

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

ABSTRACT

Aims: The study was designed to investigate the antioxidant and membrane stabilizing potentials of the leaf of *Globimetula oreophila*, 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 anti-oxidant 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 anti-oxidant capacity and ferric reducing antioxidant power)

Results: The results revealed that the extracts and the fractions of *G. oreophila* exhibited considerable and potent antioxidant activities. Fraction 1 of the methanol extract exhibited the highest antioxidant activity while fraction 7 of the same extract was the most potent in stabilizing stressed red blood cells. The activities of both the fractions and the extracts of *G. oreophila* compared favourably with the standard anti-oxidant 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 anti-oxidant 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.

35 The plant *G. oreophila* (Loranthaceae family) has the potential of providing lead and new drugs that
 36 could be employed to combat the menace of cardiovascular diseases such as cancer, heart attack,
 37 hypertension, diabetes and infectious diseases [1, 2]. It is acclaimed by the traditional health
 38 practitioners in Nigeria that the extracts of this plant are employed in the prevention, treatment and
 39 management of cardiovascular related diseases and ailments.

40 Reactive oxygen species (ROS) have been demonstrated to play prominent roles in the development
 41 of oxidative stress and etiogenesis of various forms cardiovascular and related diseases or disorders.

42 Recently, there has been an increased interest in the therapeutic potentials of medicinal plants owing
 43 to their renowned abilities to scavenge free radicals (i.e. antioxidant power) [3, 6]. In healthy
 44 individuals, the production of free radicals is balanced by the anti-oxidative defense system, however,
 45 oxidative stress is developed when equilibrium favours free radical generation as a result of a
 46 depletion of antioxidant levels [7, 8]. The oxidation of macromolecules such as lipid, DNA, protein,
 47 carbohydrate, and other biomolecules by toxic ROS cause mutation and damage to target cells or
 48 tissues, which often results in cell senescence and death [9]. The knowledge and application of such
 49 potential antioxidant activities in reducing oxidative stress *in vivo* has prompted many investigators
 50 across the globe to search for potent and cost-effective antioxidants from various plant sources [7, 8].

51 Inflammation is a common and major cause of sufferings always. The drugs available for the
 52 alleviation inflammation are nonsteroidal anti-inflammatory drugs (NSAID), which act by inhibiting the
 53 function of prostaglandin. Prostaglandin is an autocoid that is released extracellularly and initiate pain.
 54 Anti-inflammatory agents block this autocoid synthesis by either inhibiting COX (cyclooxygenase)
 55 enzyme or protecting lysosomal membrane from breakdown. [10].

56 Inflammatory is the responses to an injurious stimulus which is evoked by a wide variety of noxious
 57 agents. The ability to mount an inflammatory response is essential for survival in the face of
 58 environmental pathogens and injury; in some situations and diseases, the inflammatory responses
 59 may be exaggerated and sustained without apparent benefit and even with severe adverse
 60 consequences [11].

61 The present study is aimed at evaluating the antioxidant and membrane stabilizing activities of the
 62 extracts and fractions of the leaves of *G. oreophila*, to establish the scientific evidence for the use of
 63 the plant as anti-inflammatory agent as acclaimed by the traditional healers. In addition, there is
 64 dearth of scientific information on the biological and chemical activities of the extract, fraction and

65 bioactivity of the plant from the science community so this study could serve as a baseline for further
66 study on the plant.

67 **2.0: Materials and Methods**

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69 **2.1: Materials**

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71 **2.2: Collection and Identification of Plant Material**

72 Fresh leaves of *G. oreophila* were collected from Iwo, Osun State, Nigeria. The leaves were identified
73 and authenticated by Mr. Gabriel Ibhanesebhor and a voucher specimen deposited at IFE Herbarium
74 (ID no. 17089) at the Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria.

75 **2.3: Reagents and Chemicals**

76 All the reagents and chemicals used were of analytical grades, and were obtained from various
77 sources such as Sigma-Aldrich Chemical Company, Germany and United States of America and
78 British Drug House (BDH) England. All solutions, buffers and reagents were prepared using glass
79 distilled water. Diclofenac (standard anti-inflammatory drug) was purchased from the Pharmacy Shop
80 at the main Campus of Obafemi Awolowo University, Ile-Ife, Nigeria.

81 **2.4: Methods**

82 **2.4.1: Preparation of Plant materials and Extraction**

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

86 Powdered plant material (500 g) was extracted using dichloromethane (3L) and methanol separately
87 overnight in a large Soxhlet apparatus. The extracts were concentrated under reduced pressure to
88 afford dichloromethane and methanol respectively. Both extracts were weighed and kept in the
89 desicator until used for biochemical analyses.

90 The extracts were fractionated on Silica gel 60 (40-60 mesh size) column using gradient solvent
91 mixtures petroleum ether/ethyl acetate. Typically, each extract (1 g) was loaded onto the packed
92 Silica gel 60 column and washed with petroleum ether. The elution was conducted sequentially with
93 petroleum ether/ethyl acetate, 1:1v/v; 3:7v/v; ethyl acetate (100%); ethyl acetate/methanol (1:1v/v);
94 and methanol (100%). Fractions (15 ml) were collected at a flow rate of 1 ml/min. A total of 78
95 fractions were collected for DCM extract and 65 fractions for methanol extract. Various fractions were
96 chromatographed on pre-coated silica gel F254 plates. The plates were air dried, stained with specific

stained reagents and heated at 105°C for spot development. The Rf values were calculated and the fractions were pooled together based on their Rf 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 [12] as slightly modified (Brand-Williams, 13). 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:

$$I\% = [(A_{\text{blank}} - A_{\text{sample}})/A_{\text{blank}}] \times 100;$$

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 14] as modified Marcocci [15]. 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 [16, 17].

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

2.5.1.4: Evaluation of Ferric Reducing Antioxidant Power (FRAP)

The evaluation of ferric reducing antioxidant power (FRAP) of the extracts/fractions was carried out as reported [19] with slight modification. The extract/fraction (50 µl) and standard (50 µl) (20, 40, 60, 80, 100 µg/ml) was added to 1ml of FRAP reagent. The absorbance measurement was read at 593 nm exactly 10 minutes after mixing against reagent blank that contained distilled water. The reducing power was expressed as ascorbic acid (mg/g AAE).

2.6: Evaluation of Anti-inflammatory Properties

The evaluation of anti-inflammatory activity of the extracts and the fractions of the leaf of *G. oreophila* was based on red blood cell membrane stabilizing activity that was exposed to both heat and hypotonic induced lyses as previously described [Oyedapo *et al.*, 20]. The extracts and fractions with high antioxidant activities were selected for the membrane stability assay.

2.6.1: Preparation of Bovine Erythrocytes

Bovine erythrocyte was prepared according to the procedure reported by [Oyedapo and Famurewa, 21]. Fresh blood sample was collected into anticoagulant bottle containing trisodium citrate (3.8% w/v) and mixed thoroughly to prevent lysing. The blood was poured into clean centrifuge tubes and centrifuged at 3000 rpm for 10 min. The supernatant was carefully removed with sterilized Pasteur pipette. The packed rbc (erythrocytes) was re-suspended in fresh isosaline, mixed gently and centrifuged at 3000 rpm for 10 min. The process was repeated five more times until a clear supernatant was obtained. Then, 2% (v/v) erythrocyte suspension was prepared by diluting 2 ml of packed red blood cells to 100 ml with normal saline.

2.6.2: Assay of Red Blood Cell Membrane Stabilizing Activity

The membrane stabilizing activity of the extracts and fractions of *G. oreophila* was assayed according to a modified procedure [Oyedapo *et al.*, 20]. 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 anti-oxidant activities of DCM and extracts and fractions of *G. oreophila*. It was observed that the extracts and the fractions exhibited potent and appreciable anti-oxidant activities. These were demonstrated in their ability to react with the stable free radical, thus the odd electrons of DPPH radical gave a strong absorption band at 517 nm in the visible spectroscopy (deep violet colour). As the electron became paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolourised product is stoichiometric with the number of electron taken up. The ability was also demonstrated their percentage inhibition which is concentration dependent. At the highest concentration, the DCM extract (GODE) has a percentage inhibition of 51.97% with an IC_{50} value of 910 $\mu\text{g/ml}$. In a similar way the percentage inhibition of the fractions showed a dose dependent trend with fraction 8 (GDF8) having the highest percentage of 71.92% and an IC_{50} value of 490 $\mu\text{g/ml}$.

216 **Table 1: Antioxidant Activities** of DCM Extract and the Fractions of *G. oreophila*.

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	6650 ± 970	9690 ± 2140	1170 ± 60	1510 ± 50	1290 ± 130	490 ± 10	1380 ± 110

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218 GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.
219 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 [22]. On the other hand fraction 7 (GMF1) has the highest antioxidant activity having 79.42% and 140 µg/ml as percentage inhibition and IC₅₀ value respectively.

Table 2: Antioxidant Activities 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)	140 ± 10	NA	520 ± 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 [23]. 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. These results show that the DPPH and NO assays relatively correlate with each other.

245 **Table 3:** Antioxidant Activities 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	290 ± 00	2740 ± 260	1220 ± 10	420 ± 10	990 ± 10

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 247 GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.
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 249 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 50 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 [24]. These findings are in agreement with the phytoconstituents obtained by the GC-MS analysis of the extracts where phenolic compounds were detected [25]

Table 4: Antioxidant Activities of MeOH Extract and the Fractions of *G. oreophila*.

Conc(µg/ml)	Percentage Inhibition				
	GO2	GMF1	GMF2	GMF7	GMF8
1000	65.77 ± 0.15	65.41 ± 2.45	30.56 ± 0.26	72.08 ± 1.24	90.07 ± 0.15
500	53.36 ± 1.17	53.41 ± 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)	630 ± 10	50 ± 10	1680 ± 30	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.

In Table 5 is the antioxidant activities of the standard used, ascorbic acid.

276 **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)	10 ± 0.00	IC ₅₀ (µg/ml)	64.15 ± 2.52

277 Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically
 278 significant.
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280 3.2.3: Evaluation of Total Antioxidant Capacity (TAC) of DCM Extracts and Fractions

281 The concept of total antioxidant capacity (TAC), which originated from chemistry and then was applied
 282 to biology and medicine, and further to nutrition and epidemiology [26] was also used to assess the
 283 antioxidant activities the extracts and fractions as shown in Tables 6 and 7. The extract and the
 284 fractions possess some antioxidant activities. Fractions 4 and 8 (GDF4 and GDF8) with 4.55 and 5.11
 285 mgAAE/g showed the highest antioxidant activities when compared to the standard. In the manner,
 286 the extract and its fractions were subjected to the same TAC evaluation. In this evaluation it was only
 287 the fraction 2 GMF2 that showed an appreciable activity with 5.84 mgAAE/g when compared with the
 288 standard Table 7. The numerous available TAC assays correlate poorly with each other because
 289 various antioxidants react differently in each assay [21]. For example, the results of some assays
 290 include a substantial contribution from protein thiols, which do not react at all in other methods.
 291 Therefore, to interpret the results it is essential that one understands fully the relative contribution of
 292 the individual antioxidants to the methodology being used. Again, the total antioxidant capacity of a
 293 solution will depend on the nature of the oxidative abuse. TAC assays measure antioxidant capacity
 294 only in the defined conditions of the particular technique used.

295 3.2.4: Evaluation of Ferric Reducing Antioxidant Power (FRAP) of DCM Extracts and Fractions

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

298 antioxidants Individual members of the antioxidant defense team are deployed to prevent generation
299 of ROS, to destroy potential oxidants, and to scavenge ROS. In this perspective, antioxidant power
300 may be referred to analogously as reducing ability [27]. With this in mind, a method using reductants
301 in a redox-linked colorimetric method employing an easily reduced oxidant in stoichiometric excess
302 could offer a simple way of assessing this ability hence the use of FRAP to assess the reducing power
303 of the extracts and the fractions in this study. The DCM extract (GODE) and fractions 3, 5 and 8
304 (GDF1, GDF3 and GDF8) showed appreciable antioxidant activities with 622, 603 and 752 mgAAE/g
305 when compared with the standard of 826 mgAAE/g Table 6. Likewise the extract, (GME) exhibited
306 potent antioxidant activity with 673 mgAAE/g compared to the standard. Fractions 2 and 7 showed
307 reasonable activities Table 7. This is in agreement to the observation of Young, [28]. This
308 investigation is similar to the research of other investigators who worked on similar species such as
309 *Globimetula braunii* [29, 30], *Globimetula cupulata* [31].

310

311 **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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313 **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 [32, 33]. 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 [34, 35].

Erythrocytes have been used as a model system by numerous researchers for the study of interaction of drugs with membranes [36, 37, 38]. Drugs like anesthetics tranquilizers (mood stabilizers) and nonsteroidal anti-inflammatories stabilize erythrocytes against hypotonic haemolysis at low concentration. 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 [39].

In Table 8, is the list of the extracts and fractions employed for membrane stabilizing activity assays.

The selection was based on the anti-oxidant potency of the extracts and fractions.

Table 8: Extract/Fractions Selected for Membrane Stability Assays

S/NO	Extract/Fraction	Percentage Inhibition	IC ₅₀ (µg/ml ± SEM)
1	GODE	51.97 ± 1.57	910 ± 50
2	GDF8	71.92 ± 1.19	490 ± 10
3	GOME	75.42 ± 0.11	140 ± 10
4	GMF1	65.41 ± 2.45	50 ± 10
5	GMF2	70.98 ± 0.72	520 ± 10
6	GMF7	79.42 ± 0.72	140 ± 10
7	GMF8	76.92 ± 0.94	110 ± 30

In figures 1a - g are 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.

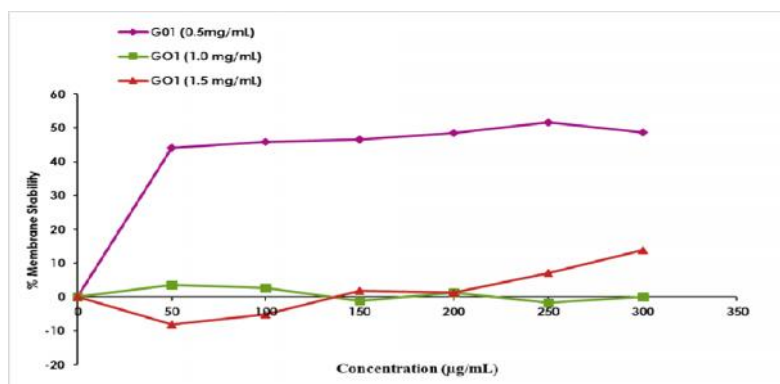


Figure 1a: Membrane stabilizing profiles of DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

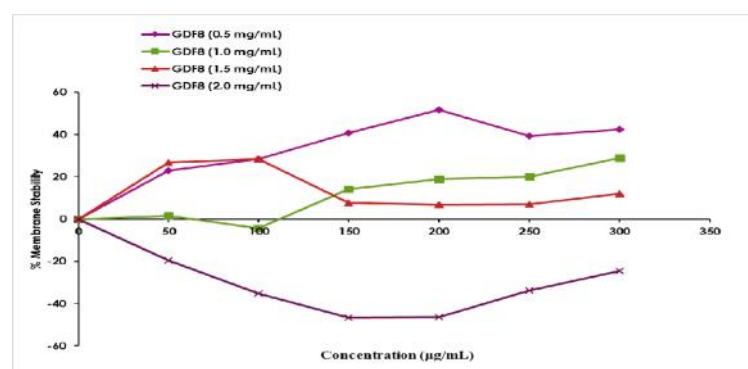


Figure 1b: Membrane stabilizing profiles of fraction 8 (GDF8), DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

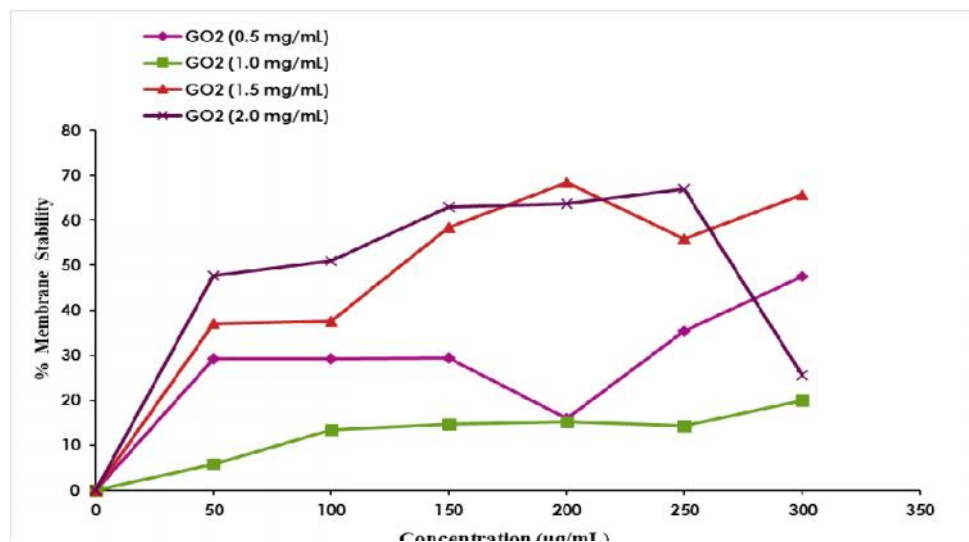


Figure 1c: Membrane stabilizing profiles of MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

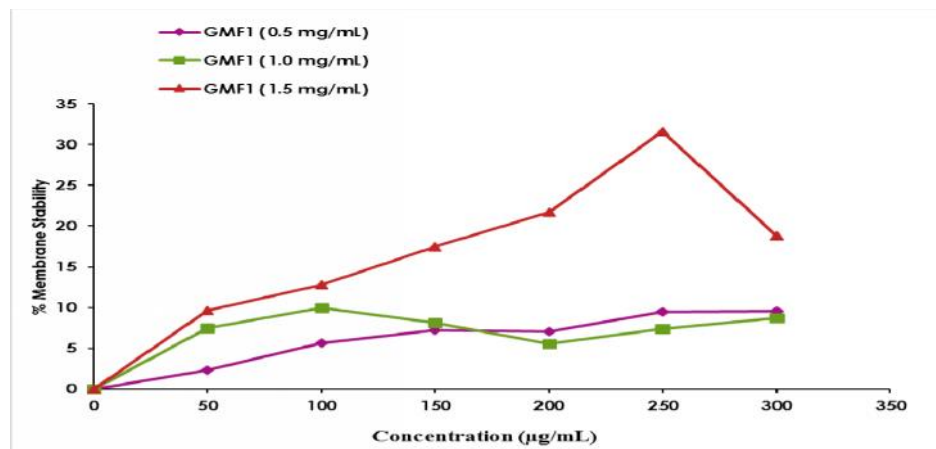


Figure 1d: Membrane stabilizing profiles of fraction 1 (GMF1), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

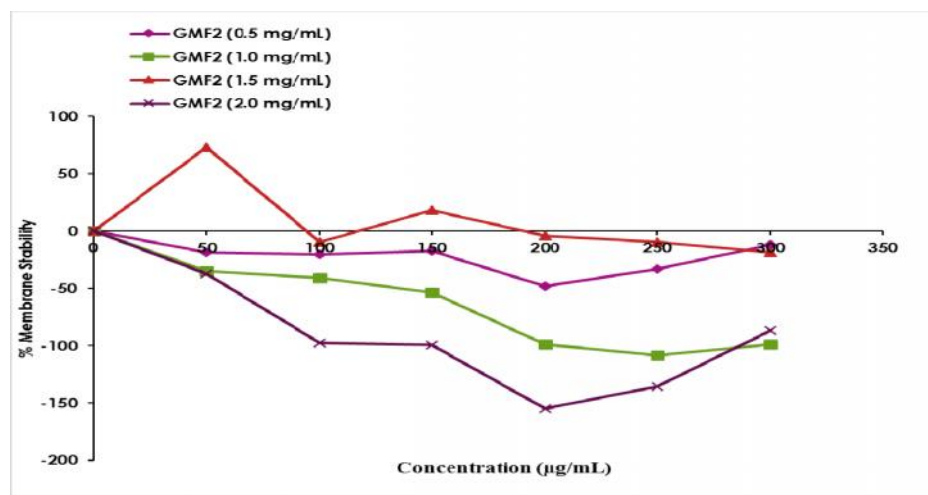


Figure 1e: Membrane stabilizing profiles of fraction 2 (GMF2), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

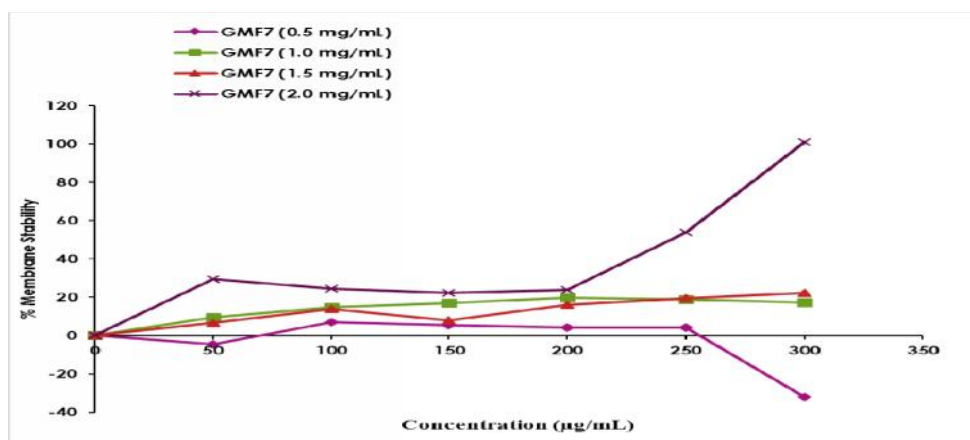


Figure 1f: Membrane stabilizing profiles of fraction 7 (GMF7), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings.

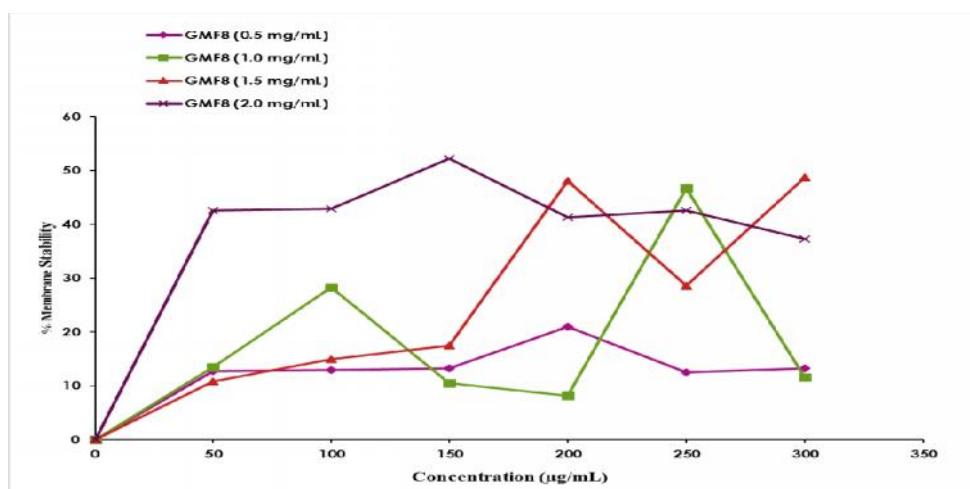
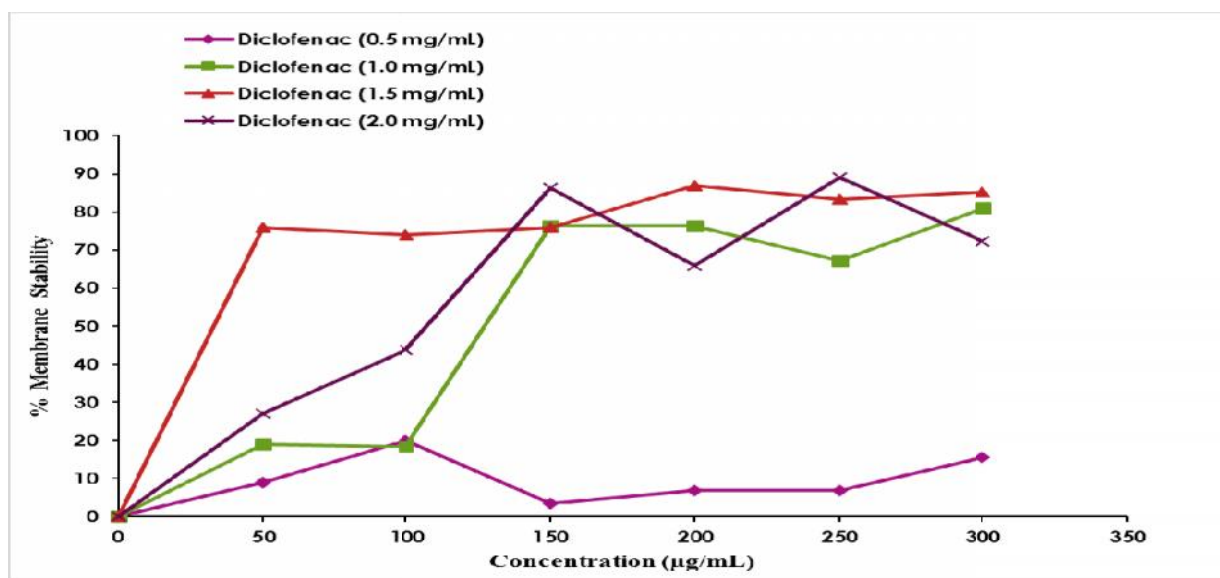


Figure 1g: Membrane stabilizing profiles of fraction 8 (GMF8), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean $\pm$ SEM of 3 readings

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.

428



429

430

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

433

434 4.0: Conclusion

435 The present study indicates that the extracts and fractions of *G. oreophila* have strong free radical
436 scavenging activity and reducing power. This anti-oxidative effectiveness of the extracts and the
437 fractions was evidenced in all the methods used for the anti-oxidant assays through their percentage
438 inhibition and their IC_{50} values.

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

ETHICAL APPROVAL

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

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