

In Vitro* Evaluation of Antioxidant and Anti-inflammatory Properties of Methanol and Dichloromethane Extracts of the Leaf of *Globimetulaoreophila

ABSTRACT

Aims: The study was designed to investigate the antioxidant and membrane stabilizing potentials of the leaf of *Globimetulaoreophila*, with a view to providing scientific support for the acclaimed ethnomedicinal or traditional uses of the plant.

Methodology: The study involved collection, identification, drying, pulverizing, extraction and fractionation of extracts of the dried leaves of *G. oreophila*. Anti-inflammatory and antioxidant activities of the extracts and fractions were evaluated using red blood cell membrane stabilization technique, while assays (2,2-diphenyl-1-picrylhydrazyl hydrate, nitric oxide, total antioxidant capacity and ferric reducing antioxidant power)

Results: The results revealed that the extracts and the fractions of *G. oreophila* exhibited potent antioxidant activities. Fraction 1 of the methanol extract exhibited the highest antioxidant activity with IC_{50} of $45.83 \pm 0.13 \mu\text{g/ml}$ while fraction 7 of the same extract was the most potent in stabilizing stressed red blood cells having $100 \pm 0.00\%$ membrane stability. The activities of both the fractions and the extracts of *G. oreophila* compared favourably with the standard antioxidant and anti-inflammatory drugs. Moreover, the activities of the fractions and extracts are concentration dependent.

Conclusion: The study revealed that the extracts and fractions of *G. oreophila* possess and exhibit anti-inflammatory and antioxidant activities which were concentration dependent and compared favourably with those of nonsteroidal anti-inflammatory drugs.

Keywords: antioxidant; membrane stabilization; anti-inflammatory; red blood cells; *G. oreophila*

1. INTRODUCTION

Medicinal plants have been demonstrated to be the valuable treasures which the nature has bestowed on mankind for food and medicine. In the scientific community, the diverse medicinal values

of these plants have aroused the interests of researchers across various disciplines all over the world. The plant *G. oreophila* (Loranthaceae family) has the potential of providing lead and new drugs that could be employed to combat the menace of cardiovascular diseases such as cancer, heart attack, hypertension, diabetes and infectious diseases [1, 2]. It is acclaimed by the traditional health practitioners in Nigeria that the extracts of this plant are employed in the prevention, treatment and management of cardiovascular related diseases and ailments.

Distinctively, the constituents of most European and Asian mistletoes are proteins, viscotoxins, lectins and carbohydrates. The low molecular weight compounds including flavonoids and phenylpropanoids of varying structural types which contribute to the antioxidative properties of various *Loranthaceae* extracts have been examined. Many reports of the presence of lower molar mass compounds like alkaloids, flavonoids, tannins and other plant constituents in the African *Loranthaceae* have not been substantiated by isolation and proper identification [3]. Faboro *et al.*, 2016 [4] carried out a phytochemical profile of dichloromethane and methanol extracts as their TMS and FAME derivatives using GC-MS on *G. oreophila*. There were thirty four (34) compounds detected which have not been reported in any other mistletoe species. They are: cis-dihydro-3,4-bis[TMS2]-2(3H)-furanone, dodecanol, octadecanol, nonacosane, pentacosanol, hexatriacontane, octacosanol, triacontanol, dotriacontanol, cis-dihydro-3,4-bis[(trimethylsilyl)oxy]-2(3H)-furanone, butanedioic acid, glyceric acid, fumaric acid, 3,4-dihydroxybutanoic acid, malic acid, tetrahydroxybutane, 2,3,4-trihydroxybutanoic acid, tartaric acid, arabinol, ribofuranose, isocitric acid, inositol, hexo-pyranose, inosose, methyl dimethoxyhydroxybenzoate, 3,5,11,15-tetramethyl-2-hexadecen-1-ol, pentadecanoic acid, cis and trans phytol, eicosanoic acid, hexatriacontane, tricosanoic acid, pentacosanoic acid, nonacosane and hexacosanoic acid. These phytoconstituents were distributed among the following classes of organic compounds: ketones, monoarylphenolics, sugars, dicarboxylic acids, sugar acids, alcohols, cyclic compounds, hydroxycarboxylic acids, sugar alcohols, steroids and fatty acids. It was the first time this type of studies would be carried out on *G. oreophila*. Although, pharmacological tests have been carried out on *G. braunii* [5, 6, 7] which is closely related to *G. oreophila*, such tests have not been carried out on *G. oreophila*.

64 The present study is aimed at evaluating the antioxidant and membrane stabilizing activities of the
65 extracts and fractions of the leaves of *G. oreophila*, to establish the scientific evidence for the use of
66 the plant as anti-inflammatory agent as acclaimed by the traditional healers. In addition, there is
67 dearth of scientific information on the biological and chemical activities of the extract, fraction and
68 bioactivity of the plant from the science community so this study could serve as a baseline for further
69 study on the plant.

70 **2.0: Materials and Methods**

71

72 **2.1: Materials**

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74 **2.2: Collection and Identification of Plant Material**

75 Fresh leaves of *G. oreophila* were collected from Iwo, Osun State, Nigeria. Iwo is located on latitude
76 7.6292° N and longitude 4.1872° E. The leaves were identified and authenticated by Mr. Gabriel
77 Ibhanesebhor (a Taxonomist, the man in charge of the Herbarium), Department of Botany, Obafemi
78 Awolowo University, Ile-Ife, Nigeria, and a voucher specimen was deposited at IFE Herbarium (ID no.
79 17089)

80

81 **2.3: Reagents and Chemicals**

82 All the reagents and chemicals used were of analytical grades, and were purchased from various
83 sources. Dichloromethane, methanol, ethyl acetate, 2,2-diphenyl-1-picrylhydrazyl hydrate, sodium
84 nitroprusside, sulfanilamide, phosphoric acid, 2,4,6-tri-(2-pyridyl)-1,3,5-triazine, sodium phosphate,
85 ammonium molybdate, N-1-naphthylethylenediamine dichloride, ferric chloride from Sigma-
86 Aldrich Chemical Company. Potassium iodide, sulphuric acid, trisodium citrate, ascorbic acid
87 and trisodium citrate were purchased from British Drug House (BDH). All solutions, buffers and
88 reagents were prepared using glass distilled water. Diclofenac (standard anti-inflammatory drug) was
89 purchased from the Pharmacy Shop at the main Campus of Obafemi Awolowo University, Ile-Ife,
90 Nigeria.

91 **2.4: Methods**

92 **2.4.1: Preparation of Plant materials and Extraction**

93 Collected fresh leaves of *G. oreophila* were rinsed with clean water, drained, air-dried for 2-3 weeks in
94 shade and pulverized into powdered form using a domestic blender. The powdered plant material was
95 packed into an air-tight plastic container until extraction.

Powdered plant material (500 g) was extracted using dichloromethane (3L) and methanol separately overnight in a large Soxhlet apparatus. The extracts were concentrated under reduced pressure to afford dichloromethane and methanol respectively. Both extracts were weighed and kept in the desiccator until used for biochemical analyses. The weight and percentage yields of DCM extract was 19.4 g which represented 3.88% of the starting material. The weight of MeOH extract was 18.2 g which represented 3.64% of the starting plant material.

The extracts were fractionated on Silica gel 60 (40-60 mesh size) column using gradient solvent mixtures petroleum ether/ethyl acetate. Typically, each extract (1 g) was loaded onto the packed Silica gel 60 column and washed with petroleum ether. The elution was conducted sequentially with petroleum ether/ethyl acetate, 1:1v/v; 3:7v/v; ethyl acetate (100%); ethyl acetate/methanol (1:1v/v); and methanol (100%). Fractions (15 ml) were collected at a flow rate of 1 ml/min. A total of 78 fractions were collected for DCM extract and 65 fractions for methanol extract. Various fractions were chromatographed on pre-coated silica gel F254 plates. The plates were air dried, stained with specific staining reagent, p-anisaldehyde stain and heated at 105°C for spot development. The R_f values were calculated and the fractions were pooled together based on their R_f values as follows: F1 (fraction 1), F2 (fractions 2 and 3), F3 (fractions 4 and 5), F4 (fractions 6-12), F5 (fractions 13-48), F6 (fractions 49-57), F7 (fractions 58-63), F8 (fraction 64), F9 (fractions 65-67) and F10 (fractions 68-78) giving ten (10) fractions for DCM and were labelled as GDF1-F10 respectively. Similarly, the fractions for MeOH were pooled together as follows: F1 (fractions 1-4), F2 (fractions 5-10), F3 (fractions 11-28), F4 (fractions 29-36), F5 (fractions 37-49), F6 (fractions 50-52), F7 (fractions 53-57) and F8 (fractions 57-65) giving eight (8) fractions for MeOH extract were labelled as GMF1-F8 respectively.

2.5: Biochemical Analyses

2.5.1: Evaluation of Antioxidant Potentials of Extracts and Fractions of *G. oreophila*

The antioxidant potential of the extracts and the fractions *G. oreophila* were evaluated using four different antioxidant assays: (2, 2-diphenyl-1-picrylhydrazyl hydrate) DPPH assay; nitric oxide, NO assay; total antioxidant capacity, TAC assay and ferric reducing antioxidant power, FRAP assays.

2.5.1.1: Assay of DPPH – Radical Scavenging Activity

The DPPH radical scavenging ability of the extracts and the fractions were determined using the stable radical DPPH (2, 2-diphenyl-1-picrylhydrazyl hydrate) and the concentration that inhibits 50% the DPPH free radical was estimated according to the procedure of Blois, 1958 [8] as slightly modified

(Brand-Williams, 9). Typically, to 1ml of different concentrations (0.0, 0.3125, 0.625, 1.25, 2.5, 5, 10 µg/ml) of the extract and standard in various test tubes were added 0.3mM DPPH (1 ml) in methanol. The reaction mixture was mixed and incubated in the dark for 30 min., after which the absorbance was read at 517 nm against the blank. The standard drug ascorbic acid (AA) 1 µg/ml was employed and treated as the test extracts.

The percentage DPPH inhibition was calculated as:

$$\% \text{ Inhibition} = \frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \times 100$$
 where A_{blank} is the absorbance of the control reaction

(containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. The concentration of the sample that caused 50% inhibition (IC_{50}) was extrapolated from the graph inhibition percentage against extract concentration.

2.5.1.2: Assay of Nitric oxide (NO) Radical Scavenging Activity

The inhibition of nitric oxide radical activity of the extract was carried out according to the earlier method [Green 10] as modified Marcocci [11]. Nitric oxide, generated from sodium nitroprusside in aqueous solution at physiological pH, interacts with oxygen to produce nitrite ions which was measured by Griess reaction.

The reaction mixture, consisted of 0.1 ml of different concentrations of the tested extracts/fractions (0.3125, 0.625, 1.25, 2.5, 5, 10 µg/ml) and 0.9 ml of sodium nitroprusside (2.5 mM) in phosphate buffered- saline was incubated under illumination for 2½ hr. This was followed by the addition of 1% (w/v) sulphanilamide in 5% (v/v) phosphoric acid (0.5 ml) incubated further in the dark for 10 min., and 0.5 ml 0.1% (w/v), N-1-naphthylethylenediamine dihydrochloride (0.5 ml) was added. The absorbance of the chromophore formed was measured at 546 nm against the reagent blank. The percentage inhibition of nitric oxide radical scavenging was calculated as expressed above in DPPH radical scavenging assay [12, 13].

2.5.1.3: Evaluation of Total Antioxidant Capacity (TAC)

The evaluation of TAC of extracts/fractions was based on the reduction of Molybdenum (VI) to Molybdenum (V) by the extract and the subsequent formation of a green phosphate/Molybdenum (V) complex at an acidic pH as earlier reported [Prieto 14]. To 0.1 ml of the extract/fraction or standard solution of ascorbic acid (0, 20, 40, 60, 80, 100 µg/ml) was added 1ml of the reagent solution (0.6 M

155 sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was
156 incubated in a water bath at 95 °C for 2½ hr and allowed to cool to room temperature and the
157 absorbance was measured at 695 nm against reagent blank. The antioxidant activities of the extracts
158 were expressed as an ascorbic acid equivalent.

161 2.5.1.4: Evaluation of Ferric Reducing Antioxidant Power (FRAP)

162 The evaluation of ferric reducing antioxidant power (FRAP) of the extracts/fractions was carried out as
163 reported [15] with slight modification. The extract/fraction (50 µl) and standard (50 µl) (20, 40, 60, 80,
164 100 µg/ml) was added to 1 ml of FRAP reagent. The absorbance measurement was read at 593 nm
165 exactly 10 minutes after mixing against reagent blank that contained distilled water. The reducing
166 power was expressed as ascorbic acid (mg/g AAE).

168 2.6: Evaluation of Anti-inflammatory Properties

169
170 The evaluation of anti-inflammatory activity of the extracts and the fractions of the leaf of
171 *G. oreophila* was based on red blood cell membrane stabilizing activity that was exposed to both heat
172 and hypotonic induced lyses as previously described [Oyedapo *et al.*, 16]. The extracts and fractions
173 with high antioxidant activities were selected for the membrane stability assay.

174 2.6.1: Preparation of Bovine Erythrocytes

175 Bovine erythrocyte was prepared according to the procedure reported by [Oyedapo and Famurewa,
176 17]. Fresh blood sample was collected into anticoagulant bottle containing trisodium citrate (3.8% w/v)
177 and mixed thoroughly to prevent lysing. The blood was poured into clean centrifuge tubes and
178 centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed with sterilized Pasteur
179 pipette. The packed rbc (erythrocytes) was re-suspended in fresh isosaline, mixed gently and
180 centrifuged at 3000 rpm for 10 min. The process was repeated five more times until a clear
181 supernatant was obtained. Then, 2% (v/v) erythrocyte suspension was prepared by diluting 2 ml of
182 packed red blood cells to 100 ml with normal saline.

183 2.6.2: Assay of Red Blood Cell Membrane Stabilizing Activity

184 The membrane stabilizing activity of the extracts and fractions of *G. oreophila* was assayed according
185 to a modified procedure [Oyedapo *et al.*, 16]. Varying concentrations 0 - 300 µg/mL of the extracts,

fractions and the standard drug were prepared. The assay mixture consisted of 1.0 ml hyposaline (0.42% w/v NaCl), 0.5 ml of 0.1 M sodium phosphate buffer, pH 7.2 (0.5 ml), varying volumes of drug sample and isosaline (0.85% w/v NaCl) to make the mixture up to 2.5 ml and followed by the addition of 2% (v/v) bovine erythrocyte suspension (0.5 ml). The drug control was pipetted as above but without 2% (v/v) erythrocyte suspension while the blood control contained all the reagents, extracts and fractions. Diclofenac (1 mg/ml) was used as the standard drug. The reaction mixtures were incubated at 56 °C for 30 min on a water bath, cooled to room temperature under running water, and followed by centrifugation at 3000 rpm on Gallenkamp UK Bench Centrifuge for 10 min. at room temperature. The absorbance of the released hemoglobin was read at 560 nm.

The percentage membrane stability was estimated using the expression:

$$100 - \frac{(\text{Abs of Test Drug} - \text{Abs of Drug Control})}{\text{Abs of Blood Control}} \times 100$$

The blood control represents 100% lyses or zero percent stability.

2.6.3: Statistical Analysis

Each value represented the mean $\pm$ SEM of three consistent readings. Significance difference were analyzed using student's t-test with $p < 0.05$ as statistical significant

3.0: Results and Discussion

The study reported the antioxidant and anti-inflammatory potentials of the leaf extracts of *G. oreophila* with a view to providing scientific support for the use of the plant on the prevention, treatment and management of oxidant and inflammatory disorders. The weight and percentage yields of DCM extract was 19.4 g which represented 3.88% of the starting material. The weight of MeOH extract was 18.2 g which represented 3.64% of the starting plant material.

3.1: Biochemical Analyses

3.2: Antioxidant Assays.

3.2.1: Assay of DPPH – Radical Scavenging Activity of DCM Extracts and Fractions

In Table 1 is the summary of antioxidant activities of DCM extract and fractions of *G. oreophila*. It was observed that the extracts and the fractions exhibited potent and appreciable antioxidant activities. These were demonstrated in their ability to react with the stable free radical, thus the odd electrons of

218 DPPH radical gave a strong absorption band at 517 nm in the visible spectroscopy (deep violet
219 colour). As the electron became paired off in the presence of a free radical scavenger, the absorption
220 vanishes and the resulting decolourised product is stoichiometric with the number of electron taken
221 up. The ability was also demonstrated in their percentage inhibition which is concentration dependent.
222 At the highest concentration, the DCM extract (GODE) has a percentage inhibition of 51.97% with an
223 IC_{50} value of 910 $\mu\text{g/ml}$. In a similar way the percentage inhibition of the fractions showed a dose
224 dependent trend with fraction 8 (GDF8) having the highest percentage of 71.92% and an IC_{50} value of
225 490 $\mu\text{g/ml}$. The IC_{50} of fractions GDF1, GDF2, GDF3, GDF4, GDF5 and GDF9 were not determine
226 because the values were well over 1000 $\mu\text{g/ml}$ which cannot be determined by extrapolation.

227 **Table 1:**Antioxidant Activities in DPPH test of DCM Extract and the Fractions of *G. oreophila*.
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Conc(µg/ml)	Percentage Inhibition							
	GO1	GDF1	GDF2	GDF3	GDF4	GDF5	GDF8	GDF9
1000	51.97 ± 1.57	11.63 ± 1.23	10.07 ± 0.72	26.14 ± 0.69	35.96 ± 0.98	38.52 ± 1.42	71.92 ± 1.19	36.52 ± 2.70
500	40.21 ± 1.49	8.01 ± 1.03	10.01 ± 0.74	25.08 ± 0.70	25.39 ± 0.77	34.77 ± 1.27	67.54 ± 1.32	26.77 ± 0.88
250	26.70 ± 2.03	7.44 ± 0.88	8.26 ± 0.22	15.76 ± 0.66	16.26 ± 0.80	22.89 ± 0.41	42.71 ± 0.95	16.20 ± 0.45
125	24.95 ± 1.70	5.82 ± 0.66	6.13 ± 0.33	11.44 ± 1.13	15.95 ± 0.50	15.95 ± 0.30	27.64 ± 0.45	7.38 ± 0.38
62.5	22.89 ± 1.68	4.88 ± 0.94	4.88 ± 0.50	7.44 ± 0.38	11.01 ± 0.88	12.82 ± 0.51	19.14 ± 0.57	6.50 ± 0.74
31.25	22.51 ± 1.88	4.19 ± 0.78	3.38 ± 0.32	8.26 ± 0.68	9.82 ± 0.74	12.13 ± 0.16	9.82 ± 0.29	3.56 ± 0.91
IC ₅₀ (µg/ml)	910 ± 50	NA	NA	NA	NA	NA	490 ± 10	NA

229
 230 GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.
 231 Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.
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The MeOH extract and its fractions were subjected to the same assay. The percentage inhibition of the extract is 75.42% and the IC₅₀ value of 140 µg/ml. All the fractions exhibited appreciable percentage inhibition with the exception of fraction 1 (GMF1) that shows negligible percentage inhibition and with no IC₅₀ value Table 2. The observation might be due to the fact that plant constituents respond differently to different assays [18]. On the other hand fraction 7 (GMF7) has the highest antioxidant activity having 79.42% and 140 µg/ml as percentage inhibition and IC₅₀ value respectively.

Table 2: Antioxidant Activities in DPPH test of MeOH Extract and the Fractions of *G. oreophila*.

Conc(µg/ml)	Percentage Inhibition				
	GO2	GMF1	GMF2	GMF7	GMF8
1000	75.42 ± 0.11	3.69 ± 0.27	70.98 ± 0.72	79.42 ± 0.72	76.92 ± 0.94
500	70.61 ± 1.09		63.85 ± 2.33	78.49 ± 0.41	75.42 ± 2.50
250	64.92 ± 1.89		42.09 ± 2.06	75.86 ± 0.91	71.92 ± 2.27
125	54.47 ± 1.66		27.33 ± 2.31	49.41 ± 3.87	60.48 ± 4.42
62.5	44.97 ± 0.35		12.01 ± 1.21	31.96 ± 1.97	47.53 ± 1.55
31.25	38.09 ± 2.95		2.94 ± 1.44	15.45 ± 2.35	28.33 ± 3.80
IC ₅₀ (µg/ml)	91.10 ± 10	NA	358.16 ± 10	140 ± 10	110 ± 30

GO2: MeOH Extract; GMF1, 2, 7, 8; MeOH Fractions
Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.

3.2.2: Assay of Nitric oxide (NO) Radical Scavenging Activity of DCM Extracts and Fractions

The antioxidant content of medicinal plants may play a vital role in the defense against diseases [19]. Another assay used to evaluate the free radical scavenging potential is NO. The percentage inhibition obtained for DCM extract (GODE) was 59.00% with an IC₅₀ of 250 µg/ml. All the fractions exhibited antioxidant activities with fractions 1 and 2 (GDF1 and GDF2) that showed extremely low percentage inhibition of 5.7% and 10.75% respectively with no IC₅₀ values Table 3. Again fraction 8 has the highest percentage inhibition of 65.20% and IC₅₀ of 420 µg/ml.

255 **Table 3:** Antioxidant Activities in NO test of DCM Extract and the Fractions of *G. oreophila*.

Conc(µg/ml)	Percentage Inhibition							
	GO1	GDF1	GDF2	GDF3	GDF4	GDF5	GDF8	GDF9
1000	59.00 ± 2.16	5.74 ± 0.16	10.75 ± 0.23	49.07 ± 0.91	22.91 ± 1.13	41.62 ± 0.26	65.20 ± 0.11	47.67 ± 0.29
500	55.12 ± 0.88			48.35 ± 1.21	18.05 ± 0.08	22.91 ± 0.18	54.03 ± 0.69	35.57 ± 0.00
250	49.53 ± 2.41			41.37 ± 0.29	12.87 ± 1.50	15.93 ± 0.15	43.43 ± 0.37	26.27 ± 0.66
125	35.83 ± 0.77			34.49 ± 2.74	10.50 ± 1.72	10.65 ± 0.58	14.01 ± 0.66	23.37 ± 0.37
62.5	29.01 ± 0.11			16.91 ± 2.60	8.17 ± 0.80	4.86 ± 0.26	6.05 ± 0.33	13.60 ± 0.33
31.25	18.30 ± 1.54			9.41 ± 2.41	0.46 ± 0.26	3.52 ± 0.95	2.69 ± 0.07	9.31 ± 0.22
IC ₅₀ (µg/ml)	250 ± 20	NA	NA	NA	NA	NA	420 ± 10	NA

256

257 GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.

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259 Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.

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Similarly the MeOH extract and its fraction showed a dose dependent activity with fraction 8 (GMF8) having 90.07% and IC₅₀ 440 µg/ml respectively. Fraction 1 (GMF1) showed the highest antioxidant activity based on the IC₅₀ of 45 µg/ml. Fraction 7 also showed an appreciable activity of 72.08% percentage inhibition and IC₅₀ 250 µg/ml respectively Table 4.

It is a known fact that plant phenolic compounds, also called polyphenols, are a large and diverse class of compounds with one or more aromatic rings bearing hydroxyl substituent(s) and have an antioxidant potential due to their possibility to act as radical scavengers or free radical terminators. Antioxidant activity is significantly correlated with phenolic and flavonoid contents of plant materials [20]. These findings are in agreement with the phytoconstituents obtained by the GC-MS analysis of the extracts where phenolic compounds were detected [4]

Table 4: Antioxidant Activities in NO test of MeOH Extract and the Fractions of *G. oreophila*.

Conc(µg/ml)	Percentage Inhibition				
	GO2	GMF1	GMF2	GMF7	GMF8
1000	65.77 ± 0.15	85.09 ± 2.45	30.56 ± 0.26	72.08 ± 1.24	90.07 ± 0.15
500	53.36 ± 1.17	75.13 ± 0.69	25.08 ± 1.13	64.89 ± 3.77	67.63 ± 2.71
250	28.90 ± 0.34	68.98 ± 0.15	13.44 ± 0.29	51.24 ± 0.04	39.14 ± 0.13
125	24.25 ± 1.65	60.91 ± 3.22	9.72 ± 0.29	48.14 ± 1.50	19.18 ± 0.11
62.5	17.32 ± 0.26	55.53 ± 1.54	7.55 ± 0.29	39.25 ± 0.11	8.89 ± 0.77
31.25	4.60 ± 1.43	43.43 ± 0.00	6.20 ± 0.22	26.16 ± 0.22	7.81 ± 0.48
IC ₅₀ (µg/ml)	472.12 ± 10	45.83 ± 0.13	NA	250 ± 20	440 ± 30

GO2: MeOH Extract; GMF1, 2, 7, 8; MeOH Fractions.

Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.

The MeOH extract (GOME) and fractions (GMF) in the DPPH and the NO assays showed more reasonable antioxidant activities when compared to the DCM extract and its fractions. We could make this statement, because in our previous studies of GC-MS analysis on this plant the MeOH extract contained more polyhydroxyl compounds than the DCM extract [4]. It is also a known fact that polyhydroxyl compounds such as flavonoids are good antioxidants. In Table 5 is the antioxidant activities of the standard used, ascorbic acid.

287 **Table 5: Antioxidant Activities of the Standard, Ascorbic Acid.**

DPPH		NITRIC OXIDE	
Conc (µg/ml)	Percentage Inhibition	Conc (µg/ml)	Percentage Inhibition
10	40.05 ± 1.89	125	86.86± 2.50
5	25.65 ± 1.67	62.5	52.86 ± 2.63
2.5	12.00 ± 1.62	31.25	34.98± 1.14
1.25	10.01 ± 0.95	15.625	16.61± 0.75
0.625	9.39 ± 0.91	7.8125	8.065± 0.58
0.3125	6.20 ± 0.25		
IC ₅₀ (µg/ml)	12.66± 0.01	IC ₅₀ (µg/ml)	4.50 ± 0.12

288 Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically
 289 significant.

291 3.2.3: Evaluation of Total Antioxidant Capacity (TAC) of DCM Extracts and Fractions

292 The concept of total antioxidant capacity (TAC), which originated from chemistry and then was applied
 293 to biology and medicine, and further to nutrition and epidemiology [21] was also used to assess the
 294 antioxidant activities the extracts and fractions as shown in Tables 6 and 7. The extract and the
 295 fractions possess some antioxidant activities. Fractions 4 and 8 (GDF4 and GDF8) with 4.55 and 5.11
 296 mgAAE/g showed the highest antioxidant activities when compared to the standard. In the manner,
 297 the extract and its fractions were subjected to the same TAC evaluation. In this evaluation it was only
 298 the fraction 2 GMF2 that showed an appreciable activity with 5.84 mgAAE/g when compared with the
 299 standard Table 7. The numerous available TAC assays correlate poorly with each other because
 300 various antioxidants react differently in each assay [17]. For example, the results of some assays
 301 include a substantial contribution from protein thiols, which do not react at all in other methods.
 302 Therefore, to interpret the results it is essential that one understands fully the relative contribution of
 303 the individual antioxidants to the methodology being used. Again, the total antioxidant capacity of a
 304 solution will depend on the nature of the oxidative abuse. TAC assays measure antioxidant capacity
 305 only in the defined conditions of the particular technique used.

306 3.2.4: Evaluation of Ferric Reducing Antioxidant Power (FRAP) of DCM Extracts and Fractions

307 Possibly damaging reactive oxygen species (ROS) are produced as a consequence of normal aerobic
 308 metabolism. These “free radicals” are usually removed or inactivated in vivo by a squad of

309 antioxidants Individual members of the antioxidant defense team are deployed to prevent generation
310 of ROS, to destroy potential oxidants, and to scavenge ROS. In this perspective, antioxidant power
311 may be referred to analogously as reducing ability [22]. With this in mind, a method using reductants
312 in a redox-linked colorimetric method employing an easily reduced oxidant in stoichiometric excess
313 could offer a simple way of assessing this ability hence the use of FRAP to assess the reducing power
314 of the extracts and the fractions in this study. The DCM extract (GODE) and fractions 3, 5 and 8
315 (GDF1, GDF3 and GDF8) showed appreciable antioxidant activities with 622, 603 and 752 mgAAE/g
316 when compared with the standard of 826 mgAAE/g Table 6. Likewise the extract, (GME) exhibited
317 potent antioxidant activity with 673 mgAAE/g compared to the standard. Fractions 2 and 7 showed
318 reasonable activities Table 7. This is in agreement to the observation of Young, [23]. This
319 investigation is similar to the research of other investigators who worked on similar species such as
320 *Globimetulabraunii* [24, 25], *Globimetulacupulata* [26].

321

322 **Table 6: TAC and FRAP Assays of DCM Extract and the Fractions of *G. oreophila***

Assays	Extracts and Fractions								
	Rutin (Std)	GO1	GDF1	GDF2	GDF3	GDF4	GDF5	GDF8	GDF9
TAC (mg AAE/g)	8.34 ± 0.01	3.73 ± 0.15	0.43 ± 0.11	3.07 ± 0.18	1.57 ± 0.14	4.55 ± 0.06	3.00 ± 0.44	5.11 ± 0.13	1.75 ± 0.09
FRAP (mg AAE/g)	826.56 ± 0.29	622 ± 11.6	70.7 ± 1.45	404 ± 4.90	603 ± 8.29	438 ± 18.9	640 ± 20.9	752 ± 7.40	394 ± 14.4

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324 **Table 7: TAC and FRAP Assays of MeOH Extract and the Fractions of *G. oreophila***

Assays	Extract and Fractions					
	Rutin (Std)	GO2	GMF1	GMF2	GMF7	GMF8
TAC (mg AAE/g)	8.34 ± 0.01	1.04 ± 0.09	1.97 ± 0.24	5.84 ± 0.05	1.59 ± 0.09	0.04 ± 0.01
FRAP (mg AAE/g)	826.56 ± 0.29	672 ± 16.2	346 ± 4.01	599 ± 24.7	549 ± 7.31	447 ± 7.89

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3.3: Membrane Stabilization Profiles of *G. oreophila* Extracts and Fractions

Membrane stabilization is regarded as a possible mechanism of action of the anti-inflammatory activity of certain medicinal plants [27, 28]. This is confirmed by several investigations that herbal preparation and their mixtures were capable of stabilizing the red blood cell membrane and exerting anti-inflammatory activity [29, 30].

Erythrocytes have been used as a model system by numerous researchers for the study of interaction of drugs with membranes [31, 32, 33]. When the RBC is subjected to hypotonic stress the release of hemoglobin (Hb) from RBC is prevented by anti-inflammatory agents because of membrane stabilization. So, the stabilization of RBC membrane by drugs against hypotonicity induced haemolysis serves as a useful in vitro method for assessing the anti-inflammatory activity of various compounds [34].

GO1DE, GDF8, GO2ME, GMF1, GMF2, GMF7 and GMF8 were selected for stability assays based on their antioxidant potency as depicted in Tables 1-4.

Figures 1a - g depicts the profiles of the membrane stabilizing potentials of (DCM and MeOH) and their various fractions of bovine red blood cells exposed to both heat and hypotonic induced lyses. It was observed that all the extracts and fractions exhibited appreciable and significant membrane stabilizing activities. The activities are mixtures of both monophasic and biphasic mode of protection; and were also observed to be concentration dependent and compared favourable with diclofenac, a potent non-steroidal anti-inflammatory drug (NSAIF). Fig. 2.

Seven samples were assayed for red blood cell membrane stabilizing activities from *G. oreophila*. Two extracts DCM and MeOH, (GO1DE, GO2ME), one fraction from DCM extract GDF8, and four fractions from the MeOH extract (GMF1, GMF2, GMF7 and GMF8). The DCM extract (GO1DE), with a biphasic response at only one concentration, exhibited a minimum activity stability of $44.07 \pm 0.01\%$ and maximum activity of $51.71 \pm 0.01\%$ at concentrations of 1.0 and 0.5 mg/mL respectively (Figure 1a). The extract protected better at lower concentration (0.5 mg/mL) than higher concentration (1.0 mg/mL). The fraction (GDF8), from the DCM extract showed a biphasic (Figure 1b) mode of protection at 0.5, 1.0, and 1.5 mg/mL while at higher concentration (2.0 mg/mL) did not stabilize bovine red blood cells. However the minimum membrane stability exerted by this fraction was $6.87 \pm 0.01\%$ and maximum activity of 51.55 ± 0.06 . It was observed that the fraction protected better at lower concentration compared to higher concentrations. The MeOH extract (GO2ME), (Figure 1c)

exerted a minimum membrane stability of $5.88 \pm 0.01\%$ and maximum stability of $68.47 \pm 0.00\%$ at 1.0 and 1.5 mg/mL respectively. It's mode of protection was biphasic at all the concentrations tested. The membrane stability profile of the fraction (GMF1) from the MeOH extract of *G. oreophila* is depicted in (Figure 1d). A biphasic mode of protection was observed in all the concentration tested with a minimum and maximum membrane stability of $2.28 \pm 0.01\%$ and $31.59 \pm 0.00\%$ at 0.5 and 1.5 mg/mL respectively. Observation showed that GMF1 protected better at higher concentration (1.5 mg/mL). Figure 1e showed the profile of GMF2 and it was noticed that the fraction did not stabilize bovine red blood cells at all the concentrations tested except at 1.5mg/mL where it exerted maximum percentage membrane stability of $72.97 \pm 0.00\%$ and minimum of $18.41 \pm 0.02\%$. The fraction GMF7 (Figure 1f) stabilizes red blood cells membrane better at higher concentrations the mode of protection was biphasic at all concentrations used. The fraction did not stabilize bovine red blood cells at 0.5 mg/mL which is the lowest concentration. The minimum membrane stability is 9.00% and 100 $\pm$ 0.00% is the maximum membrane stability at a high concentration of 2.0 mg/mL. The fraction GMF8 (Figure 1g) stabilized bovine red blood cells at all the concentrations tested with a minimum and maximum membrane stability of $8.20 \pm 0.00\%$ and $52.17 \pm 0.01\%$ at 1.0mg/mL and 2.0 mg/mL respectively and the mode of protection was biphasic all through.

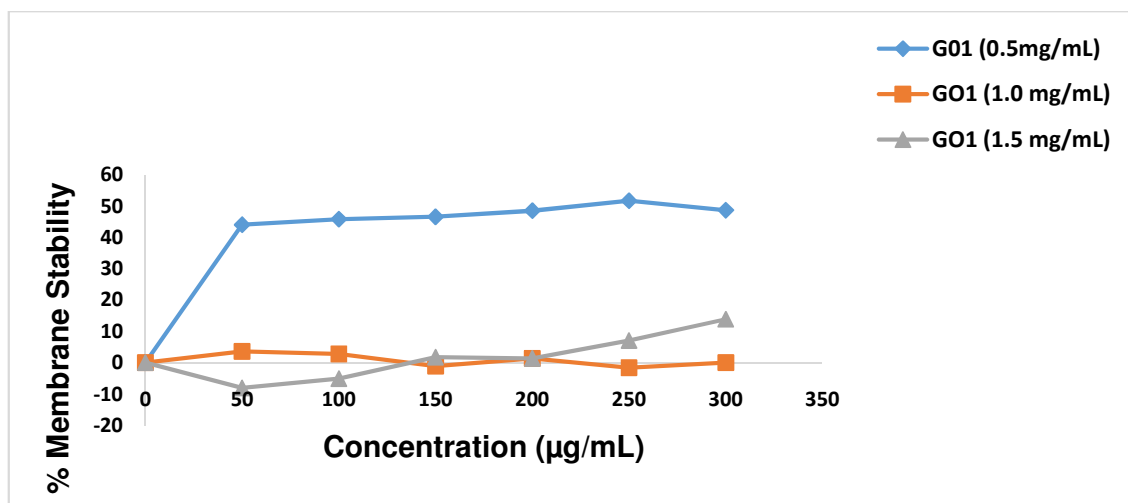


Figure 1a: Membrane stabilizing profiles of DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

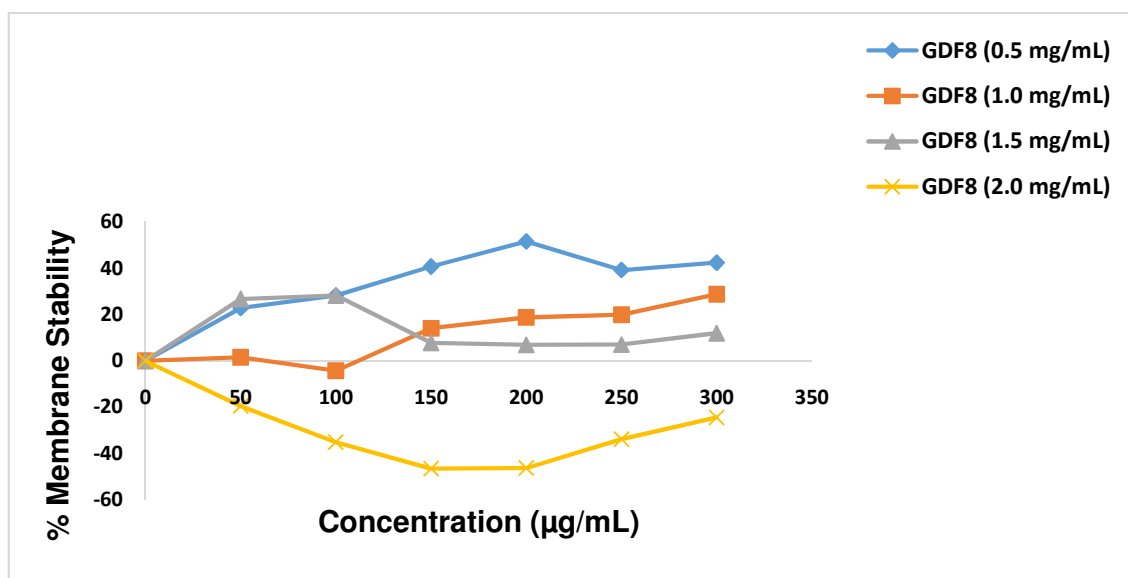


Figure 1b: Membrane stabilizing profiles of fraction 8 (GDF8), DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

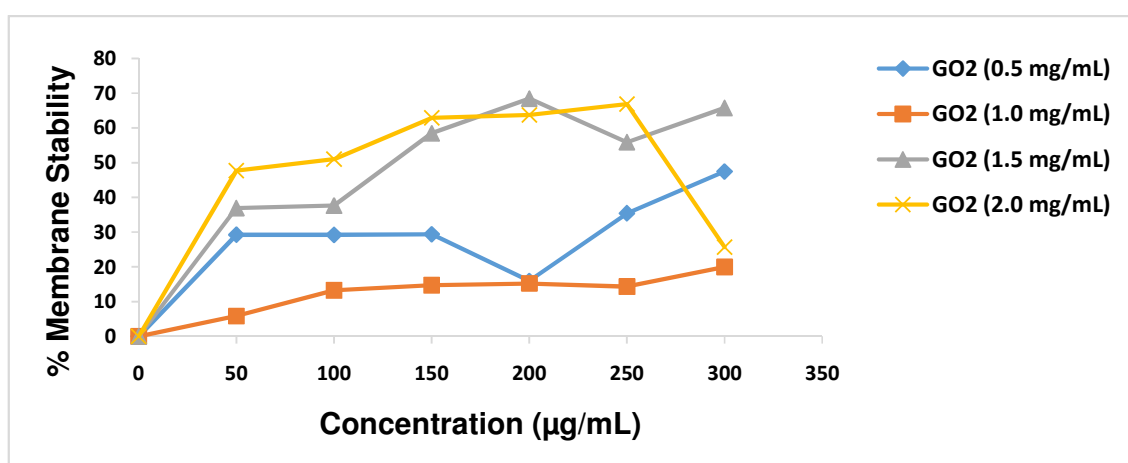


Figure 1c: Membrane stabilizing profiles of MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

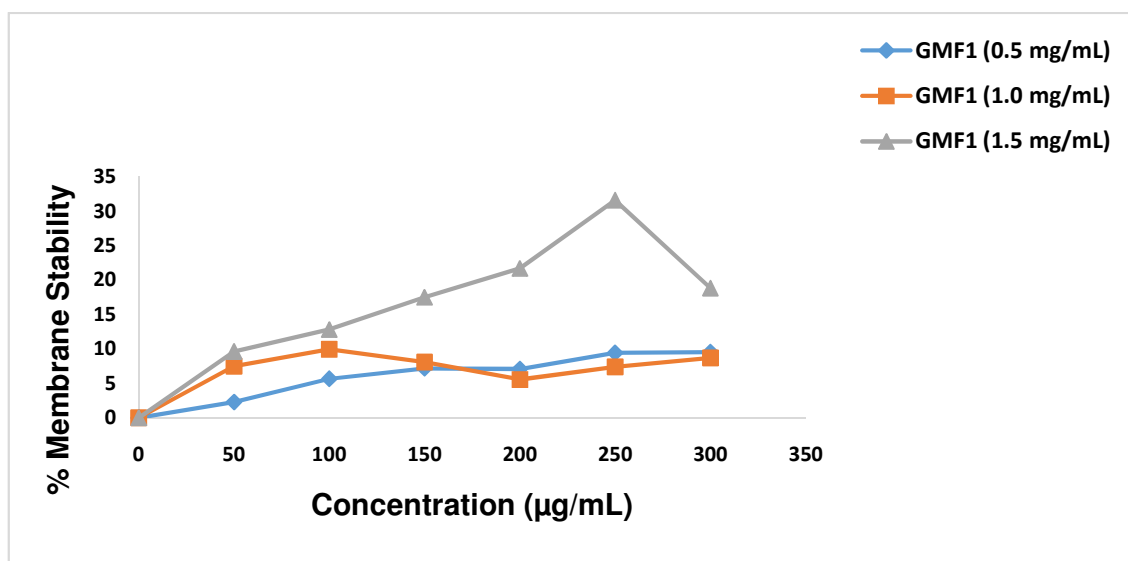


Figure 1d: Membrane stabilizing profiles of fraction 1 (GMF1), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

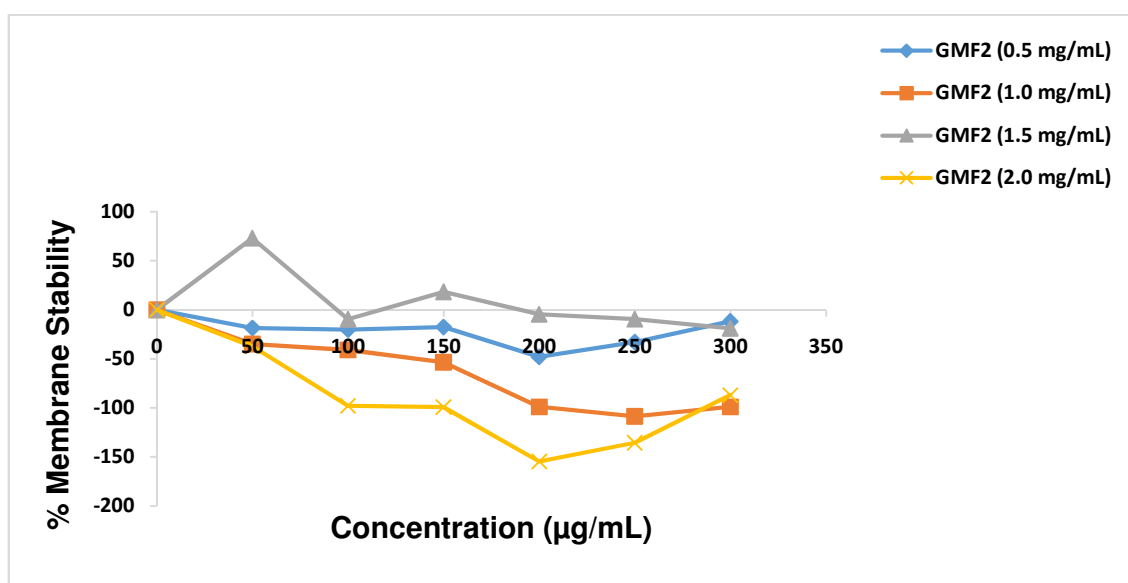


Figure 1e: Membrane stabilizing profiles of fraction 2 (GMF2), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

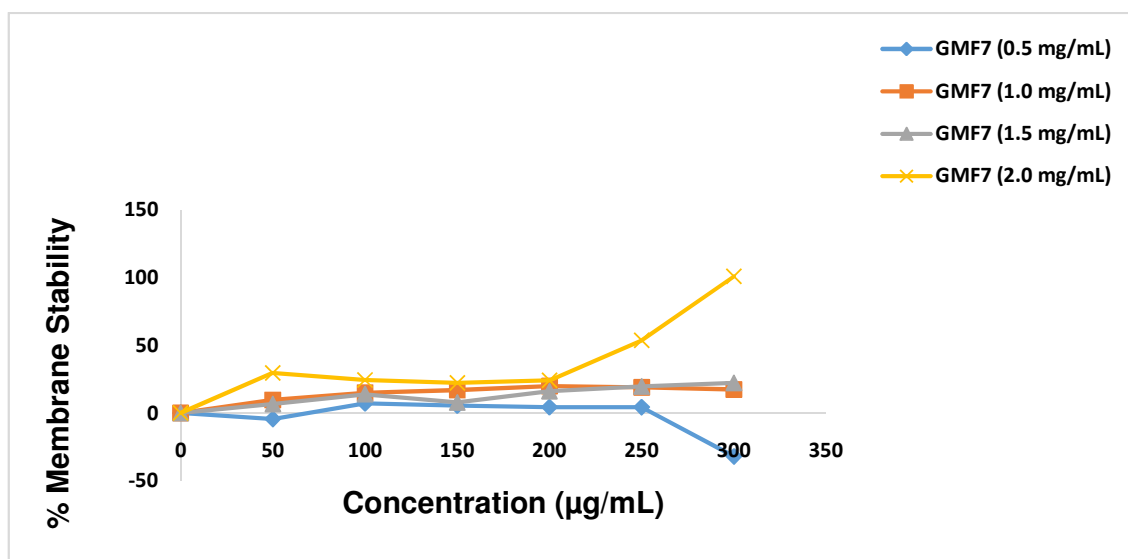


Figure 1f: Membrane stabilizing profiles of fraction 7 (GMF7), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings.

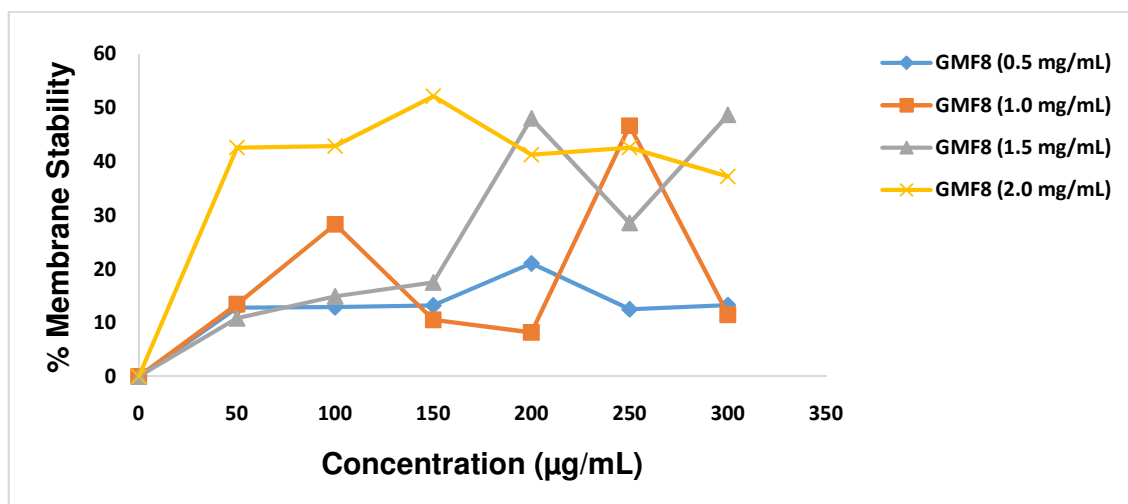
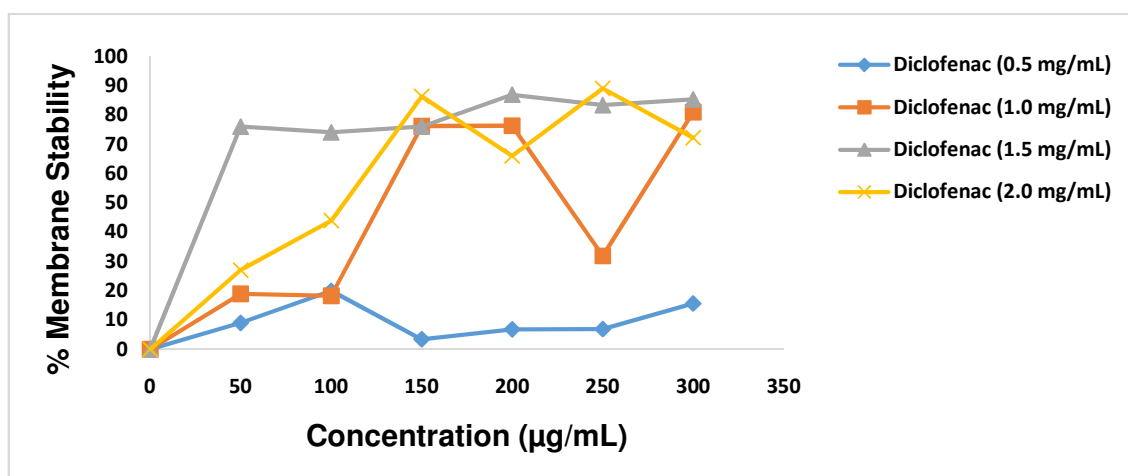


Figure 1g: Membrane stabilizing profiles of fraction 8 (GMF8), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

3.3.1: Membrane Stabilizing Profile of the Standard Drug (Diclofenac).

Diclofenac, the standard drug used has a membrane stability profile as shown in Figure 2. The mode of response is biphasic in all the concentrations tested and has a minimum of $3.45 \pm 0.00\%$ and a maximum of $89.06 \pm 0.01\%$ membrane stability.

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436 **Figure 2:** Membrane stabilizing profiles of Diclofenac on bovine erythrocytes exposed to both heat
437 and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

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439 4.0: Conclusion

440 The present study indicates that the extracts and fractions of *G. oreophila* have strong free radical
441 scavenging activity and reducing power. This anti-oxidative effectiveness of the extracts and the
442 fractions was evidenced in all the methods used for the antioxidant assays through their percentage
443 inhibition and their IC₅₀ values.

444 The study also showed that all the seven samples assayed, MeOH extract (GOME) and the fractions
445 of *G. oreophila*, protected the stressed bovine erythrocyte membrane at some of the concentrations
446 used and compare favourably with Diclofenac (standard drug). It was also noted that MeOH extract of
447 *G. oreophila* fraction 7 (GMF7), protected the erythrocyte membrane more effectively than the
448 standard drug. Although, fraction GMF7 exhibited 100% mode of protection at 2.0 mg/mL but was not
449 better in its activity at different concentrations when compared with the standard drug (diclofenac,
450 Figure 2). On the basis of these results, it could be inferred that the extracts and the fractions of *G.*
451 *oreophila* contain principles that were capable of stabilizing bovine red blood cell membrane exposed
452 to heat and hypotonic-induced lyses and could therefore serve as a scientific proof for the use of
453 these plant extracts by the local traditional health practitioners in the management and treatment of
454 inflammatory related diseases.

455 ETHICAL APPROVAL

456 No animals or human test subjects were involved in this study.

References

1. Oluwole, O., Osungunna, M.O. and Abimbola, Y., (2013). Phytochemical and antimicrobial screening of *Globimetulaoreophila* (Oliv) van Tiegh and *Phragmanthera capitata* (Spreng) Balle. International Journal of Green Pharmacy. 7: 127–130
2. Faboro, Esther O., Wichitnithad, Wisut, Fadare, Olatomide A., Akinpelu, David A., Obafemi, Craig A. (2016). Antibacterial, Antioxidant Activities and Phytochemical Screening of aqueous methanol extracts of eight Nigerian medicinal and aromatic plants. Journal of Pharmacy Research. 10(7): 523-532
3. Adesina, S. K., Illoh, H. C., Johnny, I. I. and Jacobs, I. E. (2013). African Mistletoes (Loranthaceae), Ethnopharmacology, Chemistry and Medicinal Values: An Update. *Afr. J. Tradit. Complement. Altern. Med.* **10**(4), 161-70.
4. Faboro, E. O., Wei, L., Liang, S., McDonald, A. G. and Obafemi, C. A. (2016). Characterization of dichloromethane and methanol extracts from the leaves of a medicinal plant: *Globimetulaoreophila*. *Industrial Crop and Products*. 83, 391-399
5. Le, O. and Zam, N. W. O. R. U. (2008). Oxytocic properties of the aqueous extract of *Globimetulabraunni* (Loranthaceae). *Pak J Pharm Sci*: 21: 356-60.
6. Adediwura, F. J., Temitope, O. and Oluwakemi, A. (2008). Phytochemical and laxative studies of *Globimetulabraunii* (Engle) van Tiegh growing on *Cola acuminata* (Schott & Endl). *Afr. J. Tradit. Complement. Altern. Med.* **5**:419.
7. Okpuzor, J., Ogbunugafor, H. and Kareem, G. K. (2009). Antioxidative properties of ethyl acetate fraction of *Globimetulabraunii* in normal albino rats. *J BiolSci*2009; **9**: 470-75.
8. Blois, M. S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature* 29:1199-1200
9. Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995). Use of free radical method to evaluate antioxidant activity. *LebensmWiss Technology*. 28:25-30.
10. Green, L.C., Wagner, D. A. Glogowski, J., Skipper, P. L., Wishnok, J. S. and Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite and nitrate in biological fluids. *Analytical Biochemistry*126: 131–138.
11. Marcocci, L., Maguire, J. J., Droy-Lefaix, M. T. and Packer, L. (1994). The nitric oxide-scavenging properties of *Ginkgo biloba* extract EGb 761. *BiochemBiophys Res Commun*15: 748–755.
12. Huang, D., Ou, B. and Prior, R. L. (2005). The Chemistry behind Antioxidant Capacity Assays. *Journal of Agricultural and Food Chemistry* 53: 1841-1856.
13. Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In "Food Antioxidants", Hudson BJB (ed.). Elsevier: London; 1-18.
14. Prieto, P., Pineda, M. and Aguilar, M. (1999). Spectrophometric quantitation of antioxidant Capacity through the formation of Phosphomolybdenum Complex: Specific application to the determination of Vitamin E. *Analytical Biochemistry* 269: 337-341.
15. Benzie, I. F. F. and Strain, J. J., (1999). Ferric reducing antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. *Methods Enzymology* 299: 15–27.

16. Oyedapo, O. O., Akinpelu, B. A., Akinwunmi, K. F., Adeyinka, M. O. and Sipeolu, F. O. (2010). Red blood cell membrane stabilizing potentials of extracts of *Lantana camara* and its fractions. *International Journal of Plant Physiology and Biochemistry* 2(4), 46-51.
17. Oyedapo, O. O. and Famurewa, A. J. (1995). Antiprotease and membrane stabilizing activities of extracts of *FagaraZanthoxyloidesOlaxsubscorpioidesandTetrapleuratetraptera*. *International Journal of Pharmacognosy* 33:65-69.
18. Cao, G. and Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry* 44:1309–15.
19. Akinmoladun, A.C., Ibukun, E.O., Afor, E., Akinrinlola, B.L., Onibon, T.R., Akinboboye, A.O., Obuotor, E.M., and Farombi, E.O. (2007). Chemical constituents and antioxidant activity of *Alstoniabooni*. *African Journal of Biotechnology* 6: 1197-1201
20. Zhang, R.; Zeng, Q., Deng, Y., Zhang, M., Wei, Z., Zhang, Y. and Tang, X. (2013). Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. *Food Chemistry* 136: 1169–1176.
21. Sies, H (2007). Total Antioxidant Capacity: Appraisal of a Concept. *Journal of Nutrition* 137: 1493–1495.
22. Iris F. F. Benzie and J. J. Strain (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of “Antioxidant Power”: The FRAP Assay. *Analytical Biochemistry* 239: 70 – 76
23. Young, I. S. (2001). Measurement of total antioxidant capacity. *Journal of ClinicalPathology*54:339
24. Okpuzor J., Ogbunugafor, H., and Kareem, G. K. (2009). Antioxidative Properties of Ethyl Acetate Fraction of *Globimetulabraunii* in Normal Albino Rats *Journal of Biological Sciences*. 9 (5): 470-475
25. Ja'afar, M. K., Jamil, S. andBasar, N. (2017). Antioxidant activity of leaf extracts of *Globimetulabraunii* (Engler) van tiegh parasitizing on *Piliostigmathonningii* and *parkiabiglobosa**JurnalTeknologi* (Sciences & Engineering) 79:5, 43–47
26. Cao, G. and Prior, R. L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry* 44:1309–15.
27. Sadique, J., Al-Rqobah, N. A., Bughaith, M. F. and El-Gindy, A. R. (1989). The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia* LX, 525-532.
28. Shinde, U. A., Phadke, A. S., Nair, A. M., Mungantiwar, A. A., Dikshit, V. J. and Saraf, V. O (1999). Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrusdeodarawood* oil. *Fitoterapia*70: 251-257
29. Oyedapo, O. O., Akindele, V. R. and Okunfolami. K, O. (1997). Effects of the extracts of *Olaxsubscorpioidesand Aspiliaafricana*on bovine red blood cells. *Phytotherapy Research* 11: 305-306.
30. Akinpelu, B. A., Makinde, A. M., Isa, M. O., Taiwo, O. P., Ojelabi, O. M. and Oyedapo, O. O. (2015). *In vitro* Evaluation of Membrane Stabilizing Potential of Selected Bryophyte Species. *European Journal of Medicinal Plants*6(3): 181-190
31. Sessa, G., and Weisman, G., (1968). Effect of components of the polyene antibiotic, Fillipin on phospholipids spherules (liposomes) and erythrocytes. *Journal of Biological Chemistry* 243: 4364-4371.

- 574 32. Litman, G., Litman, R.T., and Henry, C.J. (1976). Analysis of lipophilic carcinogen-
575 membrane interaction using model human erythrocytes membrane system. *Cancer*
576 *Research* 36: 438-444.
577
- 578 33. Horie, T., Sugiyama, V., Awazu, S., and Hanano, M. (1979). The correlation between drug
579 binding to the human erythrocyte and its hemolytic activity. *Journal of Pharmacology* 4: 116-
580 122.
581
- 582 34. Nambi, R.A., Sukumar, D., Sethuraman, V., Suluchana, N., and Sadique, J. (1985). "Satellite
583 symposium on Traditional Medicine as Asian Congress of Pharmacology". Tamil University
584 Thanjavur, 140.
585