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ETHNO MEDICINAL SURVEY AND EVALUATION OF TWO RECIPES USED IN MANAGING SICKLE CELL DISEASE IN ILE-IFE COMMUNITY OF OSUN-STATE, NIGERIA.

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Abstract

Background: Ethno-medicinal survey of herbal recipes used in managing sickle cell disease in Ile-Ife, Osun-State, Nigeria was carried out and two recipes selected for antisickling studies.
Materials and Methods: Information was obtained using semi-structured questionnaires and open interview from respondents consisting of herb sellers, traditional medical practitioners, artisans and traders in two local government areas of Ife. Two recipes from the survey were selected for antisickling studies. Aqueous extract of each recipe was obtained by boiling the constituents in water for 1 h while the hydro ethanolic extracts were obtained by maceration in 70% ethanol for 72 h. Inhibitory and reversal antisickling properties were assessed using sodium metabisulphite as deoxygenating agent, vanillic acid (inhibitory agent), para-hydroxybenzoic (reversal agent) acid as positive controls while phosphate buffered saline was employed as negative control.
Results: Fifty four recipes comprising forty six plants were obtained from the ethno-medicinal survey. The respondents comprised of 44% men and 56% women. The most frequently and commonly used plants belong to family Fabaceae. The inhibitory and reversal activities of the aqueous extract of recipe 1 (81.37± 1.09%, 88.56 ±1.38% respectively) were significantly ($p < 0.05$) higher than recipe 2 (78.51 ± 0.78% inhibition and 79.8 ± 2.16% reversal) at same concentration. The hydro-alcoholic extracts of recipes 1 and 2 gave highest inhibitory activities at 0.5 mg/mL (69.25 ±1.30% and 68.28 ±2.78% respectively).
Conclusion: This study documented the medicinal plants and recipes used in Ile-Ife for managing sickle cell disease, and validated the ethno-medicinal claim of two recipes.

Keywords: Medicinal plants, Ethno-medicinal survey, Sickle cell disorder, Ile-Ife

Abbreviations: SCD: Sickle Cell Disease, Hb: Haemoglobin, PHBA: p-hydroxy benzoic acid, PBS: Phosphate buffered saline

Introduction

Sickle cell disease (SCD) is a hereditary blood disorder and the common form of it is sickle cell anaemia which was first described by Herrick in 1910 (Herrick, 2001). The gene of the individual codes for the synthesis of abnormal haemoglobin Hb S, which has beta 6 valine instead of beta 6 glutamic acid found in normal haemoglobin Hb A (Murayama 1968). The red blood cells of sickle cell individuals have "sickle" shape instead of the normal round or disc shape, which affect the movement of the red blood cells within the blood vessels causing less oxygen to be circulated (Lowe and Anderson, 2015). The clinical manifestation of the disorder is prominent in patients homozygous for the S gene (Hb SS), while heterozygotes with abnormal gene such as SC, Thalassemia or SF exhibit a milder form of the disorder (Galanello and Origa, 2010). Sickle cell anaemia individuals suffer characteristically from persistent ulcer, an enlarged spleen and painful swellings of the digits and joint (Isaac - Sodeye 1975, Ballas *et al.*, 2012).

In Africa, about 12,000 infants are born each year with sickle cell disease and in rural villages as few as 2% of individuals with SCD survive beyond age of five years (Flemming, 1989). The prevalence of sickle cell trait ranges between 10 and 45% in various parts of sub-Saharan Africa (WHO, 2013). The distribution of Hb S gene worldwide also seems to overlap with areas where the malaria parasite is endemic, especially in tropical Africa, the Middle East, and Asia (Piel *et al.,* 2010).

Ile-Ife is an ancient Yoruba city in South-Western Nigeria. The city is located in the present state of Osun and is made up of Ife central and Ife East local government areas. Ile-Ife is on latitude 7°28¹ and 7°45¹ N and on longitude 4°30¹E and 4°34¹E (Olupona, 2011). It is within the tropical savann\a climate zone of West Africa. It has average

rainfall of 1,000–1,250 mm (39–49 in) usually from March to October and a mean relative humidity of 75% to 100% (Ajala and Olayiwola, 2013). According to Yoruba mythology life began at Ile-Ife. The town is predominantly characterized with several traditional believes and traditional religion is widely and proudly practiced in it. Ife is an ancient town in Yoruba history and is regarded as the cradle of civilization. According to Yoruba tradition, Ife is the ancestral and spiritual home for all Yoruba. It is believed that the creation of the world started from Ife. Hence, it is popularly referred to as ***''Land of the Source''*** (Broadus, 2018). The people of Ile-Ife believe so much in traditional medicine and they depend on it as their primary healthcare remedy for almost all their ailments despite the presence of the Obafemi Awolowo Teaching Hospital Complex. It had been observed that hospitalized Ife people and those on orthodox medication often combine it with various herbal remedies. The inhabitants of Ile-Ife depend so much on herbs that a market popularly called Oja- Ife, located at the central part of the town almost beside the King Ooni's palace, is mainly for the sale of herbs and other traditional elements commonly used by traditional medical practitioners. There is hardly any community in Ile-Ife without at least one or more traditional practitioner who attend to people's spiritual and medical needs (Ajala and Olayiwola, 2013).

Sickle cell disease has gained prominence in its management from both traditional and orthodox medical practices. Although the only cure available is haematopoietic stem cell transplantation which is very expensive and comes with varying complications, orthodox drugs such as hydroxyurea, vitamin B complex and folic acid are commonly used as palliative (Bhatia and Sheth 2015). Traditionally, some plants such as *Mangifera indica, Adansonia digitata, Cajanus cajan, Carica papaya, Moringa oleifera, Zanthoxyllum xanthoxyloides* (Sofowora, 1979, Adesanya *et al.,* 1988, Shah *et al*., 2010, Ogunyemi *et al*., 2008, Imaga *et al*., 2009, Cyril-Olutayo *et al.*, 2018) amongst others have been found to have antisickling properties and are being used to manage the disease. In this study, ethno medicinal survey of herbal recipes used traditionally in the two local government areas of Ile-Ife, Osun State for the management of sickle cell anaemia was carried out with the aim of identifying medicinal plants and evaluate two of the recipes for antisickling properties.

Materials and Methods

Ethno medicinal Survey

Semi-structured questionnaires were used to obtain ethno medicinal data from different categories of respondents which include traditional medical practitioners, herb sellers, artisans, traders, civil servants, on indigenous plants used locally for the management of sickle cell anaemia in Ile-Ife.

Although, English language was used to prepare the questionnaire, interviews were conducted in Yoruba language where necessary. Three major sections were captured in the questionnaire; one covered demographic data like gender, age, occupational level, source of knowledge acquisition of herbal practice; Two included respondents' folk classification of sickle cell disease and other questions assessing their knowledge of the disease including diagnosis methods, and symptoms; While three involved information on medicinal plants, recipes used for managing sickle cell disease, mode of preparation and administration.

Collection of Plant Materials for antisickling assay

Two recipes, containing different plant parts, used locally for the treatment of sickle cell anaemia in Ile-Ife, Osun-State were selected and evaluated in this study. The plant materials: *Nauclea latifolia* (leaves and root), *Olax subscorpiodea* (root) *Mangifera indica (*stem bark*), Khaya senegalensis* (root*)* were collected from the Obafemi Awolowo University, Biological garden, while *Securidaca longipenduuculata* (root), *Xylopia aethiopica* (fruit) *Bulhozia coriacea* (fruit) and *Garcinia kola* (stem bark) were purchased from the Ife local market. The botanical identification was carried out by Mr. Ogunlowo and voucher specimens deposited in the Herbarium, Department of Pharmacognosy, Faculty of Pharmacy (FPI, included in the online edition of index Herbariorium), Obafemi Awolowo University, Ile-Ife with the numbers FPI 2194 (*Mangifera indica*), FPI 2195 (*Khaya senegalensis*), FPI 2151 (*Olax subscorpiodea*) and FPI 2152 (*Nauclea latifolia*).

Preparation of Extracts

Fresh leaves were air dried in a screen house, while roots and barks, after being washed and cleaned, were oven dried at 40^0C. Each recipe was constituted, weighed and prepared according to the ethno medicinal survey information. Recipe 1 contained: *Xylopia aethopica* fruit (5 g), *Nauclea latifolia root* (25 g), *Olax subscorpioidea root* (25 g) and *Mangifera indica* bark (100 g); while recipe 2 contained: *Buchhozia coriaceae* fruit (12.5 g) *Garcinia kola* bark (25 g) *Securidaca longipendunculata* root (25 g) *Nauclea latifolia* leaves (50 g) *Nauclea latifolia* root (50 g) *and Khaya senegalensis* root (100 g). Dried plant parts were grinded into powder using a grinder (Christy) and weighed appropriately. Aqueous extracts of Recipes 1 and 2 were prepared by weighing the plant parts into separate round bottom flasks and covered with distilled water in ratios one of plant materials to fifteen of distilled water (1:15) and boiled for 1 h. Both recipes were thereafter removed from the heat source and allowed to simmer for 3 h according to

Sofowora (1979). The residue was removed from the resulting decoction by filtration. Aliquot 0.1 mL and 0.2 mL of the decoctions were used for the antisickling assay.

For the hydro-ethanolic extracts, Recipes 1 and 2 (155 g and 262.5 g respectively) were soaked separately in 70% ethanol for 72 h. Extracts were concentrated *in vacuo* using the rotary evaporator and freeze dried to complete dryness. Each recipe was thereafter reconstituted in distilled water to obtain 4 mg/mL concentration. Serial dilutions were made to obtain 2 mg/mL, 1 mg/mL, 0.5 mg/mL, and 0.25 mg/mL concentrations used for the antisickling assays.

Antisickling Assay Procedures

Blood samples collected from confirmed sickle cell individuals in steady state who attend routine check-ups at the Department of Immunology and Haematology out-patient clinic, Obafemi Awolowo University Teaching Hospital Complex were used (Ethical Clearance number: IRB/IEC/0004553).

For the inhibitory model, 0.2 mL of Hb SS whole blood sample, 0.2 mL of phosphate buffered saline (PBS) solution (pH 7.0) and 0.2 mL test extract were mixed carefully in a test tube and overlaid with 1 mL liquid paraffin to prevent aeration. The mixture was incubated at 37^{o}C for 4 h in a thermostated water bath (Tecan). Freshly prepared 2% w/v sodium metabisulphite solution (0.6 mL) was carefully added under the liquid paraffin after the incubation period and mixed. This was incubated again for 90 min at same temperature. Vanillic acid was employed as positive control for inhibitory. For the reversal model, freshly prepared 2% w/v sodium metabisulphite solution (0.6 mL) was added to 0.2 mL whole blood, 0.2 mL PBS and mixed. The medium was covered with liquid paraffin and incubated for 90 min at 37^{o}C. After the incubation period, 0.2 mL of the test extract was added, mixed carefully and re-incubated for another 4 h. At the end of the experiment, the liquid paraffin layer was carefully removed using a Pasteur pipette and the solution fixed with 3 mL of 5% v/v buffered formalin solution. Both sickled and unsickled red blood cells were counted using the light microscope and photomicrograph of representative slides taken. PHBA was used as positive control for the reversal test while PBS served as the blank in the negative control (Sofowora, 1979).

Statistical Analysis

Each assay was performed in triplicates and the one way ANOVA was used to detect significant differences and standard errors of the mean values. Level of significance was set at $p<0.05$.

Results

Ethno medicinal Survey

A total of one hundred and eight (108) respondents including: 49% herb sellers, 30% artisans and traders, 11% civil servants and 10% traditional medical practitioners from different parts of Ife LGA were interviewed. Fifty six percent of respondents were females and 45% between ages 41-50 years (Table 3). Herb sellers in Ile-Ife are mostly females specializing in the treatment of febrile children hence they are called “Elewe-omo” meaning “herb specialists for children”. Sizeable number of the respondents (41%) admitted inheriting the knowledge of use of herbs and acquired more knowledge from their family members, while some 36% of respondents were trained through apprenticeship. Some respondents (14%) got the knowledge of the use of particular herbs from the media while 9% claimed they got the knowledge during hospital visits.

Table 1: Demographic features of respondents on the plants used in the management of sickle cell disease in Ile Ife.

Demographic feature	Frequency	Percentage %
Gender		
Male	48	44
Female	60	56
Total	108	100
Age		
20-30	7	6
31-40	26	25
41-50	49	45
51-60	21	19
60 and above	5	5
Occupational level		
Civil servant,	12	11
Artisans and traders	33	30
Traditional healer	11	10
Herb sellers	52	49
Acquisition of knowledge		
Radio	15	14
Relative	44	41
Hospital	10	9
Apprenticeship	39	36

Medicinal Plants used Ethno-medicinally

The survey revealed 54 recipes consisting 46 plants belonging to 29 plant families (Tables 2 and 3). The most frequently and commonly used plants belong to the Fabaceae family, followed by Cucurbitaceae, Rutaceae and Zingiberaceae. Also plants belonging to families, Rubiaceae, Bignonaniaceae, Annonaceae, Anacardiaceae, Asteraceae, Meliaceae and Poaceae are commonly used (Table 2).

The mostly used plant part for managing sickle cell disease is the leaf (47%) followed by the stem bark (17%) and then the root (14%). Other parts being used include seed (6%) rhizome (2%) and bulb (2%) (Figure 1). Medicinal plant parts are mostly bought from the market (70% sourced from the market) encouraging easy access for users. Other sources include medicinal plant gardens (10%), 7% collect from both the market and gardens, 7% from the market and the wild, while 6% source from the wild only. Most recipes are prepared in form of decoctions and taken orally. Other modes of preparation include: squeezing, fermenting, burning, powdering, and tincture in local gin while powdered herds can be taken orally with pap or mixed with shea-butter and applied topically.

Table 2: List of Medicinal Plants used Ethno-medically in Managing Sickle Cell Anaemia and their frequency of occurrence.

S/N	Local/ common names	Plant Scientific names	Family	Parts used	Frequency of occurrence
1	Egbesi	*Nauclea latifolia* (sm) bruce	Rubiaceae	Leaves, Bark, root	24
2	Pandoro	*Kigela africana* (Lam.) benth.	Bignonaniaceae	Bark and root	15
3	Khaya/Oganwo	*Khaya senegalensis* (Desr.) A. Juss	Meliaceae	Bark, leaves, Root	14
4	Eeru Alamo	*Xylopia aethiopica* (Dunal). A. Rich	Annonaceae	Fruit	13
5	Poporo/Sorgum	*Sorghum bicolor* L. Moench	Poaceae	Leaves and seed	11
6	Ibepe/Pawpaw	*Carica papaya* L.	Caricaceae	Fruit	9
7	Mangoro/mango	*Mangifera indica* L.	Anacardiaceae	Bark and leave	9
8	Ipeta	*Securidaca longipendunculata* Fresen.	Polygalaceae	Root	8
9	Epa ikun	*Cassia tora* (L.) Roxb.	Fabaceae	Seed	8
10	Ewuro/Bitter leaf	*Vernonia amygdalina* Delile	Asteraceae	Leaves	8
11	Ifon	*Olax subscorpiodea* Oliv.	Olacaceae	Root	6
12	Wonderful cola	*Buchhoizia coriaceae* Engl.	Capparaceae	Seed	6
13	Ewe Emi/ Shea butter	*Vitellaria paradoxa* C.F. Gaertn.	Sapotaceae	Leaves, fruit	6
14	Arunpale	*Chenopodium ambrosiodes* L.	Chenopodiaceae	Leaves	5
15	Osan Lemonu	*Citrus limon* L	Rutaceae	Fruit and leaves	5
16	Orogbo/Bitter cola	*Garcinia kola* Heckel	Clusiaceae	Bark	4
17	Tanson igbo	*Petiveria alliacea* L.	Petiveriaceae	Leaves and root	4
18	Asunwon Oyinbo /Senna	*Senna podocarpa* Mill.	Fabaceae	Bark	4
19	Koko oba/ lemon grass	*Cymbopogon citratus* (DC.) Stapf	Poaceae	Leaves	4
20	Tude	*Calliandra portoricensis* Benth.	Fabaceae	Leaves,	3
21	Ataare	*Aframomum melegueta* K. Schum.	Zingiberaceae	Seed	3

22	Yanrin oko	*Lactuca capensis* L.	Asteraceae	Leaves	3
23	Ata ile pupa/Tumeric	*Curcuma longa* L.	Zingiberaceae	Rhizome	3
24	Afon	*Treculia africana* Decne.	Moraceae	Bark	3
25	Egunsi bara	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Cucurbitaceae	Leaves	2
26	Ewe otili/ Pigeon pea	*Cajanus cajan* (L.) millsp.	Fabaceae	Leaves	2
27	Okuku	*Pteleiopsis suberosa* Engl. & Diels	Combretaceae	?	2
28	Ogbe ori akuko	*Heliotropium indicum* L.	Boraginaceae	Leaves	2
29	Ugu	*Telfaira occidentalis* Hook. f.	Cucurbitaceae	Leaves	2
30	Jebo	*Entandrophragma utile* (Dawe & Sprague) Sprague	Meliaceae	Bark	2
31	Efo Bishop	*Cnidoscolus aconitifolius* (Mill.) I.M. Johns.	Euphobiaceae	Leaves	2
32	Ewe Ayo	*Guilandina bonduc* L.	Fabaceae	Leaves	2
33	Laali	*Lawsonia inermis* L.	Lythraceae	Leaves	1
34	Ewe ile/Moringa	*Moringa oleifera* Lam.	Moringaceae	Leaves	1
35	Ato	*Chasmanthera dependens* Hochst.	Menispermaceae	Leaves	1
36	Igi ose	*Adansonia digitate* L.	Bombacaceae	Leaves	1
37	Osun	*Pterocarpus osun* Craib.	Fabaceae	Leaves	1
38	Efinrin/Scent leaf	*Ocimum gratissimum* L.	Labiateae	Leaves	1
39	Orin ata/Fagara	*Zanthoxylum xanthoxyloides* Lam.	Rutaceae	Root	1
40	Ata ile/ Ginger	*Zingiber officinale* Roscoe	Zingiberaceae	Rhizome	1
41	Aidan	*Tetraplura tetraptera* (Schumm. & Thonn.) Taub.	Fabaceae	Fruit	1
42	Ejinrin	*Momordica charantia* L.	Cucurbitaceae	Leaves	1
43	Alubosa/Onion	*Allium cepa* L.	Amaryllidaceae	Bulb	1
44	Kasu/Cashew	*Anacardium occidentale* L.	Anacardiaceae	Leaves	1
45	Masqurade tree	*Polyalthia longifolia* Sonn.	Annonaceae	Fruit	1
46	Osan wewe/Lime	*Citrus aurantium* L.	Rutaceae	Fruit	1

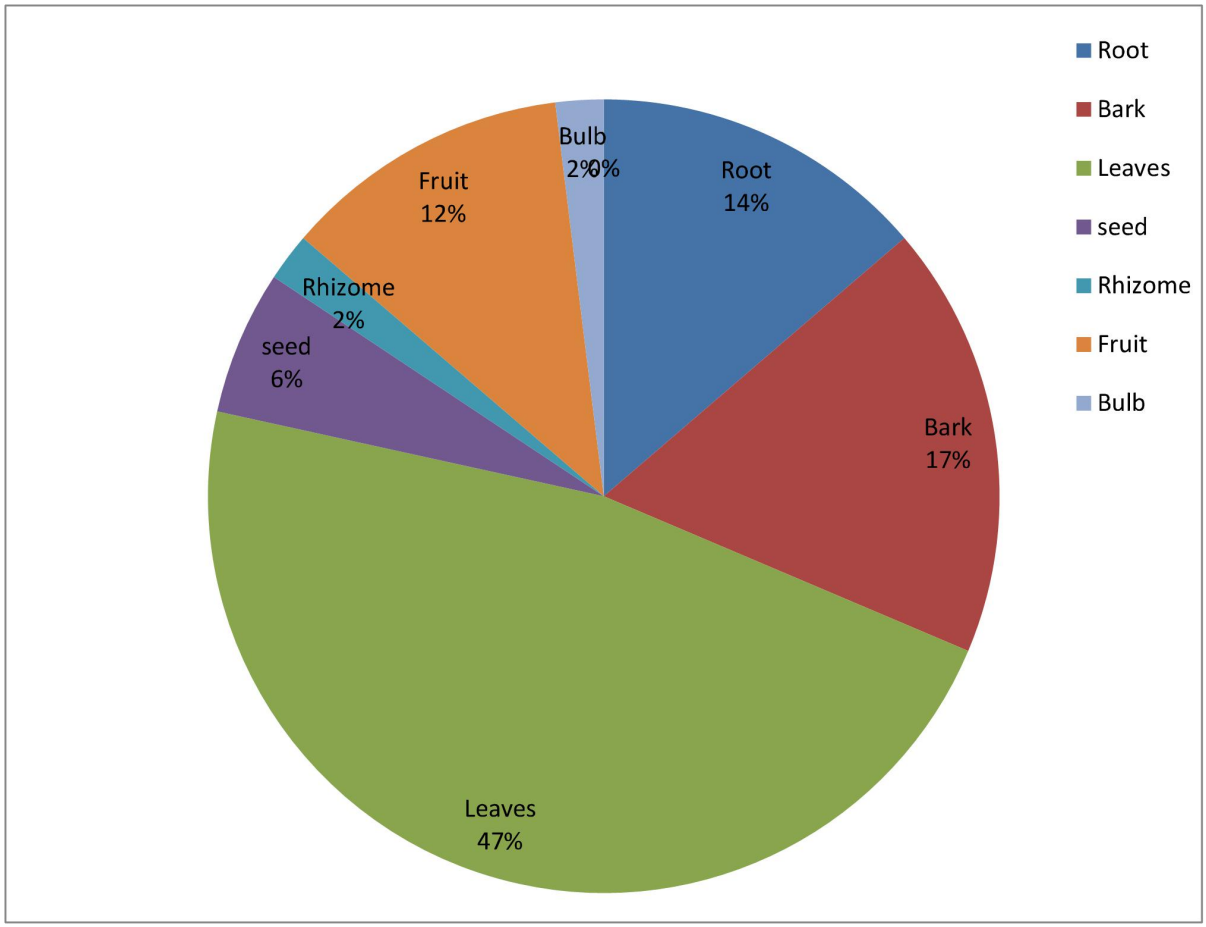

Figure 1: Pie chart showing the percentage use of different plant parts in the treatment of sickle cell disease in Ile-Ife

Table 3: List of recipes used in managing sickle cell anaemia including plant parts used, mode of preparation and mode of administration

Recipe	Local Names	Scientific names	Parts used	Preparation	Mode of administration
1	Egbesi Ipeta Wonderful Cola Orogbo Oganwo	*Nauclea latifolia* *Securidaca longipendunculata* *Buchhoizia coriaceae* *Garcinia kola* *Khaya senegalensis*	Root, Leaf Root Fruit Fruit Bark	Decoction	Oral
2	Eeru alamo Mangoro Egbesi Ifon	*Xylopia ethiopica* *Mangifera indica Nauclea latifolia* *Olax subscorpiodea*	Fruit Bark Root Root	Decoction	Oral
3	Ibepe Poroporo	*Carica papaya* *Sorghum bicolor*	Fruit Leaf	Ferment for 5 days and boil	Oral
4	Egbesi Ewe Otili Oganwo	*Nauclea latifolia* *Cajanus cajan* *Khaya senegalensis*	Bark Leaf Bark	Decoction	Oral
5	Ewe Ayo Ewe Otili Ewe Poroporo Ewe Koko Oba	*Guilandina bonduc* *Cajanus cajan* *Sorghum bicolor* *Cymbopogon citratus*	Leaf Leaf Leaf Leaf	Decoction	O r a l
6	Ewe Emi Ata Ile Pupa Eso Ibepe	*Vitellaria paradoxa* *Curcuma longa* *Carica papaya*	Leaf Rhizome Fruit	Decoction	Oral
7	Oganwo Poroporo Ewe Emi	*Khaya senegalensis* *Sorghum bicolor* *Vitellaria paradoxa*	Bark Leaf Leaf	Decoction	Oral
8	Egbesi Ipeta	*Nauclea latifolia* *Securidaca longipendunculata*	Root Root	Decoction	Oral

	Pandoro Mangoro	*Kigelia africana* *Mangifera indica*	Bark Bark		
9	Ata Ile Pupa Ewe Emi Ewe Ayo Ewe Poroporo	*Curcuma longa* *Vitellaria paradoxa* *Guilandina bonduc* *Sorghum bicolor*	Rhizome Leaf Leaf Leaf	Decoction	Oral
10	Egbesi Pandoro Oganwo	*Nauclea latifolia* *Kigelia africana* *Khaya senegalensis*	Bark, Root Bark Bark	Decoction	Oral
11	Eeru Alamo Wonderful Cola Pandoro	*Xylopia ethiopica* *Buchhoizia coriaceae* *Kigelia africana*	Fruit Fruit Root	Decoction	Oral
12	Eso Ibepe Wonderful Cola Poroporo	*Carica papaya* *Buchhoizia coriaceae* *Sorghum bicolor*	Fruit Seed Leaf	Ferment and boil with wonderful Cola	Oral
13	Egbesi Yanrin Ewuro	*Nauclea latifolia* *Launaea taraxacifolia* *Vernonia amygdalina*	Leaf Leaf Leaf	Decoction	Oral
14	Wonderful Cola Egbesi Eeru Alamo Orogbo Bark	*Buchhoizia coriaceae* *Nauclea latifolia* *Xylopia ethiopica* *Garcinia kola*	Seed Leaf, Bark Fruit Bark	Decoction	Oral with milk
15	Ugu Poroporo	*Telfairia occidentalis* *Sorghum bicolor*	Leaf Leaf	Soak Sorghum leaf in hot water overnight and squeeze with Ugu	Oral
16	Mangoro Ipeta Oganwo	*Mangifera indica* *Securidaca longipendunculata* *Khaya senegalensis*	Leaf Bark Bark Leaf	Decoction	Oral

	Epa Ikun	*Cassia tora*			
17	Egbesi Ipeta Eeru Alamo Mangoro	*Nauclea latifolia* *Securidaca longipendunculata* *Xylopiaa ethiopica* *Mangifera indica*	Leaf, Root Roots Fruits Bark	Decoction	Oral
18	Pandoro Epa Ikun Wonderful Cola	*Kigela africana* *Cassia tora* *Buchhoizia coriaceae*	Bark, Root Leaf Fruit	Decoction	Oral
19	Epa Ikun Eeru Alamo Ifon Ipeta	*Cassia tora* *Xylopiaa ethiopica* *Olax subscorpiodea* *Securidaca longipendunculata*	Leaf Fruit Root Roots	Decoction	Oral
20	Ugu Ibepe Poroporo	*Telfairia occidentalis* *Carica papaya* *Sorghum bicolor*	Leaf Fruit Leaf	Decoction	Oral
21	Pandoro Eeru Alamo Egbesi	*Kigelia africana* *Xylopiaa ethiopica* *Nauclea latifolia*	Bark Fruit Leaf, Bark	Decoction	Oral
22	Egbesi Ipeta Pandoro Oganwo	*Nauclea latifolia* *Securidaca longipendunculata* *Kigelia africana* *Khaya senegalensis*	Leaf, Root Root Root Bark	Decoction	Oral
23	Ibepe Tude	*Carica papaya* *Calliiandia portoricensis*	Fruit Leaf, Root	Dry and grind into powder	Oral
24	Arunpale	*Chenopodium ambrosiodes*	Leaf Leaf	Decoction	Oral

	Ewe Emi Egesi	*Vitellaria paradoxa* *Nauclea latifolia*	Leaf, Bark		
25	Orin Ata (Fagara)	*Zanthoxylum xanthoxyloides*	Root	Powder	Oral
26	Egbesi Eeru Alamo	*Nauclea latifolia* *Xylopiaa ethiopica*	Leaf, Bark Fruit	Decoction	Oral
27	Ataare Tansan Igbo	*Aframomum maleguata* *Petiveria alliaceae*	Seed Leaf	Burning dried leaves and seed together	Oral (Mix powder with pap)
28	Ifon Ipeta Mangoro Epa Ikun	*Olax subscorpiodea* *Securidaca longipendunculata* *Mangifera indica* *Cassia tora*	Root Root Bark Bark	Decoction	Oral
29	Egbe ori akuko Yanrin Ewuro	*Heliotropium indicum* *Lactuca capensis* *Vernonia amygdalina*	Leaf Leaf Leaf	Tincture in local gin	Oral
30	Wonderful cola Epa ikun Pandoro Eeru alamo Orogbo Mangoro	*Buchhoizia coriaceae* *Cassia tora* *Kigelia africana* *Xylopiaa ethiopica* *Garcinia kola* *Mangifera indica*	Seed Leaf Root Seed Root Bark	Decoction	Oral
31	Tanso Arunpale	*Petiveria alliaceae* *Chenopodium ambrosiodes*	Leaf, Root Leaf	Tincture in local gin	Oral
32	Eru Alamo Egbesi Ewuro	*Xylopia ethiopica* *Nauclea latifolia* *Vernonia amygdalina*	Fruit Root Leaf	Decoction	Oral
33	Oganwo Eru alamo Igi iba Koko oba	*Khaya senegalensis* *Xylopia ethiopica* *Cymbopogon citratus*	Bark Fruit Bark Leaf	Decoction	Oral

34	Egbesi Ipeta Oganwo	*Nauclea latifolia* *Securidaca longipendunculata* *Khaya senegalensis*	Leaf, Root Root Leaf, Bark	Decoction	Oral
35	Jebo Tude Asunwo Afon	*Entandrophragma utile* *Calliiandia portoricensis* *Senna podocarpa* *Treculia africana*	Bark Leaf, Bark Bark Bark	Decoction	Oral
36	Oganwo Egbesi Koko oba Eru Alamo	*Khaya senegalensis* *Nauclea latifolia* *Cymbopogon citratus* *Xylopia ethiopica*	Bark Bark Leaf Fruit	Decoction	Oral
37	Ifon Oganwo Egbesi	*Olax subscorpiodea* *Khaya senegalensis* *Nauclea latifolia*	Root Root Leaf, Root	Decoction	Oral and bathing
38	Arunpale Egbesi Pandoro Oganwo	*Chenopodium ambrosiodes* *Nauclea latifolia* *Kigelia africana* *Khaya senegalensis*	Leaf Leaf, Root Leaf Bark	Decoction	Oral
39	Egbe ori akuko Ejinrin Yanrin	*Heliotropium indicum* *Momodica charantia* *Lactuca capensis*	Leaf Leaf Leaf	Squeeze leaves with salt	Oral
40	Egbesi Pandoro Poroporo	*Nauclea latifolia* *Kigelia africana* *Sorghum bicolor*	Leaf, Bark Bark Leaf	Decoction	Oral
41	Egbesi Pandoro	*Nauclea latifolia* *Kigelia africana*	Bark, Root Bark Bark	Decoction	Oral

	Mangoro Poroporo	*Mangifera indica* *Sorghum bicolor*	Leaf		
42	Ewe Emi Oganwo Ibepe Ata ile pupa	*Vitellaria paradoxa* *Khaya senegalensis* *Carica papaya* *Curcuma longi*	Leaf, bark Bark Fruit Rhizome	Decoction	Oral
43	Pandoro Oganwo Ewe Emi	*Kigelia africana* *Khaya senegalensis* *Vitellaria paradoxa*	Bark Bark Leaf	Decoction	Oral
44	Ibepe Mangoro	*Carica papaya* *Mangifera indica*	Leaf, Fruit Leaf, Root	Decoction	Oral
45	Tanso Masquerade	*Petiveria alliaceae* *Polyalthia longifolia*	Root Fruit	Tincture in local gin	Oral
46	Tanson Arunpale Ori	*Petiveria alliaceae* *Chenopodium ambrosiodes* *Vitellaria paradoxa*	Leaf Leaf Butter	Dry, Powder and mix with shea butter	Topical application
47	Epa ikun Egbesi Pandoro Oganwo	*Cassia tora* *Nauclea latifolia* *Kigelia africana* *Khaya senegalensis*	Leaf Root Bark Leaf	Decoction	Oral
48	Mangoro Poroporo	*Mangifera indica* *Sorghum bicolor*	Leaf, bark Leaf	Decoction	Oral
49	Arunpale Epa ikun Egbesi Pandoro	*Chenopodium ambrosiodes* *Cassia tora* *Nauclea latifolia* *Kigelia africana*	Leaf Leaf Leaf Bark	Decoction	Oral
50	Okuku Asunwon Egbesi	*Pteleiopsis suberosa* *Senna podocarpa* *Nauclea latifolia*	Bark Bark Leaf, Root	Decoction	Oral

51	Epa ikun Pandoro Egbesi	*Cassia tora* *Kigelia africana* *Nauclea latifolia*	Leaf Fruit Leaf, bark	Decoction	Oral
52	Ewuro Ejinrin	*Vernonia amygdalina* *Momodica charantia*	Leaf Leaf	Decoction	Oral
53	Ewe laali Ewuro Kasu	*Lawsonia inermis* *Vernonia amygdalina* *Anacardium occidentale*	Leaf Leaf Leaf	Decoction	Oral
54	Ewe Ile Efo Bishop Ugu	*Moringa oleifera* *Cnidoscolus aconitifolius* *Telfairia occidentalis*	Leaf Leaf Leaf	Decoction	Oral

Antisickling Results

The aqueous extract of the two recipes tested showed high inhibitory and reversal properties. Recipe 1 showed higher antisickling properties than recipe 2 (table 4) while the hydro-ethanolic extract of both recipes exhibited high reversal properties at low concentrations (table 5).

Table 4: Anti-sickling properties of the aqueous extracts of Recipes 1 and 2

Volume of decoction (mL)	% Inhibition Recipe 1	% Reversal Recipe 1	% Inhibition Recipe 2	% Reversal Recipe 2
0.1	81.37±1.09	88.56±1.38*	78.51±0.78	79.8±7.16*
0.2	66.31±2.07	67.8±1.58	76.14±2.01	74.03±3.31
Vanillic acid (4 mg/mL)	96.71± 0.91	-	96.71± 0.91	-
p-hydroxylbenzoic acid (4 mg/mL)	-	78.97± 1.89	-	78.97 ± 1.89

*Values are presented as ± SEM (standard error of mean). p<0.05; *-significantly higher than the positive control.*

Table 5: Anti-sickling activities of the hydro-ethanolic extracts of Recipes1 and 2.

Concentration (mg/mL)	% Inhibition Recipe 1	% Reversal Recipe 1	% Inhibition Recipe 2	% Reversal Recipe 2
4.0	33.41±1.76	67.62±1.80	55.25±1.32	53.58±2.15
2.0	45.71±1.12	28.04±4.13	41.01±1.50	59.34±1.91
1.0	53.95±2.17	20.58±4.21	49.43±1.01	63.48±1.18
0.5	69.25±1.30	19.4±3.59	68.28±2.78	57.39±1.76
0.25	60.32±1.12	19.71±2.49	59.32±2.34	49.15±3.78
Vanillic acid 4mg/mL	96.71± 0.91	-	96.71± 0.91	-
p-hydroxybenzoic acid (4 mg/mL)	-	78.97± 1.89	-	78.97 ± 1.89

n=3; values are presented as ± SEM (standard error of mean). p<0.05

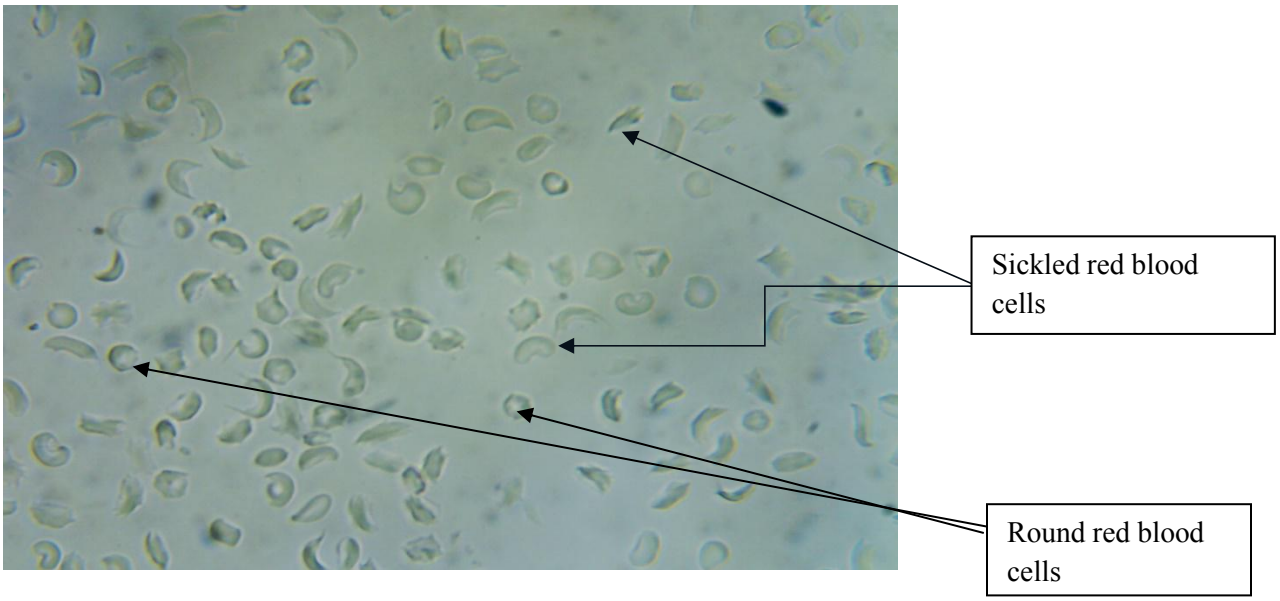

Figure 2: Representative Photomicrograph of untreated deoxygenated Hb SS red blood cells (negative control)

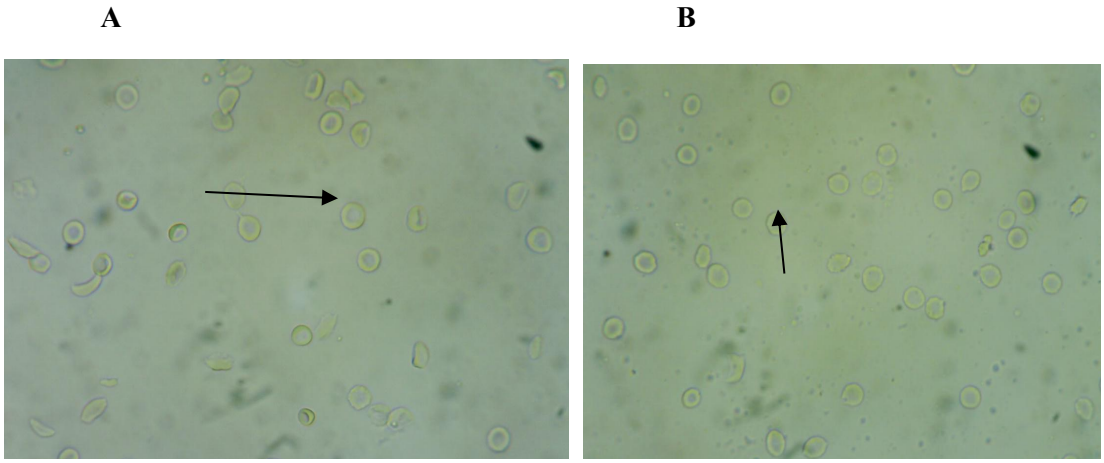

Figure 3: Representative Photomicrograph of typical A) Inhibition and B) reversal of Hb SS red blood cells. *Arrows show normal round cells after treatment with recipe 1.*

Discussion

Medicinal plants have been greatly employed in the treatment of many health conditions, especially in the Africa continent. In Ile-Ife community, sickle cell anaemia is amongst the diseases being managed palliatively with herbs and it is believed that sickling process can be hindered traditionally. The traditional healers without any scientific background have made use of abundant resources from nature to manage SCD with a promising degree of success over time. There are many plants already in use as sources of treatment for SCD in various parts of the world.

The ethno medicinal survey carried out in this study, amongst the people of Ile-Ife, revealed that the knowledge of the use of herbs reside more with herb sellers, market men and women, and traditional medical practitioners. Although the knowledge of the use of herbs is usually passed from generations and kept within the

family, interested members of the community are still being trained as herbalists (apprenticeship). Most of the respondents were adults, 45% aged between 41-50 years, 25% between 31-40 years, 19% between 51-60 years while only 6% and 5% were aged above 60 years and below 30 years respectively (Table 1). The knowledge of the use of herbs especially for the management of sickle cell anaemia in Ile-Ife resides with women more than men. Seventy percent of the plants used by the Ile-Ife people are obtained from the community market (Figure 1) indicating that most of the plants are readily accessible, acceptable by the people and affordable. These are major advantages of herbal medicine over orthodox medicine. The leaves are the most frequently used plant part (47%), in the management of sickle cell disorder while root and bark accounted for 17%, and 14% usage respectively (Figure 2).

The survey revealed 54 recipes consisting 46 plants belonging to 29 plant families (Tables 2 and 3). The medicinal plant with the highest frequency of occurrence is *Nauclea latifolia*, followed by *Kigela africana, Khaya senegalensis, Xylopia aethiopica, Sorghum bicolor, Carica papaya, and Mangifera indica.* (Table 2). These plants are mostly used in combination with other plant materials to make decoctions, tinctures or powdered drugs which are taken orally or topically (Table 3). The mode of preparation is majorly by boiling the plant parts in water to make decoctions. Some are prepared by soaking in local gin to make tincture, squeezed to extract the juice, fermented and boiled, while others are dried, powdered and taken orally with pap (Table 3). The symptoms of sickle cell crisis is often associated with malaria hence several plants used in the treatment of malaria are also being employed in managing the disease. Some plants reported by Odugbemi *et al,* 2007; and Adebayo and Kretti, 2011, such as *Nauclea latifolia, Khaya senegalensis, Cajanus cajan, Xylopia aethiopica, Carica papaya, Zingiber officinale, Vernonia amygdalina, Mangifera indica, Cymbopogon citratus* as antimalarial plants were also found in this study to be very important in managing sickle cell disease (Table 3). The dependence of the people on medicinal plants and their role in health care system will increase as they are culturally viable and expected to remain affordable. This is because the existing modern health care services is limited and expensive compared with traditional medicine.

Out of the fifty-four recipes used by the Ile-Ife people of Osun state to manage sickle cell disease, two commonest recipes (Tables 3) were selected for antisickling studies. The aqueous extracts of the two recipes were prepared as they were being prepared locally i.e. by decoction. Aliquot 0.2 mL of the decoction was used directly for the antisickling assay. Another 0.2 mL of the decoction was taken and diluted with equal volume of distilled water to reduce the concentration. The decoctions gave very high inhibitory and reversal activities on Hb SS red blood cells (Table 4, Figures 2-3), although the inhibitory activity of the positive control, Vanillic acid at 4 mg/mL was significantly ($p<0.05$) higher than that of the recipes. Recipe 1 had a better reversal activity than recipe 2 and significantly ($p<0.05$) more active than PHBA, positive control (Table 4).

For the hydro-alcoholic extracts, the highest inhibitory antisickling of recipe 1 and 2 were recorded at 0.5 mg/mL concentration (69.25± 0.30% and 68.28 ±2.78% respectively) (Table 5), inferring that the extracts were more active at lower concentrations. The EC_{50} for the inhibitory activities of recipe 1 was 1.70±0.30 mg/mL while that of recipe 2 was 1.29±0.39 mg/mL. The reversal properties of the hydro-ethanolic extracts of recipe 1 gave the highest activity of 67.62 ± 1.80% at 4 mg/mL while 63.48±1.185% reversal was recorded for recipe 2 at 1.0 mg/mL. The reversal activity of PHBA positive control, 78.97±1.89%, was significantly higher at 4 mg/mL (Table 5). The EC_{50} value for the reversal activity of recipe 1 was 2.56±0.10 mg/mL while that of recipe 2 was 0.61±2.20 mg/mL. From this EC_{50} values, it can be inferred that the hydro-ethanolic extract of recipe 2 is more active than that of recipe 1. There has been advocacy for low therapeutic dose for the treatment of SCD due to its chronic nature as well as the large amount of Hb in the body which requires large and frequent doses of drugs to effectively treat the disease (Nnamani *et al*., 2008).

The ethno-medicinal claim of the use of the decoction of recipes 1 and 2 has been validated in this study. The highest inhibitory and reversal activities were recorded with the aqueous extracts and this showed that the water soluble components of the plants are responsible for the antisickling activities. This finding is in line with literature as amino acids and other hydrophilic compounds have been implicated in antisickling activities exhibited by medicinal plants (Cyril-Olutayo *et al*, 2009; Osuagwu, 2010; Adebayo and Krettli, 2011).

The constituents of recipe 1 viz, *Xylopia aethiopica, Mangifera indica, Olax subscorpiodea* and *Nauclea latifolia* had been reported for their antisickling properties and also implicated in the treatment of Malaria (Benoit-Vical*et al*., 1998, Afsana *et al*., 2003, Uwakwe and Nwaoguikpe 2008; Abba *et al.*, 2010, Ibukunoluwa *et al.,* 2015, Azubuike *et al*., 2016). Of the six plant materials that make up recipe 2, *Nauclea latifolia* roots and leaves, *Garcinia cola* and *Khaya senegalensis* had been reported to have antisickling properties (Adejumo *et al*., 2011; Oyedapo *et al.,* 2016). The antisickling properties of some of the plants reported in literature were higher than the combinations in this study, however, it is important to note that medicinal plants are used not only for the treatment of diseases but also as potential material for maintaining good health and conditions. These medicinal plants contain phytochemicals such as tannins, saponins, and flavonoids that confer other properties. Tannins have astringent

properties which hasten the healing of wounds and inflamed mucous membrane due to their physiological activities such as anti-oxidant, antimicrobial and anti-inflammatory properties. The traditionally-held belief of the use of combination of herds is that the synergistic combination of several active principles in some herbal preparations is responsible for their beneficial effects (Taiz and Zeiger. 1991). *Buchholzia coriacea* (Wonderful cola) has antioxidant, anti-inflammatory and analgesic properties while *Securidaca longipendiculata* has membrane stability properties and has been used in various antisickling herbal recipes (Egunyomii *et al,* 2009, Adisa *et al*., 2011, Olaleye *et al.*, 2012). These plants have been reported to contain amino acids, flavonoids which have been implicated in antisickling and antioxidant properties (Bagchi *et al*., 1999, Nwakwe and Nwaoguikpe, 2008), and work in synergy to effect the high inhibitory and reversal activities.

Conclusion

Many plant species are employed singly and in combination to combat symptoms of sickle cell anaemia locally in Ile-Ife. The two recipes tested possessed antisickling properties though the aqueous extracts gave a better antisickling activities than the hydro ethanolic extracts. The use of these recipes for managing sickle cell disorder has been authenticated in this study. *In vivo* experiments and toxicology studies would still need to be carried out to ensure the efficacy and the safety of any drug formulation from the crude plant extracts.

Conflict of Interest

The Authors declare no conflict of interest.

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