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

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EOF and EOI designed the study, wrote the protocol. Author EOF wrote the first draft of the manuscript. Authors IJO and BAA performed the statistical analysis and authors OOO and CAO managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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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 antioxidant activities of the extracts and fractions were evaluated using red blood cell membrane stabilization

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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 ± 0.13 µg/ml while fraction 7 of the same extract was the most potent in stabilizing stressed red blood cells having 100±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 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, octadecenol, nonacosane, pentacosanol, hexatriacontane, octacosanol, triacontanol, dotriacontanol, cis-dihydro-3,4-bis[(trimethylsilyl)oxy]-2(3H)-furanone, butanedioic acid, glyceric acid, fumaric acid, 3,4-dihyroxybutanoic acid, malic acid, tetrahydroxy butane, 2,3,4trihydroxybutanoic acid, tartaric acid, arabitol, ribofuranose, isocitric acid, inositol, hexo-pyranose, inosose, methyl dimethoxy- hydroxybenzoate, 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*.

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

2. MATERIALS AND METHODS

2.1 Materials

The main material used in this study is the fresh leaves of *G. oreophila* which was sourced locally.

Others include appropriate chemicals purchased from recognised chemical companies.

2.2 Collection and Identification of Plant Material

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

2.3 Reagents and Chemicals

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

2.4 Methods

2.4.1 Preparation of plant materials and extraction

Collected fresh leaves of *G. oreophila* were rinsed with clean water, drained, air-dried for 2-3 weeks in shade and pulverized into the powdered form using a domestic blender. The powdered plant material was 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^{o}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 [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 were 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-naphthyl ethylenediamine 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 sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated in a water bath at 95ºC 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 [15] 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*., 16]. 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, 17]. A fresh blood sample was collected into an 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 the activity of the extracts and fractions of *G. oreophila* was assayed according 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.

2.7 Statistical Analysis

Each value represented the mean ± SEM of three consistent readings. Significance difference was analyzed using Student's t-test with $p<0.05$ as statistically significant.

3. 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

Four different antioxidant assays were carried out on the extracts and fractions of *G. oreophila* for the biochemical analyses.

3.2 Antioxidant Assays

3.2.1 Assay of DPPH – radical scavenging activity of DCM extracts and fractions

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 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 electrons taken up. The ability was also demonstrated in 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 µg/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 µg/ml. The IC_{50} of fractions GDF1, GDF2, GDF3, GDF4, GDF5 and GDF9 were not determined because the values were well over 1000µg/ml which cannot be determined by extrapolation.

The MeOH extract and its fractions were subjected to the same assay. The percentage inhibition of the extract is 75.42% and the IC_{50} 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 IC_{50} 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 IC_{50} value respectively.

Table 1. Antioxidant activities in DPPH test 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	NA	NA	NA	NA	NA	490 ± 10	NA

GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.
Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.

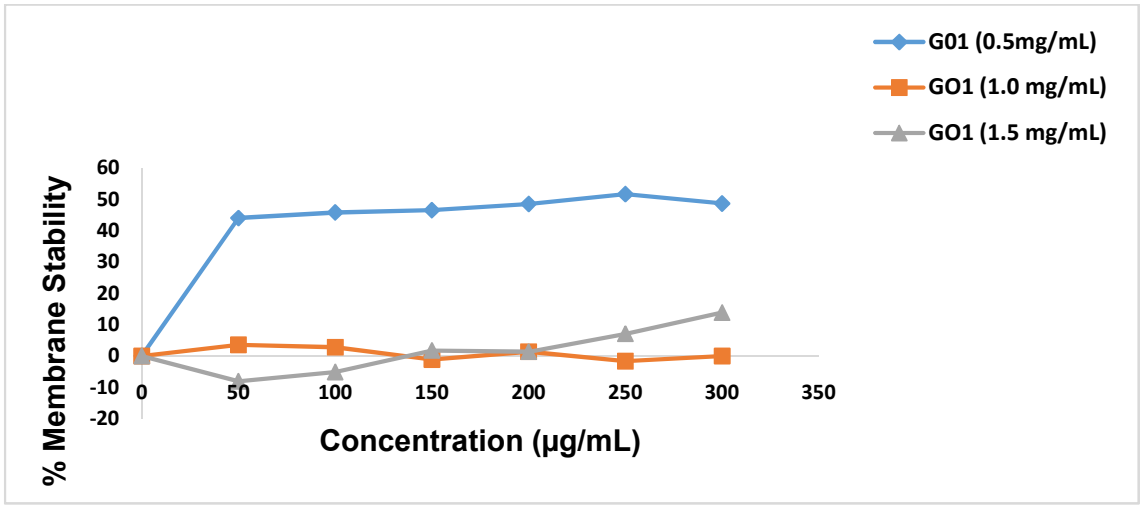

Fig. 1a. Membrane stabilizing profiles of DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses.
Each value represented the mean ± SEM of 3 readings

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 defence 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 IC_{50} of 250 µg/ml. All the fractions exhibited antioxidant activities with fractions 1 and 2 (GDF1 and GDF2) that showed an extremely low percentage inhibition of 5.7% and 10.75% respectively with no IC_{50} values Table 3. Again fraction 8 has the highest percentage inhibition of 65.20% and IC_{50} of 420 µg/ml.

Similarly, the MeOH extract and its fraction showed a dose-dependent activity with fraction 8 (GMF8) having 90.07% and IC_{50} 440 µg/ml respectively. Fraction 1 (GMF1) showed the highest antioxidant activity based on the IC_{50} of 45 µg/ml. Fraction 7 also showed an appreciable activity of 72.08% percentage inhibition and IC_{50} 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].

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

3.2.3 Evaluation of total antioxidant capacity (TAC) of DCM extracts and fractions

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

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 ± 016	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

GO1: DCM Extract; GDF1, 2, 3, 4, 5, 8, 9 DCM Fractions.
Each value represented Mean ± SEM of n=3 reading. Value of p≤0.05 was taken as statistically significant.

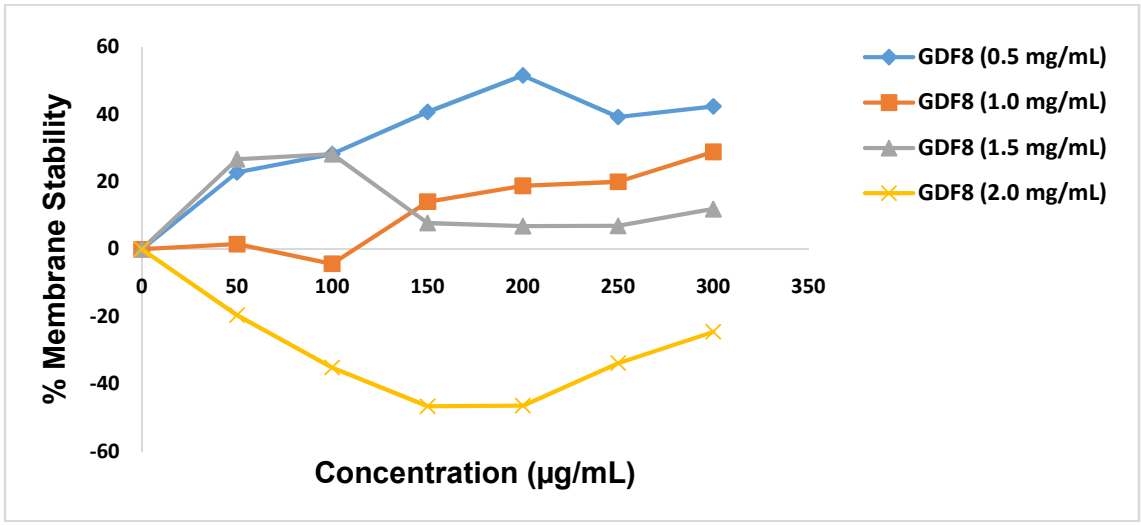

Fig. 1b. Membrane stabilizing profiles of fraction 8 (GDF8), DCM extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses
Each value represented the mean ± SEM of 3 readings

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.

Table 5. Antioxidant activities of the standard, ascorbic acid

Conc (µg/ml)	DPPH	Nitric oxide	
	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	IC_{50} (µg/ml)	56.26 ± 0.22

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

3.2.4 Evaluation of ferric reducing antioxidant power (FRAP) of DCM extracts and fractions

Possibly damaging reactive oxygen species (ROS) are produced as a consequence of normal aerobic metabolism These ‘‘free radicals’’ are usually removed or inactivated in vivo by a squad of antioxidants Individual members of the antioxidant defence team are deployed to prevent generation of ROS, to destroy potential oxidants, and to scavenge ROS. In this perspective, antioxidant power may be referred to analogously as reducing ability [22]. With this in mind, a method using reductants in a redox-linked colorimetric method employing an easily reduced oxidant in stoichiometric excess could offer a simple way of assessing this ability hence the use of FRAP to assess the reducing power of the extracts and the fractions in this study. The DCM extract (GODE) and fractions 3, 5 and 8 (GDF1, GDF3 and GDF8) showed appreciable antioxidant activities with 622, 603 and 752 mgAAE/g when compared with the standard of 826 mgAAE/g Table 6. Likewise the extract, (GME) exhibited potent antioxidant activity with 673 mgAAE/g compared to the standard. Fractions 2 and 7 showed reasonable activities in Table 7. This is in agreement to the observation of Young, [23]. This investigation is similar to the research of other investigators who worked on similar species such as *Globimetula braunii* [24, 25], *Globimetula cupulata* [26].

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 the 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. Moreover, Figs **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 extracts and fractions exhibited mixtures of the monophasic and biphasic mode of protection which are concentration dependent and compared favourably with that of standard (diclofenac) a non-steroidal anti-inflammatory drug (NSAD), Fig. 2. Also, the percentage membrane stability exhibited by the extracts and fractions ranged from 2.28 ± 0.01% (minimum) and 72.97 ± 0.12% (maximum), while the minimum and maximum membrane stabilizing activities of the standard drug was 3.45 ± 0.00% and 89.06 ± 0.01%. The findings of this study agreed with previous studies on the membrane stabilizing potentials of extracts and fractions of plants from various sources [35,36,37,38]

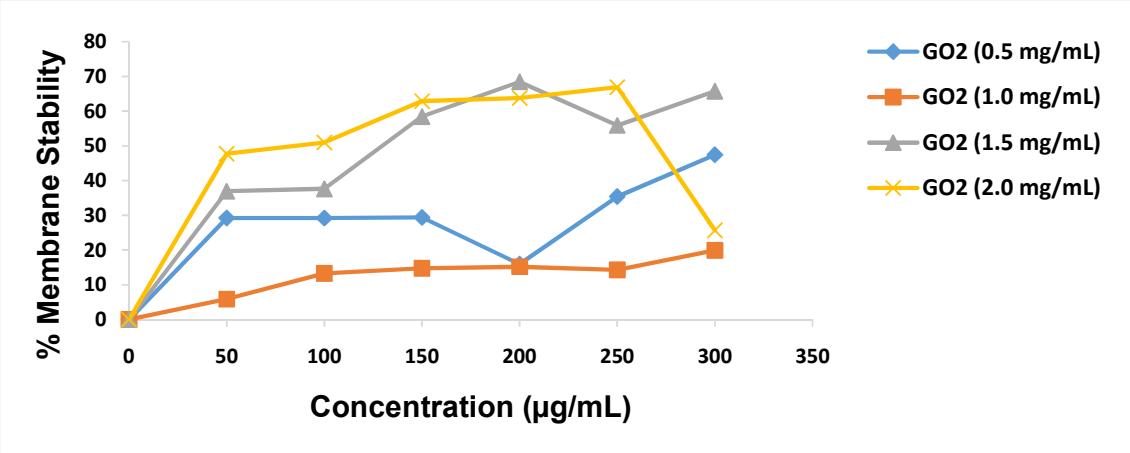

Fig. 1c. Membrane stabilizing profiles of MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses

Each value represented the mean ± SEM of 3 readings

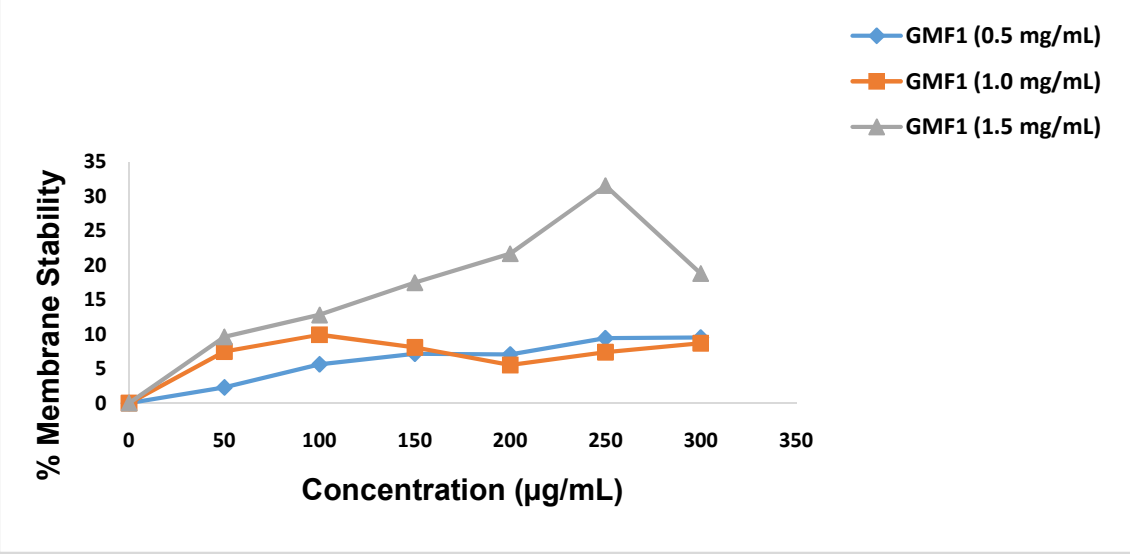

Fig. 1d. Membrane stabilizing profiles of fraction 1 (GMF1), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses

Each value represented the mean ± SEM of 3 readings

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

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

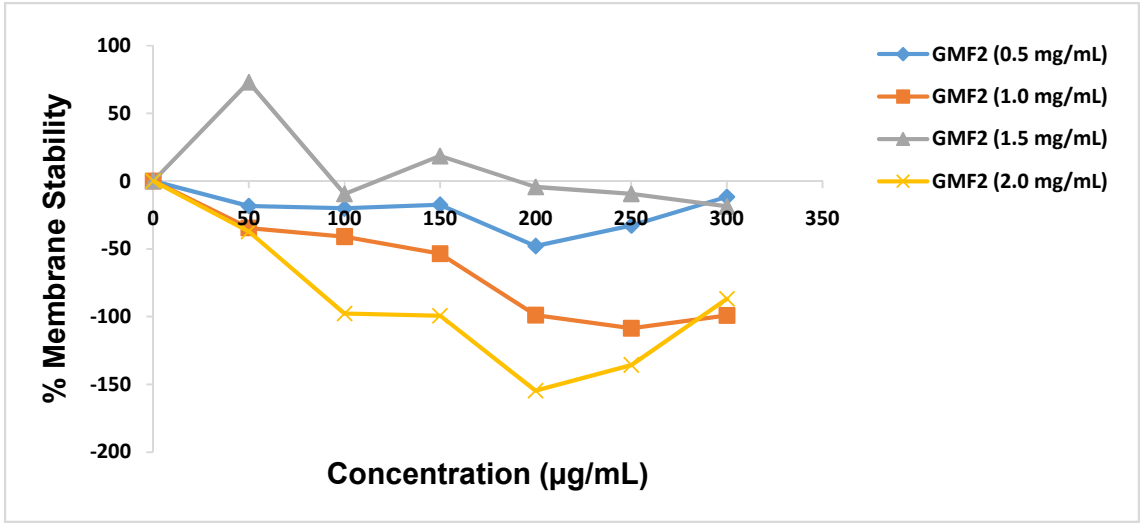

Fig. 1e. Membrane stabilizing profiles of fraction 2 (GMF2), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses

Each value represented the mean ± SEM of 3 readings

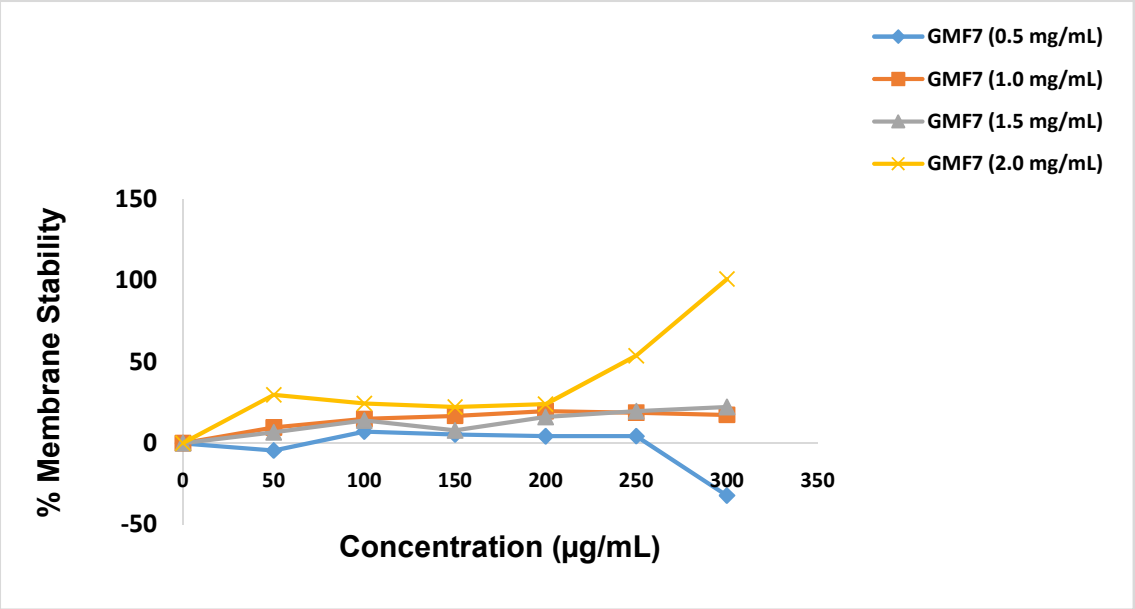

Fig. 1f. Membrane stabilizing profiles of fraction 7 (GMF7), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses

Each value represented the mean ± SEM of 3 readings.

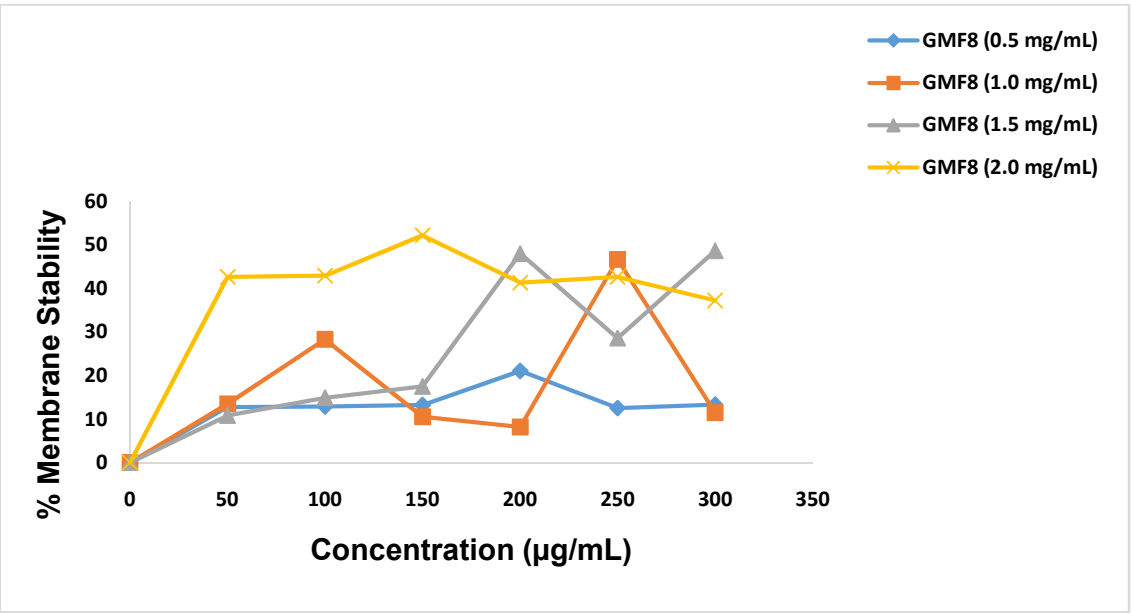

Fig. 1g. Membrane stabilizing profiles of fraction 8 (GMF8), MeOH extract of *G. oreophila* on bovine erythrocytes exposed to both heat and hypotonic induced lyses

Each value represented the mean ± 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 Fig. 2. The mode of response is biphasic in all the concentrations tested and has a minimum of 3.45± 0.00% and a maximum of 89.06 ± 0.01% membrane stability.

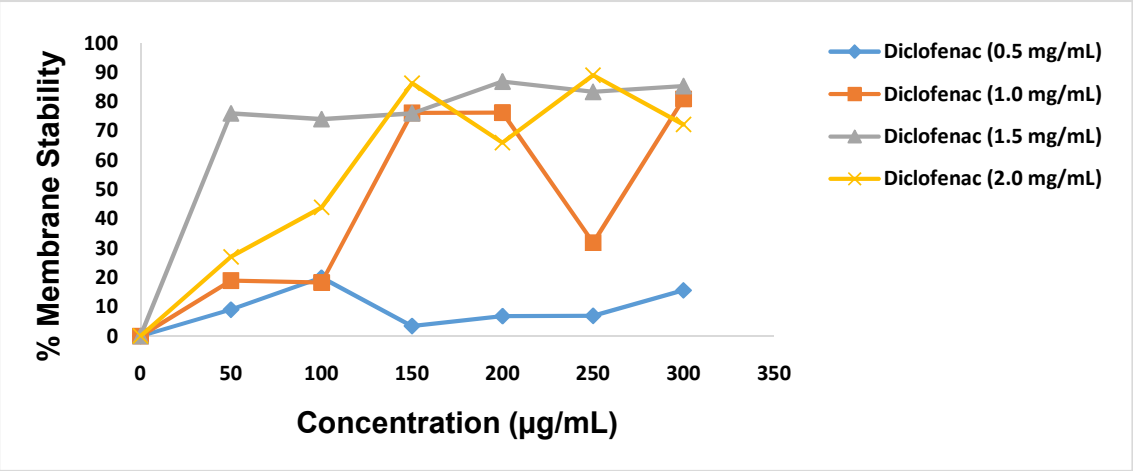

Fig. 2. Membrane stabilizing profiles of Diclofenac on bovine erythrocytes exposed to both heat and hypotonic induced lyses. Each value represented the mean ± SEM of 3 readings

4. CONCLUSION

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

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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