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Comparative *in vitro* radical scavenging and erythrocyte membrane stabilizing activities of extracts and partitioned fractions of *Artocarpus altilis* stem bark and *Senna alata* leaf

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Abstract
This study investigated the radicals scavenging and erythrocyte membrane stabilizing properties of stem bark of *A. altilis* and leaf of *S. alata*. The plants were macerated in 80% EtOH/H_2O at room temperature, evaporated under reduced pressure, lyophilized and partitioned to obtain *n*-hexane, DCM, EtOAc, *n*-BuOH and aqueous fractions respectively. *In vitro* radical scavenging and erythrocyte membrane stabilizing activities of extracts and fractions were carried out using colorimetric assay and hypotonicity and heat-induced haemolysis methods respectively. The results showed concentration-dependent radical scavenging activities. The EtOAc fractions of *A. altilis* and *S. alata* with IC_{50} values 0.68±0.07 and 1.55±0.38 mg/mL exhibited good NO radical scavenging properties and IC_{50} values of 0.25±0.05 and 0.49±0.13 mg/mL anti-lipid peroxidation activities, while the EtOAc and DCM fractions of *A. altilis* with IC_{50} 2.22±0.05 and 3.67±0.19 mg/mL OH radical scavenging activities. The crude extracts, DCM and EtOAc fractions of both plants exhibited good protective activity on erythrocyte membrane in a dose-dependent manner compared with diclofenac. The results justify the ethnomedicinal use of the plants in polyhedral formulations for the management of oxidative stress-induced diseases.

Keywords: *Artocarpus altilis*, *Senna alata*, radical scavenging, erythrocyte membrane stability, ethnomedicinal, Ijesha land South-western Nigeria

1. Introduction
The use of medicinal plants in the management of oxidative-stress related diseases such as cancer, diabetes, cardiovascular and some inflammatory diseases, is an age-long medical practice across many cultures of Africa and Asia. Generally, the curative activities of medicinal plants and other natural products have spurred renewed interest in the pharmacological investigations of plants (Pant, 2014; Pant *et al*., 2021) [1, 2]. Free radicals are continuously being generated in the body, it is only safe if the rate at which they are generated is balanced by the rate at which they are neutralized for the maintenance of normal physiological conditions (Jones, 2008) [3]. The detoxification of free radicals by the endogenous enzymes in the body is strengthened by the radical scavenging activities of exogenous antioxidants obtained from dietary sources, mainly medicinal plants (Domann, 2013) [4]. Oxidative stress can damage vital body organs over prolonged exposure to reactive oxygen, nitrogen and lipid peroxidation species (Chen *et al*., 2012; Liochev, 2013; Radi, 2018; Jakubczyk *et al*., 2020) [5, 6, 7, 8]. It has been suggested that about 95% of human pathological conditions in people above the age of 35 years are associated with the deleterious activities of free radicals (Gunalan *et al*., 2012) [9]. The leaf and stem bark of *Artocarpus altilis* and leaf of *Senna alata* are both used in ethnomedicine for the treatment of oxidative stress-related diseases, microbial infections, wounds and in the management of fungal diseases respectively (Ontong *et al*., 2019; Soifoini *et al*., 2021) [10, 11]. The fruit of *A. altilis* is a staple rich source of carbohydrates, protein, vitamins and many micronutrients, and consumed widely among the Yoruba of the South-western Nigeria as an alternative to yam (Jones *et al*., 2011) [12]. It is also very rich in flavonoidal antioxidant compounds (Ramdath *et al*., 2004; Akanni *et al*., 2014; Liu *et al*., 2015; Mausio *et al*., 2020) [13, 14, 15, 16]. The leaves of *S. alata* is used as a remedy for skin rashes, lesions, ringworm and venereal diseases (Chomnawang *et al*., 2005; Oladeji *et al*., 2020) [17, 18]. In a recent survey carried out in Ijesha land, stem bark of *A. altilis* and leaf of *S. alata* are frequently used as components of some polyherbal formulations for the treatment of oxidative stress-related diseases and cutaneous wound infections.

This study showed the *in vitro* erythrocyte membrane stabilizing and radicals scavenging potentials of hydroethanolic extracts and partitioned fractions of the stem bark of *A. altilis* and leaf of *S. alata*.

2. Materials and Methods

MeOH, EtOH, *n*-Hexane, DCM, EtOAc, *n*-BuOH and Me_2CO (BDH, England) were redistilled before use. $BaCl_2.2H_2O$, Anhydrous NaCl, Sodium Citrate, $FeSO_4$, $HCl_{(aq)}$, $FeCl_3$, Sodium Nitroprusside, Ascorbic Acid, Sulphanilamide, N-1-Naphthylethylenediammine Dihydrochloride, Deoxyribose, Ethylenediammine Tetraacetic Acid, Hydrogen Peroxide, Phosphate Buffer, 2,4,6-Tris-2-Pyridyl-1,3,5-Triazine, Sodium Phosphate, Concentrated H_2SO_4, $BaSO_4$, butylated hydroxyl toluene were purchased from Sigma-Aldrich (Darmstadt, Germany).

2.1 Plant Collection and Authentication

Stem bark of *Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg and leaf of *Senna alata* (L.) Roxb. Were harvested from the wild (Olosara and Alaro Farm Settlements, 7° 53′41.71N and 4° 58′55.26E) in July, 2019. The bark and leaf were identified and authenticated by Mr I. I. Ogunlowo, the curator at the Herbarium Section, Faculty of Pharmacy, Obafemi Awolowo University, Nigeria. Herbarium specimens were deposited and assigned voucher numbers: FPI 2253 and FPI 2258, respectively. The nomenclature of the plants was also confirmed on www.worldfloraonline.org. The plant materials were then dried in the screen house and milled.

2.2 Preparation of Extract and Fractions

The powdered bark of *A. altilis* (0.945 kg) and leaf of *S. alata* (0.850 kg) were extracted (3X) with 80% (v/v) EtOH:H_2O (10 L) at r.t. for 48 hours with continuous agitation on mechanical shaker. The combined extract of each plant was evaporated to dryness at 40 °C *in vacuo* to yield the dry crude extracts, which were independently suspended in distilled H_2O and successively partitioned to afford respective fractions: *n*-hexane, dichloromethane (DCM), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH) and aqueous residue. These fractions were evaporated to dryness at 40°C under reduced pressure and preserved.

2.3 DPPH Radical Scavenging Activity

Reactions were carried out with plant extracts and active fractions at varying concentrations of 5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL, from an initial stock solution of 10 mg/mL. A 1 mL aliquot of 0.3 mM DPPH radicals in methanol was added to 1 mL of extract and fractions in triplicates. Reactions were carried out in the dark for about 30 minutes. The same treatment was applied to ascorbic acid and methanol as positive and negative controls respectively. The absorbance was read at 517 nm. The ability of samples to detoxify DPPH free radicals was then evaluated using the equation:

$$\%\text{inhibition}=\frac{Absorbance\ of\ control-Absorbance\ of\ samples}{Absorbance\ of\ control}\times 100\%$$

The 50% inhibitory concentration (IC_{50}) of ascorbic acid, extracts and fractions of *A. altilis* and *S. alata* were obtained from a linear regression graph of% inhibition against concentrations of samples tested (Brand-Williams *et al*., 1995; Brand-Williams *et al*., 1997; Prior *et al*., 2005) [19, 20, 21].

2.4 Nitric Oxide (NO) Radical Scavenging Activity

Sodium nitroprusside is known to generate NO radicals in aqueous medium at physiological pH, the quantity of which was determined by reaction with Griess reagent. The reaction mixture containing 0.1 mL each of varying concentrations of samples (5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL) and 0.9 mL of 2.5 mM sodium nitroprusside in phosphate buffer saline was incubated under illumination for 150 minutes at r.t. After incubation, 0.5 mL of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10 minutes, followed by the addition of 0.5 mL of 0.1% N-1-napthylethylene diamine dihydrochloride. The same reaction mixture without samples served as the negative control while ascorbic acid was the positive control. The absorbance of the resulting solution was then read at 546 nm. The ability of samples to detoxify NO radicals was evaluated using the equation:

$$\%\text{ inhibition}=\frac{Absorbance\ of\ control-Absorbance\ of\ samples}{Absorbance\ of\ control}\times 100\%$$

The 50% inhibitory concentration (IC_{50}) of the standards and samples were obtained from a linear regression graph of% inhibition against concentrations of samples tested (Marcocci *et al*., 1994; Ahmed *et al*., 2016) [22, 23].

2.5 Hydroxyl Radical Scavenging Activity

The reaction mixture consists of 1.0 mL of working reagent (100 µL 3.0 mM deoxyribose, 200 µL 0.1 mM EDTA, 100 µL 2 mM H_2O_2, 100 µL 0.1 mM L-Ascorbic acid, 0.1 mM $FeCl_3.6H_2O$ in 10 mM phosphate buffer, pH 7.4) and 0.5 mL various concentrations of the samples (5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL). The reaction mixture was incubated at 37 ºC for 1 hour, followed by the addition of 1.0 ml of 1% (w/v) thiobarbituric acid (in 0.25 N HCl) and 1.0 ml of 10% (w/v) trichloroacetic acid. The reaction mixture was then incubated at 100 ºC for 20 minutes, after cooling, the pink chromogen was extracted into 1.0 ml of butanol and the absorbance was read at 532 nm against reagent blank. The ability of samples to detoxify the generated hydroxyl radicals was then evaluated using the equation:

$$\%\text{inhibition}=\frac{Absorbance\ of\ control-Absorbance\ of\ samples}{Absorbance\ of\ control}\times 100\%$$

The IC_{50} of the standards and samples were obtained from a linear regression graph of% inhibition against concentrations of samples tested (Zou *et al*., 2015) [24].

2.6 Anti-Lipid Peroxidation Activity

A 0.25 mL of 10% liver homogenate was added to 0.1 mL of 150 mM Tris-Cl buffer, pH 7.2, 0.05 mL of 1% (w/v) ascorbic acid, 0.05 mL of 0.07M $FeSO_4$ and 0.5 mL different concentrations of samples (5, 2.5, 1.25, 0.625, 0.3125, 0.15625 mg/mL). The reaction mixtures were then incubated at 37 ºC for 1 hour. After incubation, 0.5 mL of 0.1 N HCl, 0.2 mL of 9.8% sodium dodecyl sulphate, 0.9 mL of distilled water and 2.0 mL of 0.67% thiobarbituric acid were added sequentially. The reaction mixture was then incubated at 100ºC for 30 min., cooled and 2.0 mL of butanol was added and later centrifuge at 3000 rpm for 10 min. The upper organic supernatant was collected and measured at 532 nm against reagent blank. The ability of samples to neutralize the generated peroxidation products radicals was then evaluated using the equation:

$$\%\text{inhibition}=\frac{Absorbance\ of\ control-Absorbance\ of\ samples}{Absorbance\ of\ control}\times 100\%$$

The IC_{50} of the standards and samples were obtained from a linear regression graph of% inhibition against concentrations of samples tested (Medipilwar *et al*., 2015) [25].

2.7.1 Membrane Stabilization Test

This was evaluated using membrane stabilization method of heat and hypotonicity-induced haemolysis method.

2.7.1.1 Preparation of Bovine Erythrocytes Suspension

Fresh blood was collected from healthy cow into a vial containing 100 mL of 3.8% (w/v) trisodium citrate anticoagulant solution; 5 mL of the blood was transferred into centrifuge tubes and centrifuged at 3000 rpm for 15 minutes. The supernatant was carefully removed and discarded; and it was then carefully washed three times with isosaline till a clear supernatant was obtained. The volume of blood cells was then adjusted to 2 mL and re-constituted as 2% (v/v) blood suspension with normal saline.

2.7.1.2 Preparation of Working Solutions, Samples and Standard

A 5 mg/mL stock solution of the extracts and fractions of *A. altilis* stem bark and *S. alata* leaves was prepared, from which 1 mg/mL (1000 µg/mL) working solution was prepared. A 200 mL of 0.85% (w/v) isosaline was prepared by weighing 1.70 g of anhydrous NaCl and dissolving with distilled water to 200 mL. Similarly, 100 mL of 0.42% (w/v) hyposaline solution was prepared by weighing 0.42 g of anhydrous NaCl and dissolving with distilled water to 100 mL. A 100 mL of 0.15 M Na-phosphate buffer pH 7.4 was also prepared. The standard drug Diclofenac was used as positive control.

2.7.1.3 Heat and Hypotonicity-Induced Haemolysis

Briefly, different concentrations (0, 50, 100, 150, 200, 250, 300 µg/mL) of extracts and fractions were added to test-tubes, followed by appropriate volume of hyposaline solution, phosphate buffer solution, isosaline solution and the erythrocytes suspension respectively (Table 1). The erythrocyte suspension was added gently down the side of the test-tube. The entire set-up was incubated at 56 °C for 30 minutes. It was cooled for 20 minutes and centrifuged at 3000 revolutions per minute for 10 minutes and absorbance was read at 560 nm. This experiment was carried out in triplicate and repeated for the drug control and standard drug (Diclofenac) serving as positive control. The blood control contains no test samples and was taken as 100% haemolysis, while the drug control contains no blood suspension but equivalent volume of isosaline. Activity was evaluated using the equation:

$$\%\ \text{Membrane Stability}=[100-\{\frac{ABSTest\ Drug-ABSDrug\ Control}{ABSBlood\ Conrol}\}]\times 100$$

Table 1: Assay protocol of membrane stability activity

Reagent/Test-tube	1 – 3	4 – 6	7 – 9	10 – 12	13 – 15	16 – 18	19 - 21
Samples (mL)	0.00	0.15	0.30	0.45	0.60	0.75	0.90
Hyposaline (mL)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Phosphate buffer (mL)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
n-saline (mL)	1.50	1.35	1.20	1.05	0.90	0.75	0.60
Blood Suspension (mL)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Total Assay Volume	3.00	3.00	3.00	3.00	3.00	3.00	3.00

2.8 Statistical analysis

All experiments were performed in triplicates and the results are expressed in mean% inhibition ± SEM. Linear regression analysis was used to calculate the IC_{50} values of standard (ascorbic acid), extracts and fractions.

3. Results and Discussion

Tables 2 showed the mean percentage inhibition of ascorbic acid at various concentrations obtained for the DPPH, NO, OH radicals scavenging activities, anti-lipid peroxidation activities while Tables 3-9 show the antioxidant activities of the crude extracts and the active fractions of *A. altilis* stem bark and *S. alata* leaf, while the mean IC_{50} values of the extracts and active fractions are presented in Table 10. Figures 1 showed the graph of the percentage erythrocyte membrane stability of crude extract and active fractions of *A. altilis* and *S. alata*, respectively at various concentrations. Diclofenac, a standard anti-inflammatory drug was also tested at the same concentrations. Diclofenac and the crude extracts of *A. altilis* and *S. alata* exhibited a biphasic protective activity on erythrocyte membrane, while the fractions of the two plants showed a typical monophasic protection on the blood cells membrane.

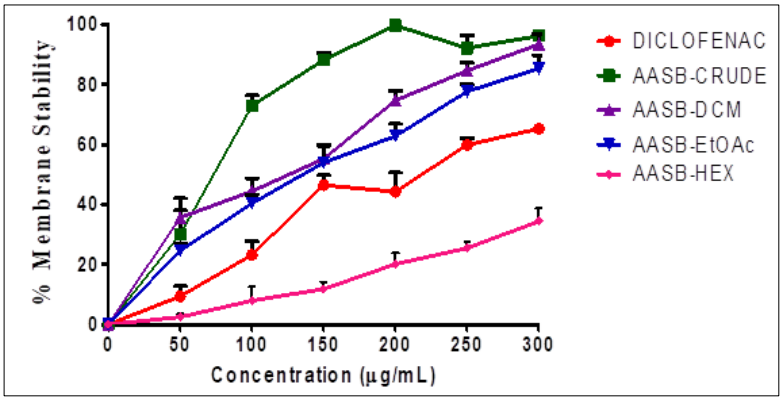

Fig 1: Percentage Erythrocyte Membrane Stability against Concentrations of Diclofenac, Crude Extracts and Fractions of *A. altilis* Stem Bark. AASB-HEX, AASB-DCM, AASB-EtOAc stands for hexane, dichloromethane and ethyl acetate fractions of *A. altilis* Stem Bark Extract respectively. AASB-CRUDE is the Crude Extract of *A. altilis* Stem Bark

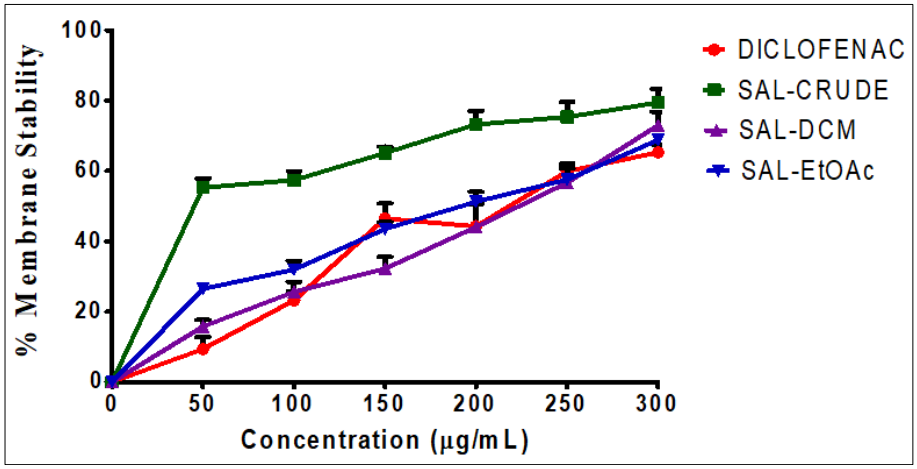

Fig 2: Percentage Erythrocyte Membrane Stability against Concentrations of Diclofenac, Crude Extract and Fractions of *S. alata* Leaf. SAL-CRUDE, SAL-DCM, SAL-EtOAc stands for the Crude Extract, dichloromethane and ethyl acetate fractions of *S. alata* leaf respectively

Table 2: Antioxidant Activities of Ascorbic acid (Standard Antioxidant agent)

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
0.10	58.41±3.01	93.37±3.25	56.29±0.41	26.30±0.51
0.08	53.79±1.42	98.29±1.33	46.00±2.03	23.31±0.27
0.06	49.80±1.00	86.85±2.49	42.29±1.82	11.57±0.10
0.04	28.93±2.97	50.34±4.39	26.85±0.24	6.81±0.85
0.02	20.73±0.83	26.96±6.79	7.71±0.35	1.74±0.16

Inh is the Inhibition of ascorbic acid for each of the antioxidant activity, SEM is the standard error of the mean.

Table 3: Antioxidant Activities of Crude Extract of *A. altilis* Stem Bark

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	38.34±1.83	50.37±0.05	50.75±0.75	81.95±0.29
2.5	35.95±0.43	49.55±1.39	46.88±0.43	63.39±1.22
1.25	25.31±1.10	48.04±1.71	44.88±2.55	58.22±1.43
0.625	18.21±2.26	39.20±0.48	41.56±4.04	47.05±2.43
0.3125	18.08±6.03	35.95±3.41	37.80±0.75	31.74±2.22
0.15625	16.23±0.77	30.29±0.59	32.53±0.21	28.50±0.22

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean.

Table 4: Antioxidant Activities of Hexane Fraction of *A. altilis* Stem Bark

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	34.72±4.10	49.17±3.15	65.66±0.85	56.60±0.43
2.5	32.47±3.18	43.13±0.91	49.70±0.32	49.09±0.43
1.25	26.19±0.57	42.45±3.20	43.83±0.32	47.97±0.65
0.625	22.44±1.01	38.75±3.26	42.17±2.77	41.68±1.79
0.3125	15.89±0.34	38.82±4.27	41.42±1.17	41.00±0.00
0.15625	13.85±0.72	33.76±2.18	38.70±0.75	32.96±0.35

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean.

Table 5: Antioxidant Activities of Dichloromethane Fraction of *A. altilis* Stem Bark

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	46.57±0.43	54.61±2.19	61.15±0.21	73.94±0.07
2.5	37.31±2.46	52.71±2.03	44.13±1.38	70.89±0.50
1.25	34.52±1.54	48.94±0.96	41.56±4.03	59.63±0.14
0.625	28.79±3.28	46.98±0.64	38.10±0.53	51.12±0.61
0.3125	25.31±1.01	27.04±3.52	35.99±0.75	44.52±2.25
0.15625	21.42±0.48	21.53±0.05	34.65±2.98	34.79±1.51

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean

Table 6: Antioxidant Activities of Ethyl acetate Fraction of *A. altilis* Stem Bark

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	44.41±2.46	61.34±2.13	65.66±0.85	76.75±0.57
2.5	35.40±1.50	59.87±1.65	49.70±0.32	68.85±0.50
1.25	33.08±1.01	44.71±1.71	43.83±0.32	54.77±0.43
0.625	28.44±0.53	42.98±0.37	42.17±2.77	52.25±0.65
0.3125	28.17±1.30	42.22±0.91	41.42±1.17	46.81±2.58
0.15625	15.00±0.57	34.51±2.72	38.70±0.75	40.57±0.29

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean

Table 7: Absorbance and Percentage Inhibition of Crude Extract of *S. alata* Leaf

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	44.82±1.30	57.55±0.43	42.17±1.28	53.44±1.79
2.5	43.32±0.14	55.97±0.05	37.65±2.98	49.39±1.51
1.25	35.74±0.48	50.38±1.21	37.35±2.61	39.25±2.08
0.625	30.42±1.83	41.92±0.48	33.89±4.36	32.15±1.36
0.3125	29.26±0.34	36.40±0.53	28.46±0.75	27.83±0.65
0.15625	26.46±1.64	23.94±2.51	14.31±1.81	26.67±3.94

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean

Table 8: Antioxidant Activities of Dichloromethane Fraction of *S. alata* Leaf

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	38.40±2.75	58.76±2.13	43.37±0.21	72.31±1.94
2.5	34.72±4.58	49.24±0.53	23.19±1.92	67.75±2.15
1.25	32.47±3.28	48.11±2.29	22.44±0.32	57.71±1.29
0.625	28.10±0.87	43.20±0.32	19.28±2.17	51.20±3.15
0.3125	26.81±1.88	41.62±1.34	13.75±0.95	48.07±1.53
0.15625	20.94±0.05	34.15±1.77	5.57±1.17	37.72±1.83

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean

Table 9: Antioxidant Activities of Ethyl acetate Fraction of *S. alata* Leaf

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Conc (mg/mL)	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**	**Mean% Inh ± SEM**
5.0	42.77±2.55	64.58±1.22	57.08±0.75	76.63±0.57
2.5	39.61±3.25	64.50±1.71	37.05±2.37	68.85±0.50
1.25	37.86±2.46	60.27±0.21	31.65±0.85	54.77±0.41
0.625	26.73±0.48	49.06±0.42	29.37±4.05	52.25±0.65
0.3125	22.78±2.60	45.85±0.80	20.78±4.11	46.85±2.58
0.15625	20.17±2.12	44.94±3.26	10.10±0.11	40.57±0.29

Inh is the Inhibition of crude extract of *A. altilis* for each of the antioxidant activity, SEM is the standard error of the mean.

Table 10: IC_{50} (mg/mL) Values of Ascorbic acid, Extracts and Fractions of *A. altilis* Stem Bark and *S. alata* Leaf

	DPPH Radical Scavenging Activity	NO Radical Scavenging Activity	OH Radical Scavenging Activity	Anti-lipid Peroxidation Activity
Samples	**Mean IC_{50}**	**Mean IC_{50}**	**Mean IC_{50}**	**Mean IC_{50}**
Ascorbic acid	0.015±0.00	0.07±0.01	0.09±0.01	0.17±0.00
Extract (AA)	7.38±0.18	2.37±0.22	4.52±0.64	0.67±0.11
Hexane (AA)	9.96±1.57	5.41±0.34	4.50±0.09	2.93±0.06
DCM (AA)	5.59±0.02	2.21±0.25	3.67±0.19	1.21±0.38
EtOAc (AA)	6.13±0.50	1.55±0.38	2.22±0.05	0.25±0.05
Extract (SA)	5.64±0.19	1.41±0.29	6.03±0.52	3.82±0.30
DCM (SA)	6.99±0.29	1.34±0.73	6.11±0.17	0.72±0.11
EtOAc (SA)	5.90±0.69	0.68±0.07	3.99±0.14	0.49±0.13

IC_{50} is the 50% inhibitory concentration, Extract (AA), Hexane (AA), DCM (AA) and EtOAc (AA) is the crude extract, hexane fraction, dichloromethane and ethyl acetate fraction of *A. altilis* stem bark respectively, while Extract (SA), DCM (SA) and EtOAc (SA) is the crude extract, dichloromethane fraction and ethyl acetate fraction of *S. alata* leaf respectively.

3.1 *In vitro* Antioxidant Activities of Extracts and Fractions

NO Radical Scavenging Activity: The extracts and fractions showed antioxidant activity by neutralizing the NO radicals formed in solution. The results showed that the NO radicals scavenging activity increased with increasing concentration. Ascorbic acid had 93.37±3.22 NO neutralized while EtOAc, DCM fractions, crude extract and hexane fraction of *A. altilis* stem bark had 61.34±2.13, 54.61±2.19, 50.37±0.05 and 49.17±3.15 NO inhibition, respectively. This shows that the NO radical scavenging activity decreases from EtOAc, DCM, crude extract through hexane fractions of *A. altilis*. Similar trend was also observed in the IC_{50} values, EtOAc fraction exhibiting the highest IC_{50} value of 1.55±0.38 mg/mL followed by DCM fraction with 2.21±0.25 mg/mL, crude extract with 2.37±0.22 mg/mL and 4.60±0.34 mg/mL for the hexane fraction. The NO inhibition activity of *S. alata* leaves extract and fractions were also tested at same concentration of 5 mg/mL, with percentage of NO inhibited at maximum dose of 5 mg/mL for the crude extract and fraction given as crude extract with 57.55±0.43, DCM fraction of *S. alata* leaves with 58.76±2.13 and EtOAc fraction with mean percentage NO inhibited as 64.58±1.22. The mean IC_{50} which is the dose of the sample that would cause a 50% inhibition in the NO radicals generated in solution decreases from EtOAc fraction with 0.68±0.17 mg/mL, DCM fraction with 1.34±0.33 and 1.41±0.29 for the crude extract of *S. alata* leaves. The whole results showed that most chemical compounds responsible for the NO radical scavenging activity were concentrated in moderately polar solvents. The extracts and fractions of the two plants displayed good activity, however, their activities do not compare with that of ascorbic acid as positive control. The difference in the IC_{50} values between the extracts and fractions of the two plants and ascorbic acid was significant at $P<0.05$, but the extracts and fractions of both plants offer natural ways of neutralizing excess NO radicals released into the body system. (Edge and Truscott, 2018) [26]. Nitric Oxide (NO) radical however, is an important chemical mediator in the body, it is involved in the chemical signalling reactions in the body and controls the erectile muscles of the penile organ in males, but it is required only in minute amount (Abraham, 2014) [27]. Moreover, unregulated production of NO radicals results in the formation of toxic nitro fatty acids and nitro proteins molecules in the body. NO is the one of the most poisonous forms of reactive nitrogen species, it plays a role in lipid biology and disrupts the brain lipid transduction signal (Juan *et al*., 2002) [28]. Studies have also revealed that key enzymes involved in the electron transport chain of glycolytic pathways and activates other peroxynitrite forms causing degeneration of injuries (Oliveira *et al*., 2004) [29].

Anti-lipid Peroxidation Activity: The results obtained showed the potency of the crude extracts and active fractions of *A. altilis* and *S. alata* to prevent lipid peroxidation reactions and other toxic products generated in membranes. All the samples exhibited varying ability to inhibit lipid peroxidation. The anti-lipid peroxidation was found to be dose-dependent. The ability of the samples at 5 mg/mL to inhibit lipid peroxidation increases from hexane fraction with mean inhibition of 56.59±0.43, DCM fraction with 73.94±0.07, EtOAc with 74.55±0.22 and 81.95±0.29 for the crude extract of *A. altilis* stem bark. The crude extract of *A. altilis* showed the highest mean percentage lipid peroxidation inhibited at 5 mg/mL while extract and fractions of *S. alata* leaves had 76.5±0.57, 72.3±1.94 and 53.4±1.79 average inhibition for EtOAc, DCM and crude extract at same concentration respectively. The IC_{50} values showed that EtOAc fraction of both *A. altilis* and *S. alata* was the most effective in preventing lipid peroxidation. The EtOAc fraction of *A. altilis* with IC_{50} of 0.25±0.07 mg/mL, crude extract of *A. altilis* with 0.67±0.11 mg/mL, DCM and hexane fractions of *A. altilis* had 1.21±0.38 and 2.93±0.06 mg/mL respectively. The EtOAc fraction of *S. alata* had 0.49±0.13 mg/mL, DCM with 0.72±0.11 and crude extract of *S. alata* leaves had 3.82±0.30 mg/mL. Ascorbic acid used as a positive control had IC_{50} value of 0.17 mg/mL. The results obtained are in agreement with the finding of Akanni *et al*., (2014) [30]. There is a significant difference between the IC_{50} values of samples and ascorbic acid at $P<0.05$. Polyunsaturated fatty acids and their metabolites have many physiological roles such as energy generation and protecting the integrity of cellular and subcellular membrane structure and functions (Negre-Salvayre *et al*., 2008) [31]. Lipids are the main targets of reactive nitrogen and oxygen species (Nurbudu *et al*., 2019) [32]. An attack on the membranes causes loss of transport signals, integrity, transport potential viability and loss of osmotic balance in the cells (Carla *et al*., 2009) [33]. The peroxidation of fatty acids leads to the formation of membrane-toxic carbonyl products believed to be responsible for cytotoxic and other molecular damages in the body (Ayala *et al*., 2014) [34].

OH Radicals Scavenging Activity: The crude extracts and fractions showed varying OH radicals scavenging activity in a dose-dependent manner as was observed in the other antioxidant assays. Butylated hydroxyl toluene control showed 56.29±0.40 inhibition at 0.1 mg/mL compared with the samples. EtOAc fraction of *A. altilis* had 65.6±0.85 mean percentage inhibition of OH radicals (IC_{50} of 2.22±0.05 mg/mL) followed by DCM fraction with 61.15±0.21 mean inhibition (IC_{50} of 3.67±0.19 mg/mL), while hexane fraction had 54.0±1.81 inhibition (IC_{50} of 4.50±0.09 mg/mL) and the crude extract had 50.75±0.75 OH inhibition with IC_{50} of 4.52±0.64 mg/mL. There is no significant difference in the IC_{50} values of hexane fraction and crude extract of *A. altilis* at $P<0.05$, but the difference is significant at $P<0.01$. Extracts and fractions of *A. altilis* have well been reported to be rich in polyphenolic compounds, the results obtained agrees with the reports of Akanni *et al*., (2014) [30]. Similar trend was observed in the OH radicals scavenging activity of extract and fractions of *S. alata* leaf, where EtOAc fraction displayed the highest mean OH inhibition with 57.08±0.75 inhibition (IC_{50} of 3.99±0.14 mg/mL) followed by DCM fraction with 43.37±0.21 OH inhibition (IC_{50} of 6.11±0.14 mg/mL) and crude extract of *S. alata* leaf with 42.17±1.28 mean OH inhibition and IC_{50} of 6.03±0.52 mg/mL. There is no significant difference at $P<0.05$ in the OH radical scavenging ability of DCM fraction and crude extract of *S. alata*. OH radicals are the most reactive and abundant radicals in the human body, they catalyse the transport of nerve impulses in the cell. OH radicals occupies a central position in the pathology of wound chronicity and other oxidative-stress related diseases. It can cause an irreversible modification on biomolecules such as proteins, sugar groups of nucleic acids and fatty acids (Pham-Huy *et al*., 2008) [35].

DPPH Radicals Scavenging Activity: the results showed that the DCM fraction of *A. altilis* exhibited the good mean inhibition of radicals (46.57±0.43%) with IC_{50} value of 5.59±0.92 mg/mL), while the EtOAc fraction of *S. alata* displayed the highest mean (42.77±2.55%) and IC_{50} of 5.90±0.69 mg/mL.

Oxidative stress in wounds is a critical pathological condition where various reactive forms of oxygen and nitrogen species

overwhelm the wound-healing antioxidative mechanisms of the body thereby leading to tissue damage and inability of devitalized tissues to regrow. The healing of wounds requires basic macromolecules of protein, carbohydrates and nucleic acids. Reactive nitrogen (RNS) and oxygen (ROS) species react with these molecules by nitration, carbonylation and oxidation reactions. Natural products have been found to be effective in the neutralization of oxidative activities of these RNS and ROS species (Warren *et al*., 2010) [36]. Flavonoids of various classes exhibited pronounced antioxidant activities, especially flavonoids with ortho-dihydroxy moiety on the ring B of flavonoid skeleton, 2, 3-double bond in conjugation with a 4-oxo functionality forming an αβ-unsaturated carbonyl fragment, and one or two hydroxyl groups on ring A in meta relationship to each other have been found to be very important to strong antioxidant activities of flavonoids (Shahidi and Zhong, 2011) [37]. These structural fragments are found in the isolated compounds from the dichloromethane and ethyl acetate fractions of *A. altilis* stem bark extract and accounts for their strong radical scavenging activities (Rice-Evans *et al*., 1996; Vitaglione *et al*., 2008) [38, 39].

3.2 Erythrocyte membrane stabilizing Activity of Extracts and Fractions

Figs. 2 showed the percentage erythrocyte membrane stabilizing activity against varying concentrations of extracts and fractions of *A. altilis* stem barks and *S. alata* leaves. The results showed that DCM and EtOAc fractions and crude extract of *A. altilis* stem barks were more effective in protecting the erythrocytes cell membrane from heat and hypotonicity-induced haemolysis, with their membrane erythroprotective activities more effective at all the tested concentrations than the standard drug diclofenac, while the hexane fraction of *A. altilis* exhibited the lowest inhibition of heat and hypotonicity-induced haemolytic damage of the red blood cells. The results further showed that diclofenac displayed a biphasic protection mechanism on the blood cells membrane. Diclofenac has been found to be active at the early phase of inflammation processes mediated by serotonin, histamines and other cytoplasmic enzymes and also at the late phase, that is regulated by kinnin-like molecules (Okunrobo *et al*., 2014) [40]. The results also showed that percentage erythrocyte membrane stability increases with increasing concentration for all the tested samples and the relationship is dose-dependent. The biphasic nature of diclofenac and crude extracts of *A. altilis* stem bark and *S. alata* leaves is evident at 150 µg/mL to 250 µg/mL and 200 µg/mL to 300 µg/mL for diclofenac and crude extract of *A. altilis* stem bark, while 50 µg/mL to 300 µg/mL for crude extract of *S. alata* leaves. Heat and hypotonicity-induced erythrocyte membrane damage was more efficiently and effectively inhibited by the crude extracts of both plants than the standard drug diclofenac. The maximum inhibition of erythrocyte membrane disintegration (99.71±0.05%) was achieved at 200 µg/mL for crude extract of *A. altilis*, while at 300 µg/mL, *S. alata* crude extract and diclofenac exhibited 79.45±0.93% and 65.27±1.31% membrane damage inhibition, respectively. The DCM and EtOAc fractions of both plants extract and hexane fraction of *A. altilis* showed a typical monophasic membrane damage inhibition, which increases with increasing concentration of the tested fractions and is therefore dose-dependent. The DCM, EtOAc and hexane fractions of *A. altilis* showed the following minimum and maximum cell membrane erythroprotective activity of 35.69±1.36 to 93.42±3.43%, 24.61±2.54 to 85.36±1.41 and 2.47±1.30 to 34.38±1.39%, respectively for DCM, EtOAc and hexane fractions of stem bark extract of *A. altilis*. It is evident from the results that among the *A. altilis* stem barks treatments, the crude extract exhibited the highest protection of the cell membrane from heat and hypotonicity-induced haemolysis, and this is followed by the DCM, EtOAc, diclofenac and hexane fractions. However, at the early phase of 0-50 µg/mL, the DCM fraction of *A. altilis* showed a better red blood cell protection than the crude extract. Similar trend in inhibition of haemolysis of the erythrocytes was observed in the activities of crude extract and fractions of *S. alata* leaves where the activity was also dose-dependent at all concentrations tested. The crude extract of *S. alata* leaves exhibited the highest membrane stabilizing activity with minimum and maximum value of 55.29±0.64% and 79.47±0.95%. The response of the crude extract was also biphasic. This is followed by EtOAc fraction with minimum and maximum erythroprotective activity of 26.51±1.21% and 68.84±1.42% while DCM fraction exhibited 15.83±0.88% and 73.03±0.78%. The crude extracts of both *A. altilis* stem and *S. alata* leaves had a better erythrocyte membrane protection than diclofenac at all concentrations, though they both showed a biphasic stabilizing mechanisms.

Inflammation is part of the body's repair response to mechanical injuries, wounds, burns and microbial infections leading to the release of chemical mediators, free radicals and lysosomal hydrolytic enzymes that results in the tissue breakdown with attendant pain, swellings and loss of function (Usman *et al*., 2017) [41]. The inhibition of erythrocyte haemolysis by the crude extracts and fractions of both plants could be related to the binding affinity between the red blood cells and the active erythroprotective phytoconstituents present in the extracts and fractions of *A. altilis* and *S. alata* or alteration of the erythrocytes cellular surface charges thereby preventing the interaction of the blood cells with aggregating agents of haemolysis (Oyedapo *et al*., 2010) [42]. These plant extracts therefore offer a way of arresting the release of lysosomal enzymes of activated neutrophils in wounds which is responsible for tissue deterioration by stabilizing the erythrocyte membranes, thereby preventing haemolysis. This also shows the ability of the plant extracts and fractions to stabilize the erythrocytes membranes and therefore could be a way of protecting the blood cells from erythrocytes degrading microbial infections mediated by coagulase negative *Staphylococcus aureus* and haemolytic *Streptococcus* species, both of which have been found to be main wound colonisers (Tucker *et al*., 2015; Attiq *et al*., 2018) [43, 44].

This study highlights the antioxidant and membrane stabilising potentials of *Artocarpus altilis* stem bark and *Senna alata* leaf and validates the ethnomedicinal use of the plants as remedies for free radicals implicated human diseases.

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5. Declaration of Competing Interests

The authors declare no conflicts of interest regarding this article.

6. Author's Contribution

Conceptualisation, Study design, and Administration of Research (JMA), Data curation, Methodology, Investigation of Experimental work, Interpretation and Analysis of Results and Writing the original draft (SBO), Supervision (FOO).

7. Abbreviations

DCM, dichloromethane; EtOAc, ethyl acetate; TMPs, traditional medical practitioners; r.t., room temperature; TLC, thin layer chromatography; NaCl, sodium chloride; $FeSO_4$, iron (II) sulphate; EDTA, ethylene diamine tetraacetic acid; H_2O_2, hydrogen peroxide; HCl, hydrochloric acid; $FeCl_3.6H_2O$, iron (III) chloride hexahydrate.

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