2.13	17.55 ± 0.69
X	23.50 ± 0.37	29.35 ± 0.65

Key: Values expressed as mean ± SEM (n = 3). Data analysis by One-way ANOVA followed by Student's t- test where *** showed comparable antisickling activity with the positive control at P>0.0001

Table 6: Antisickling activities of prep-TLC bands of *K. africana* fruit

BAND (4mg/mL)	% Inhibition	% Reversal
PHBA	81.47 ± 2.68^{e}	80.23 ± 3.57^{e}
M_1	20.46 ± 0.64^{a}	10.54± 0.82^{a}
M_2	78.90 ± 0.66^{e}	70.80 ± 1.45^{d}
M_3	35.35 ± 0.520^{d}	25.05 ± 3.06^{c}
M_4	30.35 ± 3.25^{c}	10.50 ± 2.36^{a}
M_5	25.25 ± 1.52^{b}	16.30 ± 0.64^{b}

Key: M_1 – M_5 prep-TLC band from the EtOAc column bulked fraction **M**. Data expressed as mean ± SEM (n = 3). Data subjected to One-way ANOVA, followed by Student-Newman-Keul post-hoc test. Values with the different alphabets in superscript are significantly different (P<0.05), while same superscript along the column are comparable (P > 0.05).



Key: M2- prep. TLC band

Plate 1: Free radical scavenging property of PTLC band M_2

EXPERIMENTAL

Ethnomedicinal survey of Southwestern Part of Nigeria for SCD Plants

Semi-structured questionnaire was administered to 210 respondents across 23 major markets in 3 three states within the Southwest zone of Nigeria: Ilaje (GPS Coordinates: Latitude 6.2032° North and Longitude: 4.7679° East) and Okitipupa (6° 30' 0" N and 4° 48' 0" E) in Ondo State; Ile-Ife (7°332 003 N and 4°332 003 E), Osogbo (7° 46' 57.6156'' N and 4° 32' 30.5304'' E), Ilesa (7° 37' 0" N and 4° 44' 0" E) in Osun State; and Ibadan (7° 24' 7.0632'' N and 3° 55' 2.3268'' E) in Oyo State (Figures 1). Information on the indigenous knowledge of use of medicinal plants for the management of SCD was obtained from 94 Traditional Medical Practitioners (TMPs), 79 herb sellers and 37 non-herb sellers (community leaders and elderly). The questionnaire sought information on which plant drug materials they commonly sold or used for the management of SCD.

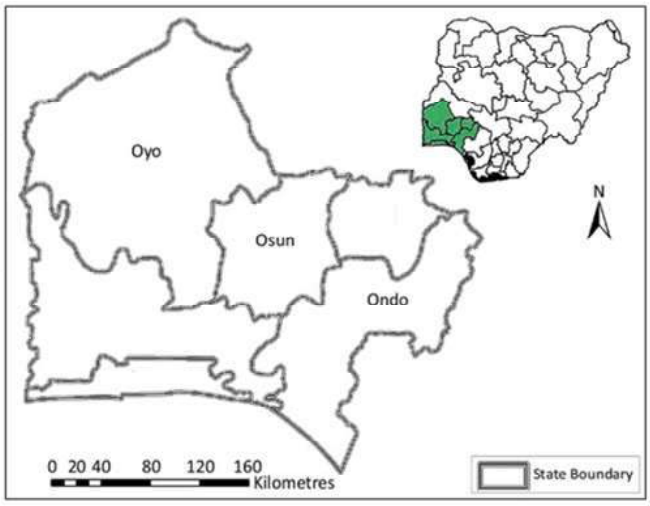


Figure 6: Map showing the ethnomedicinal survey area

Plant Materials

Twenty-seven plants with high fidelity level and with no scientific validation of use, were selected among the plants mentioned as being used for the management of SCD in the Southwestern part of Nigeria. They include *Ageratum conyzoides, Alchornea laxiflora*, *Anacardium occidentale*, *Anthocleista vogelli*, *Bambusa vulgaris*, *Baphia nitida*, *Bryophyllum pinnatum*, *Caesalpinia pucherrima*, *Cassia sieberiana*, *Chasmanthera dependens*, *Cleistopholis patens*, *Harungana madagascariensis*, *Kalanchoe crenata*, *Kigelia africana, Lecanodiscus cupanioides*, *Mangifera indica*, *Mimosa pudica*, *Morinda lucida*, *Moringa oleifera*, *Olax subscorpiodea*, *Parquetina nigrescence*, *Pyrenacantha staudtii*, *Senna alata*, *Spondias mombin*, *Tetrapleura tetraptera, Theobroma cacao* and *Vernonia amygdalina*. The plants were air-dried and powdered on a Christy Grinder (Norris, UK).

Plant Extraction

Each plant (250 g) was extracted hot with 1.0 L of distilled water under reflux for 3hrs. They were cooled, filtered, concentrated *in vacuo* and freeze-dried to obtain the extracts.

Anti-sickling Assay Methods:

Ethical Clearance and Collection of Blood Samples

An ethical clearance for the collection of blood samples from sickle cell disease patients was obtained before the study, from the Ethical Committee, Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria with protocol number ERC/2016/09/19. Fresh sickled blood samples (5 mL) were collected from confirmed sickle cell anaemia patients in steady state at the Haematology and Blood Transfusion Outpatient Unit, OAUTHC, Ile-Ife, Nigeria. The blood collection was by vein-puncture into EDTA bottles, and used within 24 hours of collection.

Inhibitory Assay

A 0.1 mL of the blood sample, 0.1 mL of phosphate buffered saline and 0.1 mL of the test extract (4 mg/mL) were mixed together in a test tube. The mixture was overlaid with 1 mL of liquid paraffin to prevent oxygenation. The mixture was then incubated in a thermostated water bath at 37 °C for 4 hours. After incubation, 0.3 mL freshly prepared sodium metabisulphite was added carefully under the liquid paraffin and then mixed gently by rolling the test tube between the two palms. The mixture was then incubated for another 1hr 30 min in the water bath at 37 °C at the end of the incubation, the liquid paraffin was removed carefully using a Pasteur pipette and the reaction mixture was fixed with 1.5 mL of 5 % buffered formalin solution, this was done in triplicates, followed by cell counting using a binocular microscope.

Reversal Assay

In triplicates, 0.1 mL of blood sample and 0.1 mL of phosphate buffered saline were mixed in a test tube. The mixture was overlaid with 1 mL of liquid paraffin and 0.3 mL of sodium metabisulphite was carefully introduced under the liquid paraffin and then incubated in a thermostatic water cabinet at 37 °C for 1 hr. 30 min. At the end of the incubation period, 0.1 mL of test extract was added carefully under the liquid paraffin and was further incubated at 37 p C for another 6 hrs. The liquid paraffin layer was removed with a Pasteur pipette and the reaction mixture was fixed with 1.5 mL of buffered formalin which was carefully mixed by rolling the test tube between the two palms, followed by cell counting.

Counting of Cells

Slides were prepared from the fixed cells after incubation. An aliquot of the fixed cells was taken with the use of Pasteur pipette and dropped on a microscope slide and carefully covered with a cover slip. The slide was placed under a microscope and red blood cells were counted at x 400 magnification. The percentage sickle red blood cells were calculated as shown below:

$$\% \text{ Sickled cell} = \frac{\text{number of sickled cells}}{\text{total number of cells}} \times 100$$

$$\% \text{ Inhibitory or Reversal} = \frac{\% \text{ sickled cells of control} - \% \text{ sickled cells of sample}}{\% \text{ sickled cells of control}} \times 100$$

Membrane Stabilisation Assay

The membrane stabilisation effect of the aqueous extract and the active ethyl acetate fraction of **KAF** was carried out on sickle cell erythrocytes suspension, according to the method of Sadique et al. [27], while ibuprofen was used as the positive control. The assay mixtures consisted of hyposaline (2.0 ml), 0.15 M Sodium phosphate buffer (pH 7.4), varying volumes of drugs (0.0 – 1.0 mL) of extract or standard drug, varying volume of isosaline, and 2% (v/v) erythrocyte suspension in isosaline (0.5 mL) to give a total assay volume of 4.5 mL. Erythrocyte suspension was absent in drug control while drugs were omitted in blood control. The reaction mixtures were thoroughly mixed and incubated at 56ºC for 30 minutes in a water bath. The tubes were cooled under running water and then centrifuged at 3000 rpm in a Gallenkamp Bench Centrifuge for 10 min at room temperature. The supernatants were collected into test tubes and absorbance (Abs) of the released haemoglobin was taken at 560 nm. The percentage membrane stabilising activity was established from the expression [27]:

$$\% \text{ Membrane stabilizing activity} = \{100 - \frac{[\text{Abs (test sample)} - \text{Abs (control)}]}{\text{Control value}} \times 100$$

Where the blood control represented 100% lysis and the value represented the average of triplicates ± Standard error of mean (SEM).

Fractionation of Six Candidate Plant Extracts

The extracts of *Moringa oleifera* leaf (**MOL**), *Alchornia laxiflora* leaf (**ALL**), *Kigelia africana* fruit (**KAF**), *Olax subscorpioidea* leaf (**OSL**), *Pyrenacantha staudtii* leaf (**PSL**) and *Parquentina nigrescense* leaf (**PNL**) were fractionated, using solvent-partitioning method [28]. Each extract (30 g) was suspended in 150 mL of distilled water inside a 2.0 L separating funnel. The aqueous phase was successively partitioned with petroleum spirit (500 mL x 3) and ethyl acetate (500 mL x 5); thus creating an organic phase. The three afforded fractions were concentrated *in vacuo* and refrigerated at 4 °C prior to further investigation.

Chromatographic purification of the active Ethyl acetate fraction of *K. africana* Fruit

The ethyl acetate fraction of **KAF** (7.30 g) was chromatographed on a Silica gel open column, using binary gradient solvent systems of increasing polarity from Hexane–EtOAc to EtOAc – MeOH, affording 150 eluates in 25 mL test tubes. They were pooled into 24 sub-fractions, **A-Z** based on their TLC profiles, and subsequently evaluated for antisickling activity. The active sub-fraction **M** (109 mg) was further purified using preparative thin-layer chromatography (Silica gel PTLC, 0.75 mm thickness). An EtOAc-MeOH (9:1) solvent system was used as the mobile phase; thus, affording five bands, $\mathbf{M_1 - M_5}$. Band $\mathbf{M_2}$ was a relatively pure spot with an R_f value of 0.69 when analysed on a Silica gel TLC developed with an EtOAc-MeOH (6:4) solvent system.

TLC-bioautography of the chromatographic fractions

The free radical scavenging property of the ethyl acetate prep-TLC band of **KAF** was done, using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) rapid radical scavenging method as described by Cuendet *et al.* [29]. A Silica gel thin-layer chromatography of the PTLC bands was developed with EtOAc-MeOH (9:1), while band $\mathbf{M_2}$ which appeared pure was developed with EtOAc-MeOH (6:4). The TLC chromatograms were sprayed with 0.25 mg/mL of DPPH in MeOH radical solution. The time taken for the scavenging action by the PTLC bands to take effect was recorded, evidenced by the bleaching of the purple DPPH radical.

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