European Journal of Medicinal Plants

24(4): 1-10, 2018; Article no.EJMP.42706
ISSN: 2231-0894, NLM ID: 101583475

Antisickling and Radical Scavenging Activities of Selected Medicinal Plants and Compounds from *Mitracarpus villosus* (Sw.) DC. Cham

Christianah A. Elusiyan[1*], Opeyemi Ayoade[1], Adewale O. Adeloye[2], Felix O. Olorunmola[1], Joseph M. Agbedahunsi[1] and Abiodun O. Ogundaini[3]

[1]Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, 220005 Osun State, Nigeria.
[2]Natural Resources and Materials, Botswana Institute for Technology Research and Innovation, Private Bag 0082, Gaborone, Republic of Botswana.
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, 220005 Osun State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author CAE conceived and designed the study, wrote the protocol and performed the analysis and also wrote the initial draft of the manuscript. Author OA managed the literature searches and conducted the experiments under the supervision of authors CAE and FOO. Author AOA ran the NMR experiments. Author JMA conducted the ethnomedicinal survey. Authors CAE and AOO analysed and interpreted the NMR. Author AOO also read and corrected the initial draft of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/42706
Editor(s):
(1) Shanfa Lu, Professor, Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Peking Union Medical College, China.
(2) Francisco Cruz-Sosa, Professor, Department of Biotechnology, Metropolitan Autonomous University, Iztapalapa Campus Av. San Rafael Atlixco, México.
(3) Marcello Iriti, Professor, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Karen Cordovil, Oswaldo Cruz Foundation, FIOCRUZ- RJ, Brazil.
(2) Idakwoji Precious Adejoh, Kogi State University, Nigeria.
(3) Otavio Augusto Chaves, SENAI Institute of Innovation in Green Chemistry, Brazil.
(4) A. Papazafiropoulou, Tzaneio Prefecture General Hospital of Piraeus, Greece.
Complete Peer review History: http://www.sciencedomain.org/review-history/25731

Original Research Article

Received 7th May 2018
Accepted 14th July 2018
Published 30th July 2018

**Corresponding author: E-mail: bimbola2009@gmail.com;*

ABSTRACT

Aims: This study investigated the antisickling and radical scavenging activities of different morphological parts of nine plants, belonging to six families. A bioassay-guided fractionation was carried out on *Mitracarpus villosus* (Sw.) DC. Cham, which was one of the most active plants.
Place and Duration of Study: Drug Research and Production Unit, Faculty of Pharmacy, Obafemi Awolowo University (OAU), Ile Ife, Nigeria between May 2013 and June 2016.
Methodology: Plant materials were collected from OAU campus and Ile-Ife town, identified and voucher specimen deposited at IFE herbarium, OAU for future reference. *In-vitro* radical scavenging and antisickling (by haemoglobin polymerization inhibitory test) activities of extracts and isolated compounds were performed using ascorbic acid and vanillic acid as positive controls respectively. Bioassay-guided fractionation was achieved by combination of various chromatographic procedures and bioassay techniques to isolate the active constituents. Structural elucidation of the isolated compounds was by spectroscopic techniques including NMR and MS.
Results: Six of the nine plant extracts showed considerable antisickling and antioxidant activities. Extracts of *Emilia praetermissa* and *M. villosus, were* however the most active. Extracts or compound which demonstrated strong radical scavenging activity were also found to demonstrate significant antisickling effects. Bioassay-guided fractionation of *M. villosus* resulted in the isolation of psychorubrin, quercetin and a mixture consisting of stigmasterol and an unidentified terpene. Quercetin was the most active antisickling and radical scavenging compound with percentage inhibition of 96.46±0.3 and 87.99±0.18 respectively.
Conclusion: *Mitracarpus villosus* ranked second best in activity among the tested plants. Antisickling activity of its isolated compounds was found to increase with increase in the antioxidant capability of the compounds. Quercetin was the most active compound while the non-antioxidant terpenoidal mixture had very weak polymerisation inhibitory effect.

Keywords: Antisickling, antioxidant, M. villosus, psychorubrin, quercetin.

1. INTRODUCTION

The inherited blood disorder, Sickle cell anaemia (SCA), is mostly prevalent among Africans especially sub-Saharan African and in some Mediterranean countries like Greece, Turkey, Saudi Arabia and India [1,2]. Millions of people are found to be affected worldwide by SCA, with three quarter of the cases occurring in Africa [3,4]. Prevalence of SCA continues to be on the increase especially in Nigeria, the most populous country in the sub-region, with at least 24% of the population as carriers of the abnormal haemoglobin gene [1,5]. An estimated 150,000 children are reportedly born annually with SCA and a greater number carries the sickle cell traits [4]. This situation calls for concern among all interest groups. In Africa, and as it is the practice among many nations across the globe, the use of plants and their preparations has continued to gain popularity in the treatment and management of many ailments affecting man and even animals. In Nigerian ethnomedicine, plants are used in the management of SCA and in the treatment of its associated symptoms. Sickle cell crisis usually starts with inflammation of joints, which is mostly as a result of oxidative stress on the affected erythrocytes (RBCs). The potential antisickling effects of some medicinal plants including *Piper guineenses*, *Pterocarpa osun*, *Eugenia caryophylla* and *Sorghum bicolor* extracts have been reported [6,7]. Herbal preparations and phytomedicines such as Niprisan and Ciklavit have undergone clinical trials and are currently being used in the management of SCA [8].

This study is a follow up on the ethnomedicinal survey conducted by the Drug Research and Production Unit in eight districts of Osun and Ondo States, Nigeria, with the associated screening of the collected plants and recipes for antisickling activities. We hereby report the antioxidant (using free radical scavenging method) and antisickling (using *in-vitro* haemoglobin polymerization inhibition) properties of nine of the medicinal plant species selected from the survey. These plants are the leaf of *Senna siamea* Lam. (SSL), whole plant of *Mitracarpus villosus* Cham. (MVW), aerial parts of *Lantana camara* Linn. (LCA), *Emilia praetermissa* Milne-Redhead (ECA), *Amaranthus viridis* Linn. (AVA), *A. hybridus* Linn. (AHA), *A. spinosus* Linn. (ASA), flower of *Lagenaria breviflora* Benth. (LBF) and fruit of *Citrullus lanatus* sub sp *mucosospermus* Thunb. (CLFM). This is with a view to providing a scientific basis for the use of these plants in the

management of sickle cell disease. The study also reports the bioassay-guided fractionation of *M. villosus* which resulted in the isolation of psychorubrin, quercetin and a mixture consisting of stigmasterol and an unidentified terpene.

2. MATERIALS AND METHODS

2.1 General Experimental Procedures

Precoated - Merck silica gel $60F_{254}$ aluminium plates were used for normal phase analytical TLC, while pre-coated Merck RP-18 plates were employed for reverse-phase TLC. Chromatogram were observed under UV light at 254 and 366 nm before spraying with vanillin/H_2SO_4 spray followed by heating at 105ºC for 1 - 2 min to reveal the components of the extracts or fractions. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) spray (0.2% in MeOH) was used to monitor the isolation of antioxidant spots. Organic solvents were re-distilled before use. Extracted plant materials, fractions and pure isolates were evaporated to complete dryness *in vacuo* using a Buchi Rotavapor R110. Absorbance values were measured on T70 UV/Vis spectrophotometer. NMR spectra was recorded on 600 MHz Bruker TOPSPIN 2.1, using $CDCl_3$ as solvent.

2.2 Collection and Identification of Plant Materials

The plant materials were collected from their natural habitats including the Medicinal plant garden of the Department of Pharmacognosy situated at the University Teaching and Research Farm, OAU. The plants were identified by Mr. Bernard Omomoh of the Herbarium Unit, Department of Botany, OAU, Ile-Ife and Mr Ifeoluwa Ogunlowo, Pharmacognosy Herbarium, OAU, Ile-Ife. Voucher specimens were deposited (Table 1).

2.3 Extraction of the Studied Plants and Isolation of Constituents of *Mitracarpus villosus* Whole Plant

Plant materials were collected within the campus of OAU and Ile-Ife Township (Table 1). Each plant (200 g) was blended and extracted, by maceration, using MeOH for 48 h at room temperature, and the filtrate was concentrated to complete dryness *in vacuo* at 40ºC to give the corresponding crude extracts. The extracts were subjected to preliminary antisickling (using polymerisation inhibitory test) and radical scavenging (using DPPH test) activities *in-vitro* and ranked according to their observed activities.

The second most active *M. villosus* whole plant (MVW) was therefore re-collected (4.5 kg), extracted as described earlier using MeOH (9.5 L) and subjected to chromatographic separation because *Emilia praetermissa* (ECA), the most active plant, was already out of season. Concentration of the filtrate to dryness gave a crude extract with an average yield of 36.5 g. An aliquot of the extract (26.5 g) was subsequently suspended in distilled water (45 ml) and successively partitioned into *n*-hexane (500 ml), DCM (550 ml) and EtOAc (550 ml) to yield the corresponding *n*-hexane (5.02 g), DCM (2.12 g) and EtOAc (3.57 g) fractions.

Portion of DCM fraction (1.57 g) was adsorbed on silica gel (60 - 120 mesh) and subjected to column chromatography (10 cm x 120 cm glass column) and eluted using gradients of *n*-hexane: EtOAc mixtures. Fractions were collected in test tubes (20 ml) and monitored by TLC (silica gel G 60 F_{254} TLC plates, Merck, layer thickness 0.2 mm) using *n*-hexane: EtOAc (1:1) and by spraying with methanolic solution of DPPH and vanillin/sulphuric acid. Fractions having similar TLC profile were combined to yield seven sub-fractions (D1 - D7). Sub-fraction D1 (fr. nos. 44 - 49), eluting with *n*-hexane: EtOAc (7: 3) was obtained as a mixture (109 mg, creamy amorphous powder, with R_f = 0.65 (*n*-hexane: EtOAc (7:3)), deeply purple colour spot (vanillin/sulphuric acid spray), not visible under UV light (wavelength 254 and 366 nm), non -antioxidant (DPPH TLC bioautography).

Partially purified sub-fraction D3 (fr nos. 51 - 56), (182 mg) was further subjected to separation by vacuum liquid chromatography (VLC) and eluted with gradients of *n*-hex: EtOAc mixtures to give D8 (fr. nos.15 - 22, (13 mg)) and D9 (fr. nos. 23 - 44, (152 mg). D8 eluting with *n*-hexane: EtOAc (8.5:1.5) was further purified by preparative TLC (20 cm x 20 cm) using solvent system 1. The major band eluted with DCM yielded pure compound D10 (3 mg). More D10 (40 mg) was obtained from D9 after repeated chromate-graphy.

Portion of the EtOAc fraction (680 mg) was subjected to silica gel column separation with a gradient system of *n*-hexane: EtOAc (100:0 - 0:100) to give 35 fractions. Collected fractions were then monitored by DPPH TLC bioautography. The combined fraction E3

Table 1. Investigated plant materials

Plant species	Family	Plant part	Collection site	Herbarium no	Code
Senna siamea Lam.	*Caesalpinioideae*	Leaf	OAU campus	FPI 2100	SSL
Mitracarpus villosus (Sw.) DC. Cham.	*Rubiaceae*	Whole plant	OAU campus	IFE 17367	MVW
Lantana camara Linn.	*Verbenaceae*	Aerial parts	OAU campus	FPI 2101	LCA
Emilia praetermissa Milne-Redhead.	*Compositae*	Aerial parts	Ile-Ife township Ile-Ife township	FPI 2102	ECA
Amaranthus viridis Linn.	*Amaranthaceae*	Aerial parts	Ile-Ife township Ile-Ife township	FPI 2103	AVA
A. hybridus Linn.	*Amaranthaceae*	Aerial parts	Ile-Ife township Ile-Ife township	FPI 2104	AHA
A. spinosus Linn.	*Amaranthaceae*	Aerial parts		FPI 2105	ASA
Lagenaria breviflora (Benth) Roberty.	*Cucurbitaceae*	Flower		FPI 2106	LBF
Citrullus lanatus sub sp *mucosospermus* Thunb.	*Cucurbitaceae*	Fruits			CLMF

(fr. nos. 21 - 31) (230 mg) eluting with *n*-hexane: EtOAc (50:50) contained the major component. Fractions 22 - 25 which eluted with *n*-hex: EtOAc (40:60) yielded compound E4 (33 mg). E4 was a yellow powder, UV-active, orange spot to vanillin/H_2SO_4 spray, R_f = 0.64 and gave an immediate discolouration of the purple DPPH solution.

2.4 Assay Methods

All the extracts were initially screened for both antisickling and antioxidant properties using established protocols.

2.4.1 Qualitative DPPH bioautography test

Qualitative DPPH test of all extracts, fractions and isolates was performed using pre-coated TLC plates, developed in appropriate solvent systems and sprayed with 0.2% methanolic solution of DPPH. Antioxidant spots were observed as yellow/whitish spots against a purple background.

2.4.2 Quantitative DPPH spectrophotometric test

For the quantitative estimation of the antioxidant activity, blank and negative control consists of each extract (1 ml) and DPPH (1 ml) respectively in MeOH (3 ml). Test extracts, isolates and ascorbic acid (positive control) concentration ranged from 3.125 - 0.781 mg/ml. Absorbance of the antioxidant assay mixture which includes methanol, the extract/ compound with or without E3 was further purified by VLC eluting with mixtures of *n*-hexane: EtOAc (80:20 - 40:60). DPPH was measured (triplicates) at 517 nm 30 mins post incubation at room temperature using T70 UV-Vis Spectrophotometer at 517 nm [9]. Ascorbic acid served as the positive control. The antioxidant activity was estimated as:

Percentage anti-oxidant activity (AA) % =

$$\frac{100 - \{(Abs_{test} - Abs_{blank}) \times 100\}}{Abs_{control}}$$

2.4.3 Antisickling (*in-vitro* haemoglobin polymerization inhibition) assay

2.4.3.1 Blood collection and preparation of erythrocyte haemolysate

Blood samples were obtained from confirmed individuals with sickle cell anaemia (HBSS genotype) as donors attending the regular weekly Sickle cell Clinic at the Department of Haematology, Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife through the puncture of the vein. Ethical approval for the use of blood samples was obtained for the study from the Ethics and Research committee, OAUTHC, Ile-Ife with clearance number IRB/IEC/0004553. Samples were used within the first 48 h of collection. The erythrocytes were separated from plasma by centrifugation (1200 × g for 10 mins) and washed three times with normal saline solution [10]. The erythrocytes were finally re-suspended in a volume of normal

saline equivalent to twice the volume of displaced plasma and used for the polymerization studies. Sodium metabisulphite (SMS) was used to induce the sickling of the blood by creating hypoxial state in the phosphate buffered medium containing the blood and the test extract or isolate. Vanilic acid served as the positive control. All the experiments were carried out in triplicates.

2.4.3.2 Assay procedure

The antisickling property was performed using the method involving sodium metabisulphite (SMS) induced polymerization of Haemoglobin (HbS) molecules [11]. The assay mixture consists of HbS haemolysate (0.1 ml), phosphate buffered saline solution (0.5 ml), distilled water (1 ml), 2% SMS (3.4 ml) and test extract or isolate at 1-4 mg/ml (1.0 ml). Absorbance of the antisickling assay mixture were recorded at 700 nm in triplicates at every 30 secs for 180 secs using a T70UV-Vis spectrophotometer at 700nm with or without the test extracts or isolated compounds. The level of polymerization was monitored by recording increase in absorbance of the assay mixture with time. Percentage polymerization was calculated according to the following formula [12]:

Percentage polymerization inhibition =
$(A_{t/c} / Ac_{180^{th}}) \times 100$

Where
$A_{t/c}$ = absorbance of test/control sample at time = t (secs)

$Ac_{180^{th}}$ = absorbance of control sample at the 180^{th} sec

2.5 Statistical Analysis

All the quantitative data were expressed as mean values and standard error of mean (± SEM) of experiments performed in triplicates. Statistical differences between the treatments and the control were evaluated by ANOVA test ($P < 0.05$).

3. RESULTS AND DISCUSSION

3.1 Antioxidant Assay

The anti-oxidant activity of the plant extracts, isolated compounds and positive control (ascorbic acid) as estimated by DPPH is presented in Fig. 1.

3.2 Antisickling Assay

The antisickling properties of the extracts and isolated compounds by sodium metabisulphite ($Na_2S_2O_5$) induced polymerization of HbS molecules using a spectrophotometer are presented in Fig. 2 and Table 2 respectively.

3.3 Isolated Compounds

Compound D1 (109 mg) was obtained as a mixture from the DCM fraction of *M. villosus* whole plant methanol extract. It was isolated as a white powder, violet colour reacting to VS spray reagent, R_f 0.65 (*n*-hexane: EtOAc; 7:3) and non-antioxidant. The mixture consists of stigmasterol and an unidentified terpene.

Compound D10 (3 mg) was also isolated from the DCM fraction as a light yellow powder; single orange colour reacting spot (VS spray), R_f value 0.57 (n-hexane: EtOAc 7:3), moderately antioxidant (DPPH bioautography); ^{1}H and ^{13}C NMR data (600 MHz, $CDCl_3$) were identical with reported data for 3-hydroxy-3,4-dihydro-1H-benzo[g]isochromene-5,10-dione and the compound was identified as psychorubrin (D10) [13, 14]. It is a naphthoquinone possessing 13 carbons: 2 methylenes, 3 methines and 6 quartenary carbons (Table 3).

Table 2. Time-course polymerization inhibitory activity of isolated compounds

		Time (secs) versus activity			
Compounds	**Concentration (mg/ml)**	**0**	**30**	**60**	**180**
Terpene mixture (D1)	A	48.56±0.34	47.47±0.54	47.02±0.77	45.81±0.99
	B	50.96±0.45	50.01±0.23	51.93±0.67	49.38±0.95
	C	65.90±0.89	64.70±0.45	63.50±0.58	59.75±0.19
Quercetin (E4)	A	87.85±0.87	86.47±0.37	85.54±0.39	81.48±0.87
	B	93.66±0.45	91.55±0.13	90.39±0.28	85.37±0.39
	C	113.13±0.34	110.70±0.25	107.8±0.10	96.46±0.30
Psychorubrin (D10)	A	62.5±0.44	62.01±0.27	61.46±0.19	59.64±0.49
	B	68.09±0.69	67.03±0.98	66.42±0.29	64.36±0.30
	C	77.00±0.76	75.80±0.95	75.20±0.38	72.04±0.68

Each value was a mean of triplicate readings ± SEM, A = 4 mg/ml, B = 2 mg/ml, C = 1 mg/ml

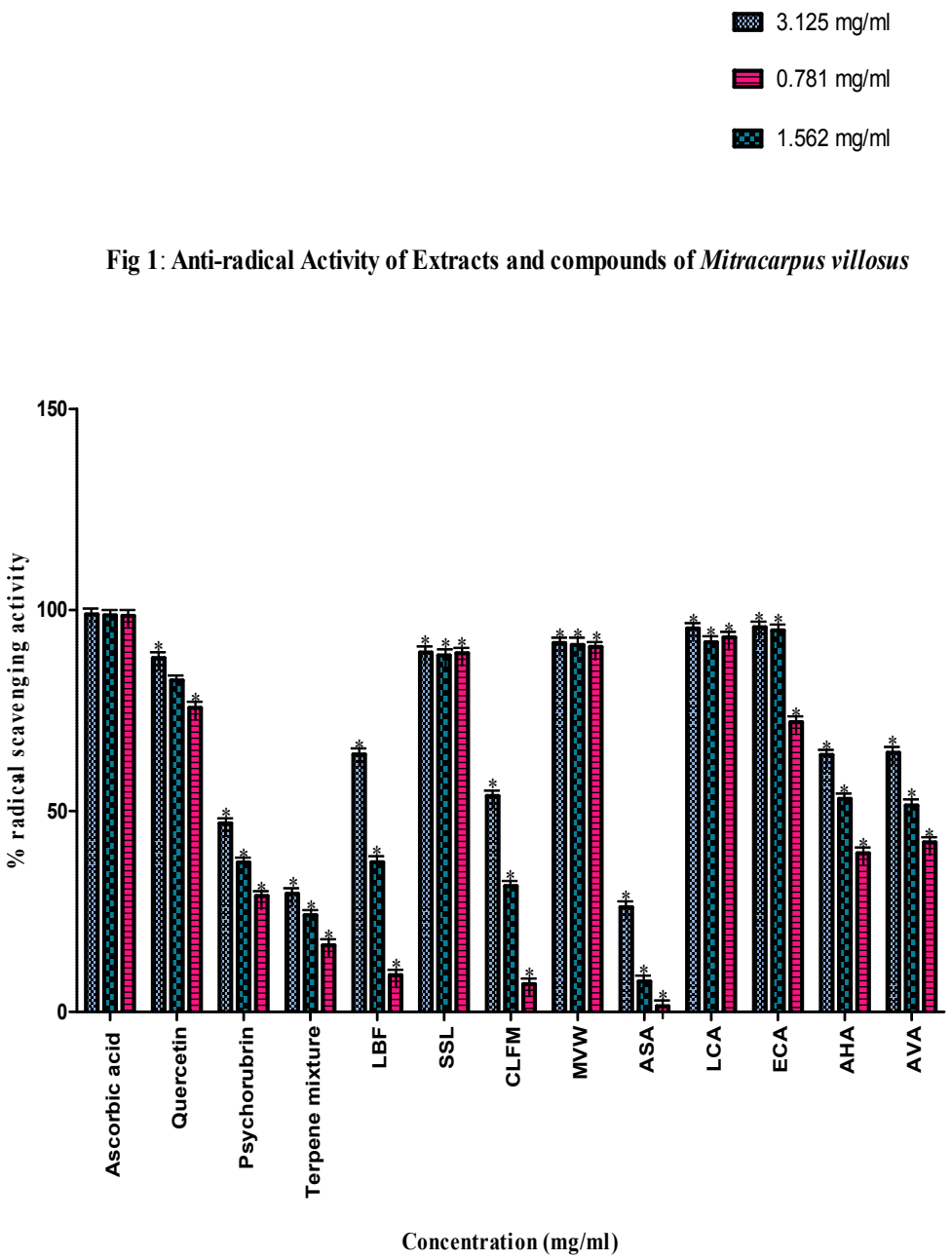


Fig 1: **Anti-radical Activity of Extracts and compounds of *Mitracarpus villosus***

Fig. 1. Antiradical activity of studied plant extracts and compounds of *Mitracarpus villosus*
Each value is mean of triplicate readings ± SEM. LBF: Lagenaria breviflora fruit, SSL: Senna siamea leaves, CLFM: Citrullus lanatus fruit, MVW: Mitracarpus villosus whole plant, ASA: Amaranthus spinosus aerial part, LCA: Lantana camara aerial part, ECA: Emilia praetermissa aerial part, AHA: Amaranthus hybridus aerial part, AVA: Amaranthus viridis aerial part

Compound E4 (33 mg) shown in Fig. 3 was obtained from EtOAc fraction as a yellow powder, golden yellow colour reacting spot (VS spray), R_f value 0.64 (*n*-hexane: EtOAc 1:1), strongly antioxidant (DPPH bioautography); FTIR (cm^{-1}): 1654.6 (C=O), 1400.6 (C=C), 1454.3, 1091.4 (–C–OH), 1160.1 (C-O). Positive ion mode ESI-MS m/z 303.23 [52%, M+1], 288.18 (100%) ^{1}H and ^{13}C NMR data (Table 4) and identified as quercetin ($C_{15}H_{10}O_7$) [13].

Fig 2: Polymerization Inhibitory Activity of Extracts of *Mitracarpus villosus*

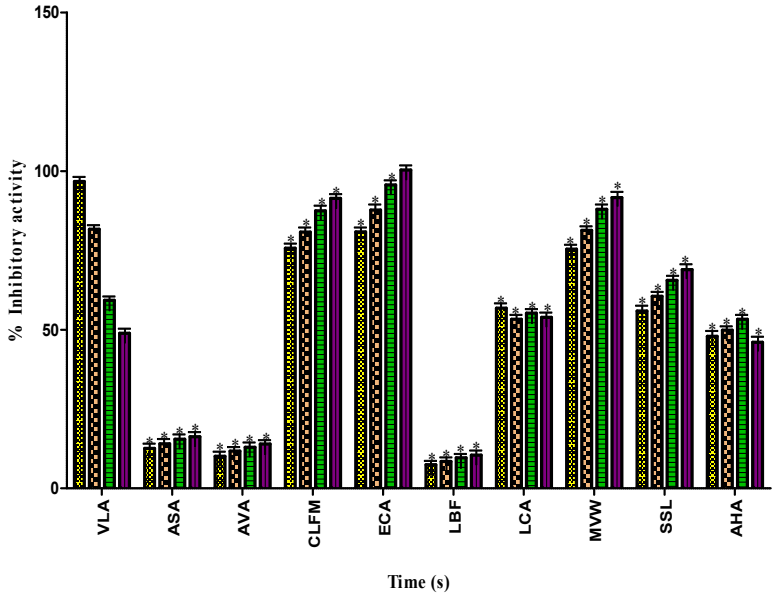
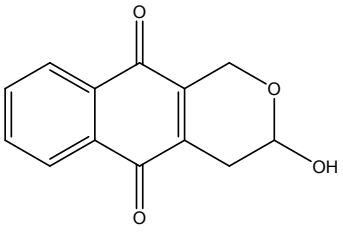
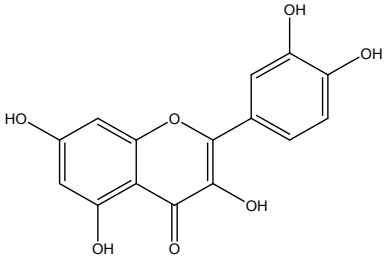


Fig. 2. Polymerisation inhibitory activity of studied plant extracts

VLA: Vanilic acid, ASA: Amaranthus spinosus aerial part, AVA: Amaranthus viridis aerial part, CLFM: Citrullus lanatus fruit, ECA: Emilia praetermissa aerial part, LBF: Lagenaria breviflora fruit, LCA: Lantana camara aerial part, MVW: Mitracarpus villosus whole plant, SSL: Senna siamea leaves, AHA: Amaranthus hybridus aerial part. Each value is mean of triplicate reading ± SEM



[D10] Psychorubrin



[E4] Quercetin

Fig. 3. Structure of compounds D10 and E4

The investigated plant extracts showed varying but dose-dependent and time-dependent activities in the two test screening models of antioxidant and antisickling when compared with the reference standards used; ascorbic acid and *para*-hyroxy benzoic acid (PHBA) respectively. The stable free radical scavenging reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), was used to estimate the ability of the crude extracts and the isolated compounds to exhibit free radical

scavenging property within the test concentration range (3.125 - 0.781 mg/ml). The antisickling activity of the extracts and compounds was determined by inhibition of polymerization of Haemoglobin (HbS) molecules.

In this study, extracts showing strong radical scavenging activities were also found to demonstrate significant antisickling effects. This initial screening was followed by ranking of the activities of the test extracts. Extracts of *Emilia praetermissa* aerial part (ECA) and *Mitracarpus villosus* whole plant (MVW) showed significant activities in both test models ($p < 0.05$). The highest antioxidant activity of 95.68% was demonstrated by ECA, followed by *Lantana camara* aerial part with 95.35% and 91.64% for MVW. Other extracts have moderate activities with *Amaranthus spinosus* aerial part having the least antioxidant activity of 26.06%. The positive control (Ascorbic acid) had the best antioxidant activity of 98.97% at the highest concentration (Fig. 1).

Table 3. ^{1}H and ^{13}C NMR Data of D10 (600 MHz, $CDCl_3$)

^{1}H position	δ_H (multiplicity)	δ_H [14]	^{13}C position	δ_C	δ_C [14]
1	4.64 – 4.85 (2H, dt) (oxymethylene group)	4.81 (1H,dt), 4.71 (1H,dt)	1	57.7	57.9
3	5.39 (1H, t) (hemi acetal proton)	5.51 (1H, t)	3	90.7	90.9
4	2.65 – 2.84 (2H, m) (methylene protons)	2.74 (1H, dq), 2.84 (1H,dq)	4	27.9	28.2
-	-	-	4a	141.3	141.5
-	-	-	5	183.0	183.3
-	-	-	5a	131.8	132.0
6,9	8.32 – 8.36 (2H, m) (aromatic protons)	8.05 – 8.13 (2H, m)	6	133.8	134.0
7,8	7.84 – 7.91 (2H, m)	7.70 – 7.76 (2H, m)	7	126.5	126.7
-	-	-	8	126.2	126.4
-	-	-	9	133.9	134.1
-	-	-	9a	132.0	132.2
-	-	-	10	183.5	183.7
-	-	-	10a	139.2	139.4

Table 4. ^{1}H and ^{13}C NMR Data of E4 (600 MHz, $CDCl_3$)

^{1}H position	δ_H	δ_H [15]	Carbon position	δ_C	δ_C [15]
2	-	-	2	148.0	147.9
3	-	-	3	137.2	137.2
4	-	-	4	177.3	177.3
4a	-	-	4a	-	-
5	-	-	5	162.5	162.5
5a	-	-	5a	-	-
6	6.20 (1H, d, *J* = 2.0 Hz)	6.17(1H, d, *J* = 2.0Hz)	6	99.2	99.3
7	-	-	7	165.6	165.7
8	6.41 (1H, d, *J* = 2.0 Hz)	6.37(1H, d, *J* = 2.0 Hz)	8	94.4	94.4
9	-	-	9	158.2	158.2
9a	-	-	9a	-	-
10	-	-	10	104.5	104.4
10a	-	-	10a	-	-
1'	-	-	1'	124.1	124.1
2'	7.75 (1H, d, *J* = 1.9 Hz)	7.73 (1H, d, *J* = 2.0 Hz)	2'	116.0	116.0
3'	-	-	3'	146.2	146.2
4'	-	-	4'	148.8	148.7
5'	6.90 (1H, d, *J* = 8.5 Hz)	6.87(1H, d, *J* = 8.0 Hz)	5'	116.2	116.2
6'	7.65 (1H, m)	7.62(1H, dd, *J* = 2.0, 7.5 Hz)	6'	121.7	121.6

All the extracts showed significant ($p < 0.05$) inhibitory effects of the polymerisation of Hbs molecules (Fig. 2) except extracts of *Lagenaria breviflora* fruit (LBF), *A. spinosus* and *A. viridis* aerial parts which showed very weak activity of 10.40, 13.90 and 16.20% respectively at the maximum time (180 secs).

In particular, extracts of ECA and MVW showed polymerization inhibitory activity of ECA 100.30% and 91.60% respectively and were therefore ranked first and second. None availability of ECA for bulk collection for bioassay-guided phytochemical study necessitated the investigation of MVW. Other extracts showed varying activities. The extract of LBF was found to exhibit the least activity and was therefore ranked ninth position.

M. villosus is locally known as Irawo ile in the Yoruba culture, Nigeria. Although the plant is generally used in ethnomedicine in the treatment of fungal diseases, wounds and ulcers; in this study, it was found to exhibit a significant ability to scavenge free radicals. Excessive generation of reactive oxygen species and the inability of the natural biological defensive system to readily detoxify the reactive intermediates or easily repair the resulting damage have been implicated in many diseases. It arises when the cellular generation of reactive oxygen species (ROS) overwhelms the antioxidant defence system. Sickle cell crisis usually starts with inflammation of joints, which is mostly as a result of oxidative stress of the affected erythrocytes (RBCs). It has been identified that a very destructive aspect of oxidative stress is the production of reactive oxygen species [9]. These species are often generated by environmental agents such as gases from exhausts, smoke from cigarette, chemicals, pollutants, dust particles etc. which individuals with SCD are exposed to from time to time.

The average yield of three successive extractions of *M. villosus* whole (MVW) plant was 2.53%. After repeated chromatographic separation and purification, three compounds were isolated from MVW. The dichloromethane fraction yielded a naphthoquinone, psychorubrin (3-hydroxy-3,4-dihydro-1H-benzo[g] isochromene -5,10-dione) and a mixture from which stigmasterol was identified was the least active. Quercetin - 2-(3, 4-dihydroxyphenyl)-3,5,7-trihydroxy -4H-chromen-4-one was isolated from the ethyl acetate fraction.

The structures of the isolated compounds were elucidated by NMR spectra data and by comparisons with the literature values [13,14] (Tables 3 and 4). The stigmasterol mixture demonstrated very weak antioxidant (29.39 ± 0.53%) and antisickling (45.81 ±0.99%) activities and psychorubrin only had moderate activities (46.90 ± 0.45%, 59.64 ± 0.30%) while quercetin possessed the highest polymerization inhibitory effect (87.99 ± 0.18%, 3.125 mg/ml) as well as free radical scavenging property (87.85 ± 0.85%, 4 mg/ml).

4. CONCLUSION

Extracts or compound which demonstrated strong radical scavenging activity were also found to demonstrate significant antisickling effects. *Mitracarpus villousus* whole plant extract afforded quercetin, psychrorubrin and a mixture containing stigmasterol with strong, moderate and weak activities respectively.

DECLARATION

The authors wish to state that this manuscript contains original unpublished work which has not been submitted or accepted for publication elsewhere.

ACKNOWLEDGEMENTS

The support of the Nigeria government through the Tertiary Education Trust Fund (TetFund) for TETF/NRF/OAU grant is duly acknowledged. The assistance of Mr. I. I. Ogunlowo of the Department of Pharmacognosy, OAU, for the collection of plants, is also acknowledged.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization. Sickle-cell anaemia. Fifty-ninth World Health Assembly A59/9 Provisional Agenda Item. 2006;11(4):1-5.
2. Frenrtte PS, Atweh GF. Sickle cell disease: Old discoveries, new concepts and future promise. J Clin Invest. 2007;117:850-858.
3. Sickle cell disease: Data & statistics. Centers for Disease Control and Prevention; 2011.

4. World Health Organisation. Sickle-cell anaemia. Report by the Secretariat. Fifty-ninth World Health Assembly. A59/9 Provisional Agenda Item. 2006;11(4).
5. Kutlar A. Sickle cell disease: A multigenic perspective of a single-gene disorder. Med Princ Pract. 2005;14:15-19.
6. Okpuzo J, Adebesin O, Ogbunugafor H, Amadi I. The potential of medicinal plants in sickle cell disease control: A review. Int J Biomed Health Sci. 2008;4(2):47-55.
7. Obodozie OO, Ameh SJ, Afolabi EK, Oyedele EO, Ache TA, Onanuga CE, Ibe MC, Inyang US. Standardization of the components of niprisan: A phytomedicine for treating sickle cell disease. J Med Plants Res. 2009;3(4):284-289.
8. Ameh SJ, Tarfa FD, Ebeshi BU. Traditional herbal management of sickle cell anemia: lessons from Nigeria. Anemia. 2012;1-9. DOI: 10.1155/2012/607436
9. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res. 2001; 15(2):127-130.
10. Tsakiris S, Giannoulia-Karantana A, Simintzi I. Schulpis KH. The effect of aspartame metabolites on human erythrocyte membrane acetylcho-linesterase activity. Pharmacol Res. 2005; 53:1-5.
11. Iwu MM, Igboko AO, Onwubiko H, Ndu UE. Effect of cajaminose from *Cajanus cajan* on gelation and oxygen affinity of sickle cell haemoglobin. J Ethnopharmacol.1988; 20:99-104.
12. Chikezie PC, Chikezie CM, Amaragbulem PI. Effect of antimalarial drugs on polymerisation of sickle cell haemoglobin (Hbs). Turkish Journal of Biochemistry. 2010;35:14-44.
13. Hayashi T, Smith FT, Lee K. Antitumor agents. 89. Psychorubrin, a new cytotoxic naphthoquinone from *Psychotria rubra* and Its Structure activity-relationship. J Med Chem. 1987;30:2005-08.
14. Fabri RL, Grazul RM, De Carvalho LO, Coimbra ES, Cardoso GMM, De Souza-Fagundes EM, Da Silva AD, Scio E. Antitumor, Antibiotic and antileishmanial properties of the pyranonaphthoquinone psychorubrin from *Mitracarpus frigidus*. Ann Brazil Acad Sci. 2012;84(4):1081-89.
15. Guvenalp Z, Demirezer LO. Flavonol glycosides from *Asperula arvensis* L. Turk J Chem. 2005;29:163-69.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25731