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In Vitro Antioxidant Properties of Dichloromethanolic Leaf Extract of *Gnidia glauca* (Fresen) as a Promising Antiobesity Drug

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Abstract

The acquisition of ethnobotanical information from traditional practitioners remains an empirical aspect of understanding the ethnopharmacology research. However, integration of information on chemical composition of plant extracts and their pharmacological activities forms a key resource for synthesis of new and effective therapeutics. In traditional African medicine, *Gnidia glauca* has folkloric remedies against obesity and its associated oxidative stress-mediated complications. However, the upsurge in its use has not been accompanied with scientific validations to support these claims. The present study aimed to determine the antioxidant potential of *G glauca* as a promising antiobesity agent. The antioxidant effects of the extract were assessed against 1,1-diphenyl-2-picrylhydrazyl, hydroxyl, hydrogen peroxide, nitric oxide, and superoxide radicals as well as lipid peroxidation, iron-chelating effect, and ferric-reducing power. Phytochemical analysis was conducted using gas chromatography linked to mass spectrophotometry. The results revealed that *G glauca* exhibited scavenging activities against all radicals formed. Besides, the extract showed iron chelation and ferric reducing abilities. The extract indicated a lower half maximal inhibitory concentration value than the standards used. For instance, the extract inhibited 50% of the formation of 2,2-diphenyl-1-picrylhydrazine at the concentration of 1.33 $\pm$ 0.03 mg/mL relative to 1.39 $\pm$ 0.06 mg/mL of the standard, vitamin C at 1% confidence limit. Similarly, the extract scavenged 50% of hydroxyl radical at 204.34 $\pm$ 10.64 μg/mL relative to 210.05 $\pm$ 8.80 μg/mL of gallic acid. The extract also contained various phytochemicals that have been associated with antiobesity effects. The synergistic effects of these phytocompounds increase their bioavailability and action on multiple molecular targets thereby correcting obesity-induced oxidative stress.

Keywords

obesity, oxidative stress, antioxidants, *Gnidia glauca*, reactive oxygen species, reactive nitrogen species, free radicals, pH

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The contribution of medicinal plants to the therapeutic arsenal in the fight against diverse ailments is evidence of human ingenuity since time immemorial.[1] The rationale behind the vast usage and greater dependence on herbal medicines as preferred prescription agents rests upon their long-term clinical experience.[2] Medicinal plants provide a major reservoir of effective chemotherapeutics essential for the maintenance of human health.[3] These phytocompounds are idiosyncratic in terms of their mechanism of actions, biological properties, and chemical structures.[4] They possess an enormous potential in ameliorating many diseases among which are anemia,[4] diabetes mellitus,[5] obesity,[6] liver and kidney disorders,[7,8] wounds,[9] and steatosis.[10,11] These bioactive compounds have been associated with minimal cytotoxicity, are biodegradable, easily available, and affordable to many people especially those in poor resource economies unlike the chemically synthesized drugs.[12]

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Current studies have shown that oxidative stress plays a significant role in the pathogenesis of many diseases worldwide.[6,11] Oxidative stress is a state of compromised redox equilibria due to an altered antioxidants–pro-oxidants balance in favor of pro-oxidants attributed to endogenous or exogenous stressors.[13] It represents a consequence of increased production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) as well as the attenuated capacity of antioxidant defenses.[14,15] The RNS and ROS interact with biomolecules (nucleic acids, proteins, lipids, carbohydrates) and exacerbate oxidative damage through carbonylation, peroxidation, nitration, and nitrosylation reactions.[16,17] These ultimately result in the depletion of endogenous antioxidant capacity, activation of the inadvertent enzymes and oxidative injury to cellular systems.[18] The compromised integrity of cellular systems by ROS and RNS serves as the prerequisite for the pathogenesis of many degenerative diseases and disorders such as aging, obesity, inflammation, cognitive impairment, cardiovascular diseases, cancer, and Alzheimer's disease.[3,19]

Oxidative stress has been shown to be a metabolic consequence of obesity.[3] Obesity is a phenotypic consequence of an energy imbalance between calories consumed and calories expended.[20] Fundamentally, it represents an energy-rich state due to chronic exposure to lipid-rich diets.[21] Under obesogenic states, the accumulation of triglycerides in the adipose tissues exacerbates oxidative stress by stimulating the production of pro-inflammatory cytokines (such as interleukin [IL]-1β, IL-6, and tumor necrosis factor–α [TNF-α]), chemokines, prostaglandins, and immune cells.[20] The hyperplastic and hypertrophied adipocytes secrete increased amounts of proinflammatory cytokines, which further perpetuates the inflammation of the adipose tissues.[20,21] The increased circulating levels of proinflammatory cytokines stimulate the production of ROS and RNS by monocytes and macrophages, which further precipitates inflammation and oxidative stress.[22,23] Obesity has also been shown to decrease endogenous antioxidant defenses.[24] Moradi et al[21] reported that mice fed with high-fat diets for 8 weeks showed decreased levels of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). Similarly, it was shown that chronic exposure to lipid-rich diets stimulates the production of ROS through peroxisomal and mitochondrial oxidation of fatty acids.[15,25]

Humans have evolved a complex and sophisticated antioxidant system to maintain the redox homeostasis and protect cells and organ systems against ROS and RNS.[26] Components of antioxidants defense systems are of either exogenous or endogenous in origin which function synergistically and interactively to neutralize RNS and ROS.[27] These components include dietary antioxidants like tocotrienols (vitamin E), glutathione, ascorbic acid (vitamin C), β-carotene, α-tocopherol, and uric acid.[28] The antioxidant enzymes such as superoxide dismutase, glutathione reductase, catalase, glutathione peroxidase also catalyze free radical scavenging activities.[27] Other antioxidant components include metal-binding proteins (like ceruloplasmin, albumin, lactoferrin, and ferritin), which sequesters free Cu^{+} and Zn^{2+} ions in the cytoplasm or Mn^{2+} ions in the mitochondrial matrix that acts as catalytic components in redox reactions.[29]

Synthetic antioxidants such as *tert*-butylhydroxyquinone, propyl gallate, butylated hydroxytoluene, and butylated hydroxyanisole have been associated with adverse effects among which are hepatic damages, malignancies as well as limited potency in animal models.[30,31] Currently, there is increased interest to substitute synthetic antioxidants for naturally occurring antioxidants from plants to act as either antioxidant additives or as nutritional supplements.[32,33]

Medicinal plants possess important antioxidant components with eminent potential in ameliorating oxidative stress–related degenerative ailments with minimal cytotoxicity.[34] Their relative potency is largely proportional to their interactions and synergistic effects with endogenous antioxidants in the eradication of free radicals.[35,36] The aqueous extract of *Allium saralicum,*[4] *Falcaria vulgaris*,[5] and *Thymus kotschyanus*[37] were found to exhibit antioxidant effects through the degradation of free radicals. They were found to increase the serum concentrations levels of SOD, CAT, GPx, and malondialdehyde (MDA) while reducing levels of glutathione (GSH). Furthermore, *A saralicum*, *F vulgaris*, and *T kotschyanus* contain phytocompounds such as alkaloid, anthraquinone, flavonoid, phenolic, saponin, steroids, and tannin, which have been reported to confer antioxidant effects in cellular systems.[4,5,37] Many antioxidant phytochemicals identified in *Alpinia zerumbet* such as essential oils, quercetin, rutin, kavalactones, phenolic acids, and flavonoids were found to be responsible for its free radical scavenging activities.[6]

In traditional African medicine, many herbs have been therapeutically applied against various ailments. One such medicinal plant is *Gnidia glauca*, which is a genus that belongs to the family of Thymelaeaceae.[38] *G glauca* has been traditionally used for the treatment of obesity and associated oxidative-related diseases such as cognitive impairment, anxiety, atherosclerosis and some cancers.[39] It has been a useful adjuvant and a key adjunct to dietary control in obese and diabetic patients.[39,40] Because of the infectivity, unaffordability, unavailability, and the potentially hazardous side effects associated with conventional antiobesity drugs, *G glauca* has received considerable acceptance.[39] However, its upsurge in use has not been accompanied by scientific evidence to validate these claims. Moreover, the hypothesized ability of *G glauca* to quench free radicals and its capacity to enhance and restore the endogenous antioxidant systems remains elusive. The authentication of its chemical constituents is not only essential for the discovery of new therapeutic agents but also discloses the new source of economic phytocompounds as well as the appreciation of the significance of its folklore use. The present study, therefore, sought to determine the in vitro antioxidant potential, free radical scavenging activities, and phytochemical profiles of dichloromethanolic (DCM) leaf extract of *G glauca* as a potential antioxidant and antiobesity supplement.

Materials and Methods

Collection and Preparation of the Medicinal Plant Material

Fresh leaves of the plant used in this study, *G glauca*, were collected from its natural habitat in Siakago Division, Mbeere North Sub-County, Embu County, Kenya. An acknowledged taxonomist authenticated the botanical identity of the plant and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi for future reference. The specimen was assigned a voucher specimen number as WAM-V1. The current study was undertaken in Biochemistry Laboratories of the Department of Biochemistry, Microbiology and Biotechnology in Kenyatta University. The collected fresh leaves of *G glauca* were shade-dried at room temperature for 21 days. The dried leaves were then milled into fine powder by use of an electric mill. The powdered plant material was kept at room temperature away from direct sunlight in a dry airtight plastic container ready for extraction.

Extraction

Five hundred grams of the powdered *G glauca* leaves were soaked in 1 L of dichloromethane and swirled regularly for 24 hours. The extract was decanted, filtered using muslin cloth into a different dry clean conical flask. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40°C to obtain a semisolid residue.[41] The percentage yield of the plant extract was determined and subsequently refrigerated at −20°C awaiting use in the bioassay.

Determination of Ferric-Reducing Antioxidant Power (FRAP)

Principle. This method is based on the conversion of the Fe^{3+}/ferricyanide complex to its ferrous form to form a violet-colored solution, whose intensity is proportional to the sample concentration. A higher absorbance of the reaction mixture is indicative of a greater reducing power of the extract.[42]

Procedure. The ferric-reducing power of the plant extract was determined by the method described by Athukorala et al.[43] A reaction mixture containing 1 mL of a solution of 2.5 mL of phosphate buffer (200 mM, pH 6.6) and 2.5 mL of potassium ferricyanide (30 mM), and the extract at different concentrations (50-250 µg/mL), was incubated at 50°C for 20 minutes. Thereafter, 2.5 mL of trichloroacetic acid (TCA; 600 mM) was added to the reaction mixture and centrifuged at 3000 rpm for 10 minutes. The supernatant of about 2.5 mL was collected and mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (6 mM). The absorbance was then determined at a wavelength of 700 nm. The blank contained all the reactants except the extract. Ascorbic acid was used as a standard. All tests were run in triplicates.

Determination of DPPH Free Radical Scavenging Activity

Principle. This method is based on an antioxidant compounds' hydrogen donating or radical scavenging ability to reduce 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical to 2,2-diphenyl-1-picrylhydrazine resulting in a pale-yellow solution. The decrease in the absorbance as the color of the solution fades (from deep violet to light yellow) is monitored at 517 nm.[44]

Procedure. The plant extract was prepared at various concentrations ranging from 0.05 to 5 mg/mL in methanol. The reaction mixture consisted of 1 mL of sample, 3 mL of methanol and 0.5 mL of 1 mM methanolic solution of DPPH. The reaction mixture was then vortexed and left to stand for 5 minutes. The absorbance of the resulting solution was measured at 517 nm. A mixture of methanol and DPPH solution served as a blank while a reaction mixture of methanol, DPPH, and standard (vitamin C) served as the positive control. All tests were run in triplicates. The percentage radical scavenging activity was calculated according to the following formula:

$$\%\ \text{DPPH Radical Scavenging activity} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100 \quad (1)$$

where control OD = optical density of the blank and sample OD = optical density of the extract or standard.

The percentage radical scavenging activity was then plotted against various concentrations and the IC_{50} (half maximal inhibitory concentration) was determined graphically.

Determination of Nitric Oxide Radical Scavenging Activity

Principle. This assay is based on the theory that sodium nitroprusside (SNP) spontaneously generates nitric oxide which interacts with molecular oxygen to form nitrite ions that may be estimated using Griess reagent. Scavengers of nitric oxide in the extract compete with molecular oxygen resulting in reduced production of nitrite ions.[45]

Procedure. Nitric oxide radical scavenging activity of *G glauca* was determined according to the method described by Farhan et al.[46] The reaction mixture constituting a solution of SNP (5 mmol/L) in phosphate-buffered saline pH 7.4 and different concentrations of the extract ranging from 250 to 2500 µg/mL, prepared in methanol, was incubated for 30 minutes at 25°C. After incubation, an aliquot of the incubated solution (1.5 mL) was diluted with 1.5 mL of Griess reagent (0.1% *N*-1-naphthyl ethylene diamine dihydrochloride [NED], 1% sulfanilamide, and 2% phosphoric acid). Quercetin was used as a standard drug. Diazotization of nitrite ions with sulfanilamide and subsequent coupling with NED generated a pink chromophore, whose absorbance was measured spectrophotometrically at 546 nm against a blank.[46] The blank contained all the reactants except the extract. All the tests were performed in triplicate. The percentage of radical scavenging activity was computed using the formula below:

$$\%\ \text{Nitric oxide radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

where A_0 = control reaction absorbance (blank) and A_1 = extract or quercetin absorbance.

Determination of Superoxide Radical Scavenging Activity

Principle. The assay is based on the ability of the extract to inhibit formazan formation through the reduction of nitro blue tetrazolium (NBT) by scavenging the superoxide radicals generated in the riboflavin-light-NBT system.[47]

Procedure. A 3-mL reaction mixture was prepared to contain 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM ethylenediaminetetraacetic acid (EDTA) and 0.1 mg NBT and various

concentrations (50-250 μg/mL) of the plant extract or standard compound. The reaction mixture was then illuminated for 90 seconds. The illuminated reaction mixture without the extract served as the negative control. The unilluminated reaction mixture without plant extract served as the blank. Immediately after illumination, the absorbance of the reaction mixture was measured at 562 nm against a blank to determine the quantity of formazan generated. All tests were performed three times each and quercetin served as the positive control. The percentage of radical scavenging activity was calculated using the following equation:

$$\%\ \text{Superoxide radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (3)$$

where A_0 = reaction control absorbance (blank) and A_1 = extract or quercetin absorbance.

Determination of Hydroxyl Radical (˙OH) Scavenging Activity

Principle. This assay is based on the ability of the extract to inhibit hydroxyl radical–mediated deoxyribose degradation by the Fenton's reaction using Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture.[48,49]

Procedure. A reaction mixture contained 28 mM 2-deoxy-2-ribose (100 μL), 20 mM KH_2PO_4-KOH buffer (pH 7.4), 200 μM FeCl3 (1:1 v/v), 200 μL EDTA (1.04 mM), 100 μL H_2O_2 (1.0 mM), 100 μL ascorbic acid (1.0 mM), and the extract (100-500 μg/mL) to a final volume of 1 mL. The mixture was incubated at 37°C for 1 hour. After incubation, 1.0 mL of 1% thiobarbituric acid (TBA) and 1.0 mL of 2.8% trichloroacetic acid (TCA) were added and further incubated at 100°C for 20 minutes to develop pink color. After cooling, the optical density was measured at $\lambda = 532$ nm. The blank solution contained all the reactants without the extract. Gallic acid was used as a positive control (standard). All experiments were performed in triplicate.[50] The formula below was used to compute for percentage hydroxyl radical scavenging activity:

$$\%\ \text{Hydroxyl radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (4)$$

where A_0 = control absorbance (blank) and A_1 = extract or standard absorbance.

Determination of Lipid Peroxidation Activity

Principle. Malondialdehyde (MDA), an end product of lipid peroxidation (the breakdown of polyunsaturated fatty acids) reacts with TBA (thiobarbituric acid) to produce a pink-colored product with an absorption maximum at 532 nm.[51]

Procedure. MDA was measured according to the method described by Wills.[52] with slight modifications. The reaction mixture of final volume of 1.0 mL contained 2.0 mL of the TCA-TBA-HCl reagent (15% [w/v] TCA, 0.375% [w/v] TBA, and 0.25 N HCl) and the plant extract (50-250 μg/mL). The reaction mixture was heated on a water bath at 90°C for 10 minutes, cooled and centrifuged at 10 000 rpm for 10 minutes to remove the TCA precipitate forming light pink-colored supernatant (MDA). Ascorbic acid was used as a reference drug. All tests were performed 3 times. The amount of MDA formed in each of the samples was assessed by measuring the absorbance of clear supernatant at 532 nm against the reference blank. All tests were performed in triplicate. The percentage of lipid peroxidation inhibition was calculated using the following equation:

$$\%\ \text{Lipid peroxidation} = \frac{A_0 - A_1}{A_0} \times 100 \quad (5)$$

where A_0 = control absorbance (blank) and A_1 = extract or ascorbic acid absorbance.

Determination of Hydrogen Peroxide Radical Scavenging Activity

Principle. This method is based on the decrease in absorbance of H_2O_2 following reduction of H_2O_2 by the antioxidant compound.[53]

Procedure. The hydrogen peroxide scavenging assay was performed according to the modified method of Ruch et al.[54] A solution of 40 mM hydrogen peroxide (H_2O_2) was prepared in phosphate buffer pH 7.4. The plant extract (at different concentrations of 0.1-0.5 mg/mL) was added to hydrogen peroxide solution, incubated for 10 minutes, and absorbance measured at 230 nm against a blank solution containing phosphate buffer without the hydrogen peroxide. Ascorbic acid was used as a positive control. All tests were run in triplicate and hydrogen peroxide radical scavenging activity was calculated using the following formula:

$$\%\ \text{Hydrogen peroxide radical scavenging activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (6)$$

where A_0 = control absorbance (blank) and A_1 = extract or ascorbic acid absorbance.

Iron (Fe^{2+}) Chelating Activity Assay

Principle. This test is based on antioxidants capacity to inhibit the transfer of electrons by forming a coordinate complex with the metal ions thereby arresting the oxidation reactions and formation of free radicals. Therefore, the presence of other chelating agents competes with ferrozine for the ferrous ions resulting in decolorization of the red color of iron (II)–ferrozine complex.[55] The chelating activity of ferrous ions can be measured by the decrease in absorbance at 562 nm.[56]

Procedure. The chelating activity of ferrous ions was determined by a standard spectrophotometric method as described by Dinis et al.[56] Briefly, 1 mL of different concentrations of plant extract (50-250 μg/mL) was added to a solution of 1 mL of 0.125 mM ferrous sulfate. The reaction was initiated by addition of 1 mL of ferrozine (0.3125 mM), vortexed and incubated for 10 minutes at room temperature, and the absorbance measured at 562 nm. The negative control test (blank) was performed without the addition of the extract. All tests were run in triplicate and EDTA was used as a positive control. The capacity of the sample to chelate the ferrous ion was calculated relative to the control using the following formula:

$$\%\ \text{Iron II chelating activity} = \frac{A_0 - A_1}{A_0} \times 100 \quad (7)$$

where A_0 = control absorbance (blank) and A_1 = extract or EDTA absorbance

Gas Chromatography–Mass Spectrometry Analysis

Analysis of the sample was carried out using GC-MS (7890/5975 Agilent Technologies, Inc, Beijing, China) consisting of a gas chromatography interfaced to a mass spectrometer instrument (GC-MS). The GC-MS was equipped with an HP-5 MS (5% phenyl methyl siloxane) low bleed capillary column of 30 m length, 0.25 mm diameter, and 0.25 µm film thickness. For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. The carrier gas used was helium (99.99%) at a constant flow rate of 1.25 mL/min in split mode. The injector and mass transfer line temperature were set at 250°C and 200°C, respectively, and an injection volume of 1 µL was employed. The oven temperature was programmed from 35°C for 5 minutes, with an increase of 10°C/min to 280°C for 10.5 minutes, then 50°C/min to 285°C for 29.9 minutes with a run time of 70 minutes. The mass spectrometry operating parameters were as follows: ionization energy, 70 eV; ion source temperature, 230°C; solvent cut time, 3.3 minutes; relative detector gain mode; scan speed, 1666 µm/s; scan range of 40 to 550 *m*/*z*, and the interface temperature of 250°C.

Data Management and Statistical Analysis

The data on absorbance measures were entered in the Microsoft Excel spreadsheet One-Word program, where it was organized and then exported to Minitab statistical software for analysis. This was found to conform with assumptions of parametric data and expressed as means ± standard deviations (mean ± SD). Inferential statistics were performed using 1- way analysis of variance followed by Tukey's post hoc tests for pairwise separation and comparison of means at a 1% level of significance. Unpaired Student's *t* test was used to compare the percentage of free radical scavenging activity between the standard compound and the plant extract at different concentrations. Simple regression analysis was performed to calculate the concentration-response relationship of standard solutions. All statistical analyses were performed using Minitab (Minitab, version 17.1). The analyzed data were presented in tables and graphs. Phytochemicals present in the plant extract were identified based on their general fragmentation pattern and using reference spectra published by the library–mass spectral databases (National Institute of Standards and Technology [NIST] library version 2005, software, Turbomas 5.2).

Results

In Vitro Ferric-Reducing Antioxidant Power of DCM Leaf Extract of G glauca

The five tested concentrations of the DCM leaf extract of *G glauca* demonstrated a concentration-related increase in ferric reductive activity. All the *G glauca* leaf extract concentrations were statistically similar to the concentration of ascorbic acid in terms of ferric reducing capacity ($P > .01$; Figure 1). However, the ferric-reducing activity of the tested extract concentrations was significantly different from each other with the highest extract concentration being the most effective ($P \leq .01$; Figure 1).

In Vitro DPPH Radical Scavenging Activity of DCM Leaf Extract of G glauca

The DCM leaf extract of *G glauca* demonstrated a concentration-dependent increase in DPPH radical scavenging activity (Figure 2). As Figure 2 shows, in all the tested concentrations, the DPPH radical scavenging activity of *G glauca* was statistically similar to that of the standard compound, vitamin C ($P > .01$). Furthermore, the DPPH radical scavenging activity was significantly different among all the tested extract concentrations ($P \leq .01$). The lowest extract concentration showed the least DPPH radical scavenging activity while the highest extract concentration exhibited the highest activity (Figure 2). The results also revealed that the concentration of DCM leaf extract of *G glauca* required to scavenge the initial DPPH radical concentration by 50% (IC_{50} value) was 1.33 ± 0.03 mg/mL, whereas the IC_{50} value of the standard compound, vitamin C was 1.39 ± 0.06 mg/mL (Table 1).

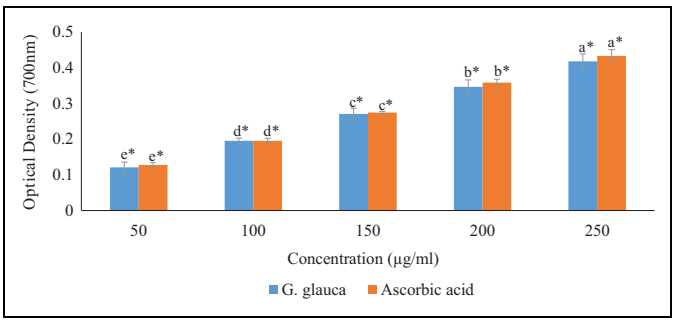

Figure 1. *In vitro* ferric-reducing antioxidant power of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

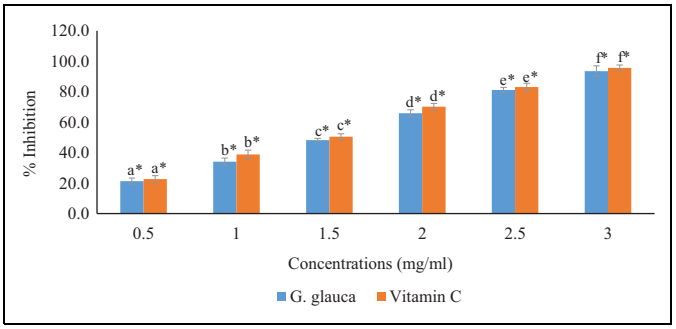

Figure 2. *In vitro* 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

In Vitro Nitric Oxide Radical Scavenging Activity of DCM Leaf Extract of G glauca

The study showed that the DCM leaf extract of *G. glauca* caused a concentration-dependent increase in nitric oxide radical scavenging activity (Figure 3). The nitric oxide radical scavenging activity of all the *G glauca* leaf extract concentrations was not significantly different from that of the standard compound, quercetin ($P > .01$; Figure 3). Across all the tested

Table 1. The Concentration of the Dichloromethanolic (DCM) Leaf Extract of *Gnidia glauca* Required to Inhibit 50% of the Radical Formed.[a]

Type of Radical Formed	Sample	IC_{50} of Sample
1. DPPH radical		
	G glauca	1.33 ± 0.03 mg/mL
	Vitamin C	1.39 ± 0.06 mg/mL
2. Nitric oxide radical		
	G glauca	665.76 ± 334.12 µg/mL
	Quercetin	748.00 ± 145.38 µg/mL
3. Superoxide radical		
	G glauca	119.73 ± 0.20 µg/mL
	Quercetin	121.16 ± 8.64 µg/mL
4. Hydroxyl radical		
	G. glauca	204.34 ± 10.64 µg/mL
	Gallic Acid	210.05 ± 8.80 µg/mL
5. MDA		
	G glauca	120.56 ± 2.51 µg/mL
	Ascorbic acid	128.53 ± 5.99 µg/mL
6. Hydrogen peroxide radical		
	G glauca	0.24 ± 0.01 mg/mL
	Ascorbic acid	0.25 ± 0.01 mg/mL
7. Iron (II)-ferrozine complex		
	G glauca	114.91 ± 1.72 µg/mL
	EDTA	119.22 ± 1.76 µg/mL

Abbreviations: IC_{50}, half maximal inhibitory concentration; DPPH, 2,2-diphenyl-1-picrylhydrazyl; MDA, malondialdehyde; EDTA, ethylenediaminetetraacetic acid.
[a]Results are expressed as means ± SD for replicate measurements n = 3.

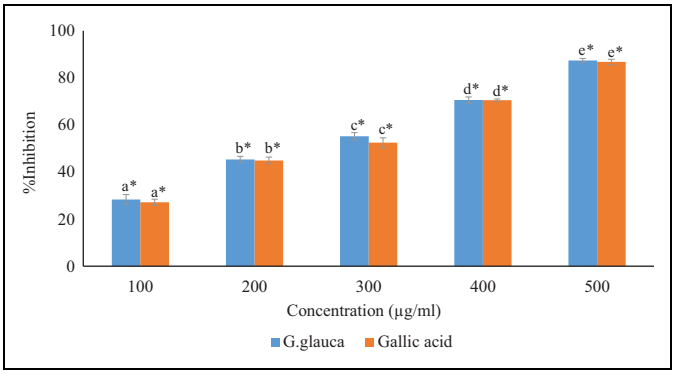

Figure 3. *In vitro* nitric oxide radical scavenging activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

concentrations, the nitric oxide radical scavenging activity of the *G glauca* was significantly different from each other whereby the highest concentration showed significantly higher activity than those of lower extract concentrations ($P \leq .01$; Figure 3). The DCM leaf extract of *G glauca* also showed a lower IC_{50} value of 665.76 ± 334.12 µg/mL than the standard quercetin, which had an IC_{50} value of 748.00 ± 145.38 µg/mL (Table 1).

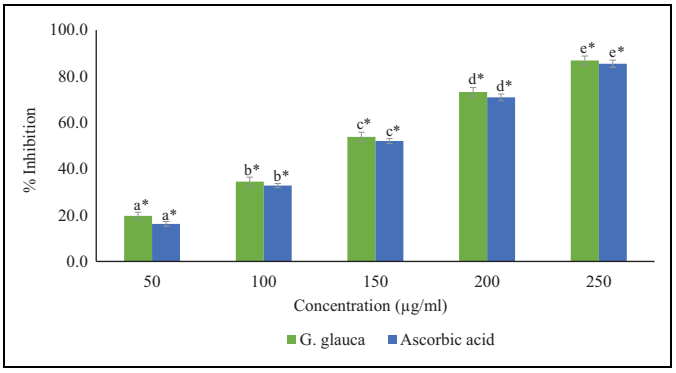

Figure 4. *In vitro* superoxide radical scavenging activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

In Vitro Superoxide Radical Scavenging Activity of DCM Leaf Extract of G glauca

As Figure 4 indicates, the DCM leaf extract of *G glauca* caused an exponential increase in superoxide radical scavenging activity from the lowest extract concentration to the highest extract concentration. The superoxide radical scavenging activities of *G glauca* and the standard compound, quercetin, were comparable ($P > .01$; Figure 4). The effectiveness of the extract in scavenging the superoxide radicals was higher in the highest extract concentration than in lower concentrations of the extract (Figure 4). The concentration of the DCM leaf extract of *G glauca* required to inhibit superoxide radical formation by 50% was 119.73 ± 0.20 µg/mL, whereas the standard, quercetin, showed a higher IC_{50} value of 121.16 ± 8.64 µg/mL (Table 1).

In Vitro Hydroxyl Radical Scavenging Activity of DCM Leaf Extract of G glauca

The DCM leaf extract of *G glauca* displayed potent efficacy of hydroxyl radical scavenging activity across all the extract concentrations (Figure 5). The ability of *G glauca* leaf extract to scavenge hydroxyl radicals occurred in a concentration-dependent manner (Figure 5). As Figure 5 shows, the hydroxyl radical scavenging activity of the standard compound, gallic acid, was statistically similar to that of *G glauca* leaf extract in all the tested concentrations ($P > .01$). Among all the *G glauca* leaf extract concentrations, the hydroxyl radical scavenging activity was significantly different from each other (Figure 5). As the concentrations increased, the capacity of the extract to scavenge hydroxyl radicals also increased significantly with the highest extract-concentration exhibiting the highest activity ($P \leq .01$; Figure 5). Findings of the present study also revealed that the DCM leaf extract of *G glauca* had a lesser IC_{50} value of 204.34 ± 10.64 µg/mL than the standard

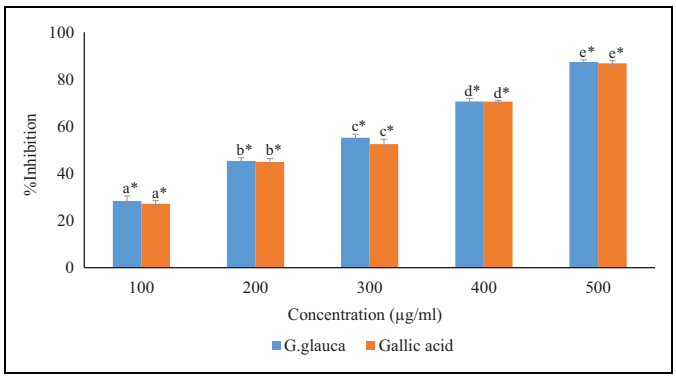

Figure 5. *In vitro* hydroxyl radical scavenging activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

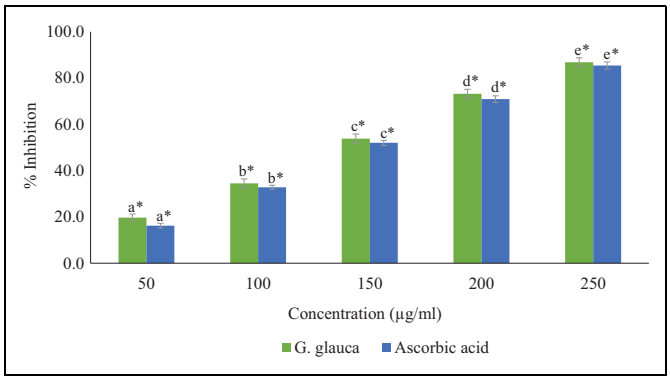

Figure 6. *In vitro* lipid peroxidation inhibition activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

compound, gallic acid, whose IC_{50} value was 210.05 ± 8.80 µg/mL (Table 1).

In Vitro Lipid Peroxidation Inhibition Activity of DCM Leaf Extract of G glauca

The DCM leaf extract of *G glauca* exhibited a concentration-dependent increase in lipid peroxidation inhibition (Figure 6). The lipid peroxidation inhibitory effect of the *G glauca* leaf extract was significantly different among the extract concentrations ($P \leq .01$; Figure 6). Furthermore, the highest extract concentration showed significantly greater inhibition (86.86%) than the lowest extract concentration, which inhibited 19.71% of MDA. The inhibition of lipid peroxidation by *G glauca* at different concentrations was statistically similar to that of the standard compound, ascorbic acid ($P > .01$; Figure 1.6). The DCM leaf extract of *G glauca* showed a lower IC_{50} value of 120.56 ± 2.51 µg/mL than the standard, ascorbic acid, which had an IC_{50} value of 128.53 ± 5.99 µg/mL (Table 1).

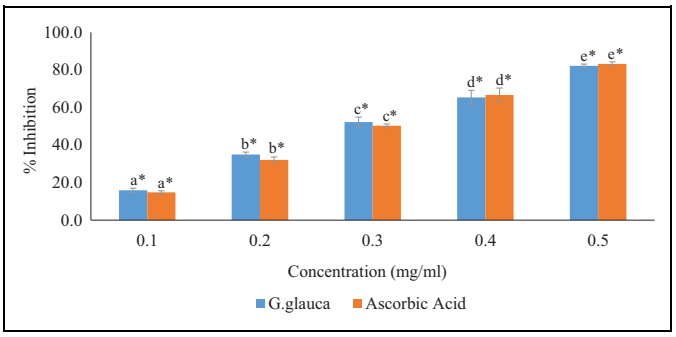

Figure 7. *In vitro* hydrogen peroxide radical scavenging activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

In Vitro Hydrogen Peroxide Radical Scavenging Activity of DCM Leaf Extract of G glauca

As observed, the activity of DCM leaf extract of *G glauca* in scavenging hydrogen peroxide radical occurred in a concentration-dependent manner (Figure 7). All the tested concentrations of the *G glauca* leaf extract showed statistically similar hydrogen peroxide radical scavenging activity to that of the standard compound, ascorbic acid ($P > .01$; Figure 7). The hydrogen peroxide radical scavenging activities among all the tested concentrations of the *G glauca* leaf extract were statistically significant from each other ($P \leq .01$). The highest extract concentration was more effective than those of lower extract concentrations (Figure 7). The concentration of the DCM leaf extract of *G glauca* required to inhibit hydrogen peroxide radical formation by 50% (IC_{50} value) was 0.24 ± 0.01 mg/mL, whereas the IC_{50} value for the standard, ascorbic acid, was 0.25 ± 0.01 µg/mL (Table 1).

In Vitro Iron-Chelating Activity of DCM Leaf Extract of G glauca

The results also revealed that there was a concentration-related increase in iron chelating activity of the DCM leaf extract of *G glauca* (Figure 8). The potential to inhibit the formation of iron (II)–ferrozine complex was significantly different among all the concentrations ($P \leq .01$; Figure 8). The highest extract concentration showed significantly higher activity than those of the lower extract concentrations ($P \leq .01$). The iron-chelating activities exhibited among the 5 extract concentrations were statistically comparable to that of the standard compound, EDTA ($P > .01$; Figure 8). Furthermore, it was observed that the IC_{50} value of the DCM leaf extract of *G glauca* was 114.91 ± 1.72 µg/mL, whereas the IC_{50} value for the standard, EDTA, was 119.22 ± 1.76 µg/mL (Table 1).

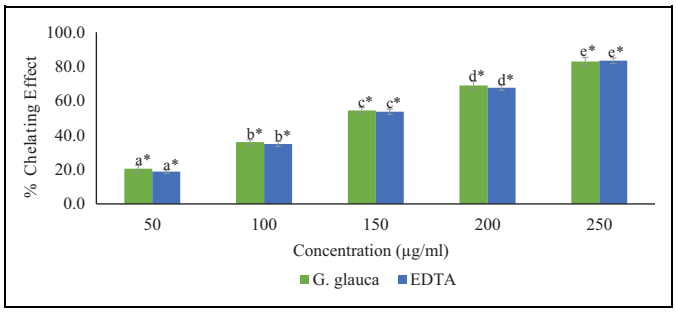

Figure 8. *In vitro* iron-chelating activity of dichloromethanolic (DCM) leaf extract of *Gnidia glauca*. Bar graphs with different letters across the tested concentrations are statistically significant ($p \leq 0.01$). Bar graphs with asterisks (*) within the same concentration are not significantly different ($p > 0.01$).

Table 2. Quantitative Analysis of Phytochemical Compounds in Dichloromethanolic (DCM) Leaf Extract of *Gnidia glauca*.[a]

RT	Compound Name	Concentration (mg/kg)
11.51	3,4,5-Trihydroxybenzoic acid (gallic acid)	10.24 ± 1.02
15.12	3,7-Dimethyl-2,6-octadiene-1-ol acetate (neryl acetate)	9.77 ± 1.81
15.19	Ferulic acid	10.18 ± 1.14
21.53	Phenol, 2,4-bis(1,1-dimethylethyl)-	10.15 ± 1.58
24.73	3,5-dihydroxy-trans-stilbene (pinosylvin)	13.39 ± 4.06
25.96	Naringenin chalcone	7.71 ± 1.63
26.98	Luteolin	9.77 ± 2.62
29.22	Curcumin	16.91 ± 2.30
30.24	Quercetin	15.74 ± 1.01
32.23	Gallocatechin-catechin flavan	10.40 ± 1.00
35.41	Vitamin E	12.25 ± 1.67
35.48	Stigmasterol	7.75 ± 2.23
36.29	Squalene	7.48 ± 0.52
36.82	α-Amyrin	5.25 ± 0.78
37.85	β-Amyrin	6.98 ± 2.47
24.92	Catechins	9.27 ± 2.05

Abbreviation: RT, retention time.
[a]Results are expressed as means ± SD for replicate measurement n = 3.

Identification and Characterization of Bio-Compounds in the DCM Leaf Extract of G glauca

The GC-MS analysis of DCM leaf extract of *G glauca* revealed the presence of the following phytochemicals that have been associated with antiobesity effects: curcumin, quercetin, stilbenes (pinosylvin), vitamin E, flavanols, neryl acetate, squalene, gallocatechin-catechin flavan, gallic acid, catechins, luteolin, naringenin chalcone, terpenoids and sterols, α-amyrin, β-amyrin, and ferulic acid (Table 2).

Discussion

A redox imbalance in favor of pro-oxidants results in overproduction of ROS, which constitutes primary catalysts that initiate bimolecular oxidation that causes oxidative stress.[57,58] Antioxidants reconcile the upshot of free radicals by directly reacting, neutralizing, or competing for substrates whose terminal electron acceptor is molecular oxygen (O_2).[59] Molecular oxygen, therefore, acts as a thermodynamic sink.[60] Several synthetic antioxidant agents commercially available are reported to be toxic and carcinogenic, offering natural antioxidants from medicinal plants as better alternatives against oxidative deterioration.[61] Herbal medicines offer useful therapeutic agents in the management and prevention of oxidative stress–related degenerative diseases.[44] In this study, the dichloromethanolic leaf extract of *G glauca* demonstrated significant in vitro antioxidant and free radical scavenging activities.

The reducing power ability of a chemical compound is based on its reductive capacity in a Fe^{3+}-Fe^{2+} system.[62,63] Usually, biologically active compounds with ferric reducing power capacity are electron donors and are able to reduce the oxidized intermediates such as those of lipid peroxidation processes.[61,64] The results obtained in this study indicated that the ferric reducing capacity of *G glauca* at various concentrations (50-250 µg/mL) conformed to Beer's law at 700 nm.[49] The reduction of Fe^{3+} to Fe^{2+} is an indicator of the extract's electron-donating ability.[46] The amount of Fe^{2+} complex (Perl's Prussian blue ferric ferrocyanide, $(Fe_4[Fe(CN)_6]_3)$] formed was directly proportional to the measured absorbance at 700 nm and is indicative of an increase in the reductive ability of the extract. Therefore, an increase in optical density indicates a higher reductive ability.[65] Previous studies on ethanolic seed extracts of *Trachyspermum ammi* also demonstrated a concentration-dependent increase in reducing power potential.[45] Besides, the reducing capabilities of the root extract of *Biophytum sensitivum* were found to be dose dependent and comparable to the reference compound, quercetin.[66]

The in vitro DPPH radical inhibitory assay is based on an antioxidant's hydrogen donating ability to reduce DPPH radical in methanol to form the nonradical DPPH-H.[67] In the present study, *G glauca* extract demonstrated a remarkable concentration-dependent DPPH radical scavenging activity. The interaction between the extract and DPPH might have occurred through the transfer of electrons and hydrogen ions to 2,2-diphenyl-1-picrylhydrazyl radical to form a stable 2,2-diphenyl-1-picrylhydrazine molecule (DPPH).[68] The DPPH radical usually has a strong absorbance at the wavelength of 517 nm. However, on acceptance of an electron or hydrogen atom from an antioxidant compound, it becomes a stable diamagnetic molecule with decreased absorbance at 517 nm.[69] The resulting color change from purple to pale yellow determines the antiradical power of an antioxidant.[69] A stable diamagnetic free radical, DPPH, has been widely applied as a sensitive and rapid tool for estimation of free radical scavenging activities of both lipophilic and hydrophilic antioxidants.[68] The IC_{50} value of *G glauca* was less than that of the standard, vitamin C. Lower IC_{50} value is an indication of a high DPPH free radical scavenging activity at low extracts concentrations.[53]

Previous researches have also demonstrated DPPH radical scavenging activities of various plant extracts. Methanolic

whole plant extract of *B sensitivum* exhibited antiradical activity in scavenging DPPH radical with a maximum inhibition of about 43.96%.[66] The leaf, flower, and stem extracts of *Thymelaea hirsuta* also demonstrated a concentration-dependent scavenging activity on DPPH radicals.[36]

Sodium nitroprusside spontaneously generates nitric oxide in aqueous solution at physiological pH, which interacts with molecular oxygen forming nitrite ions that may be estimated using Griess reagent.[45] In the present study, *G glauca* demonstrated a concentration-dependent increase in nitric oxide radical scavenging activity. The half-maximal activity (IC_{50}) of *G glauca* extract was also lower than that of the standard. Consistent with this study, *Strychnos henningsii* extract was found to cause moderate concentration-dependent scavenging of nitric oxide with an IC_{50} of 0.49 mg/mL.[44] The antioxidant potency of scavenging nitric oxide by methanolic leaf extracts of *Phyllanthus freternus*, leaves, barks, and roots of *Triumfetta rhomboidae* and barks of *Casuarina littorea* resulted in linear time-dependent nitrite production.[70] *Newbouldia laevis* was also found to inhibit nitrite formation by direct competition with molecular oxygen.[71]

Nitric oxide is a cell-signaling molecule generated by specific nitric oxide synthase through which arginine is metabolized to citrulline with the formation of NO via a 5-electron oxidative reaction.[70] Nitric oxide plays a vital physiological role in respiratory, immune, and neuromuscular systems.[72] It affects the release of neurotransmitters, neuronal excitability, enhances neurotoxin-induced cellular damage and neuronal cell death (observed in Parkinson's disease and Alzheimer's disease). It modulates spatial learning and memory retention processes (cognitive impairment).[72] Nitric oxide is also associated with inflammatory bowel syndrome, juvenile diabetes, sepsis, arthritis, carcinomas, dementia, multiple sclerosis, stroke, as well as ulcerative colitis.[58]

The riboflavin-light-NBT system generates superoxide anions that reduce the yellow dye (NBT^{2+}) to produce the blue formazan monitored spectrophotometrically at 562 nm. Antioxidants inhibit formazan formation by scavenging the superoxide radicals in the reaction mixture.[46,47] The observed decrease in absorbance caused by the *G glauca* extract at 562 nm is indicative of the ability of the extract to quench the superoxide radicals in the reaction mixture.[46] The lower IC_{50} value of the extract than that of the standard exhibits stronger free radical scavenging activity.[46] Superoxide radical scavenging activities of the *Newbouldia laevis* plant extract increased markedly with increasing concentrations.[71] Similarly, previous studies also demonstrated the abilities of fruit extracts of *Terminalia chebula*, *Terminalia belerica*, and *Emblica officinalis* to quench superoxide radicals from the reaction mixture in a concentration-dependent manner.[73]

The superoxide anion is an oxygen-centered and relatively weak oxidant with a selective reactivity generated by numerous biological and metabolic reactions in the human body.[73] Although superoxide radicals exhibit only limited chemical reactivity in biological systems, they act as potential precursors of highly ROS such as hydroxyl radical, hydrogen peroxide and singlet oxygen, which result in lipid peroxidation thereby exacerbating oxidative stress.[73,74] Therefore, this makes superoxide radical scavenging capacity as a first-line defense mechanism against oxidative damage.[73]

In this study, the ability of the *G glauca* leaf extract to inhibit hydroxyl radical–mediated deoxyribose damage was evaluated by the Fenton's reaction using iron (II)–dependent DNA damage assay.[48] The hydroxyl radicals generated by the Fenton's reaction degrade DNA deoxyribose sugar, using Fe^{2+} salts as a catalytic component.[75] *G glauca* exhibited the ability to quench hydroxyl radicals from the sugar, halting the reaction and thereby forming a fading pink chromophore as the extract's concentration increases. Similarly, *Trachyspermum ammi* seeds showed hydroxyl radical scavenging activities in a concentration-dependent manner.[45] The leaf, stem, and root extracts of *Clerodendrum viscosum* also exhibited the ability to inhibit hydroxyl radical–mediated deoxyribose degradation in a Fe^{3+}-EDTA-ascorbic acid and H_2O_2 reaction mixture.[76]

Hydroxyl radical is the most potent ROS in free radical pathology of biological systems capable of damaging exclusively all cellular components.[77] Hydroxyl radicals are usually formed from superoxide anion and hydrogen peroxide in the presence of metal cations such as Fe^{2+} and Cu^{+}. These highly reactive radicals cause oxidative damage to DNA, lipids, and proteins.[75] Hydroxyl radical oxidizes polyunsaturated fatty acid moieties of the cell membrane phospholipids and initiates lipid peroxidation.[77] The hydroxyl radical also damages nucleic acid by causing polynucleotide strand breakage and alteration of the structure of DNA bases thereby contributing to cytotoxicity, mutagenicity, and carcinogenicity.[75]

Lipid peroxidation is the free radical–mediated glycation and protein-modifying reactions in cellular components.[78] In this study, the inhibition of hydroxyl ion–induced lipid peroxidation by the *G glauca* extract resulted in a concentration-dependent decrease in MDA production estimated by TBA reaction with an absorption maximum at 532 nm. This capacity might be due to the extracts' ability to scavenge the hydroxyl radicals generated during the decomposition of hydrogen peroxide.[79] Consistent with this study, reports have indicated that essential oils derived from *Eryngium creticum* exhibited an antilipid peroxidation effect in a similar manner.[46]

In biological systems, lipid peroxidation is initiated by generation of hydroxyl and superoxide radicals, which accelerates the decomposition of lipid hydroperoxides into peroxyl and alkoxyl radicals that eventually propagate the chain reaction in lipids.[49] Several aldehyde products are eventually produced, among which MDA is the most important derivative.[80] Production of excess amount of highly ROS in the biological systems forms the hallmark of the modifications in cellular membrane function and structure through reduction of membrane lipid fluidity and increase in membrane permeability.[81]

MDA is an important biomarker of lipid peroxidation, which has been associated with the pathogenesis of various disorders among which are inflammation, cancer,[82] atherosclerosis,[83] diabetes mellitus,[84] Alzheimer's disease,[85] as well

as degradation of lysosomes and mitochondrial swellings and disintegration.[48]

Hydrogen peroxide is an important ROS with the ability to directly inactivate enzymes by oxidation of essential thiol (-SH) groups.[78] It rapidly penetrates biological membranes and once inside the cell, it interacts with redox-active transitional elements such as Fe^{2+} and possibly Cu^{+} ions via the Harber-Weiss reaction to generate the highly reactive hydroxyl radicals initiating an oxidative attack.[86,87] In this study, the DCM leaf extract *G glauca* exhibited the ability to inhibit hydrogen peroxide radical in a concentration-related manner. The activity was may be attributable to the presence of phenolic compounds in the plant extract that can donate an electron to H_2O_2 and thus neutralizing it to water.[88] In addition, this could be due to its ability to catalyze peroxidases to decompose hydrogen peroxide to water and oxygen.[78] Consistent with this study, the leaves, flowers, and fruits of *Crataegus monogyna* extract demonstrated the ability to scavenge hydrogen peroxide in an amount dependent manner.[89] Another study demonstrated that methanolic extract of *Trichodesma zeylanicum* caused a strong dose-dependent inhibition of hydrogen peroxide.[90]

Ferrozine can quantitatively react with Fe^{2+} to form a red-colored complex.[78] The presence of a chelating agent in the reaction mixture limits the formation of ferrozine-Fe^{2+} complex and results in the decrease in the intensity of the red color formed with an increase in the concentration of the chelating agent.[78] As demonstrated in this study, the plant extract caused a concentration-dependent reduction in color change due to its competition with ferrozine for the ferrous ions, thereby inhibiting the formation of a ferrozine-Fe^{2+} complex.[56] A similar study demonstrated dose-dependent inhibition of the formation of the ferrozine-Fe^{2+} complex by the action of *Clerodendrum viscosum*.[76] *Mellilotus arvensis* extract inhibited the formation of ferrous and ferrozine complex, signifying that it has chelating activity and captures ferrous ion before ferrozine.[91]

The dual oxidation state characteristic of iron enables it to accept or donate electrons through redox reactions.[78] The capacity of iron to interact with superoxide anion (O_2^{-}) and hydrogen peroxide results in the formation of reactive hydroxyl radical ($OH^{\cdot}$) through Haber-Weiss reaction, which exacerbates damage to the cell membrane, proteins, and nucleic acids.[78] Moreover, the dual oxidation state of iron enables the acceleration of lipid peroxidation through the decomposition of lipid hydroperoxides into peroxyl and alkoxyl radicals responsible for the perpetuation of the oxidative-chain reactions.[92]

Obesity is a complex metabolic disorder characterized by an increased adipose tissue mass due to positive energy balance.[93] The epidemic of obesity is currently on the rise probably due to increasingly sedentary lifestyles combined with easy availability of palatable, high-fat foods. It presents modifiable risk factors for type 2 diabetes mellitus, cardiovascular disease, dyslipidemia, hepatocellular carcinoma, and nonalcoholic fatty liver disease (steatosis).[94] Globally, its prevalence has shown a startling increase in all age-groups and has been associated with morbidity and mortality. The low-grade chronic systemic inflammation of the adipose tissue stimulates the production of ROS, which in turn precipitates oxidative stress. Besides, under obesogenic states, the compromised redox homeostatic status characterized by attenuated antioxidant defense systems serves as a prerequisite for the pathogenesis of obesity-related diseases.[25] The upsurge in the prevalence of obesity and associated morbidity has presented unmet medical needs for safe and effective therapies.[95]

Attempts to decrease fat mass via pharmacological reduction of energy intake and fat mobilization have had limited potency and/or intolerable side effects.[96] Newer insights into traditionally used medicinal plants are indispensable for exploration of their novel bioactive components.[97] Some of the postulated mechanisms of activity of herbal medicines in management of obesity is through appetite suppression via central receptors (NPY, AgRP, CB-1, and dopamine receptors), inhibition of triglyceride absorption, increase in lipolysis, improvement of glycemic control, adipose tissue differentiation as well as increase in energy expenditure and thermogenesis.[98] Earlier studies have also shown some antiobesity biomaterials exhibiting appetite-repression activity. For instance, the oral administration of 100 mg/kg of *Phaseolus vulgaris* and *Robinia pseudoaccacia* in Harlan-Wistar rats caused a 16-hour 8.25-fold decrease in food intake.[99] Moreover, administration of 20, 40, 80, and 150 mg/kg of aqueous extract of *Allium saralicumin* rats fed with high-fat diet for 4 months caused a decrease in body weight and levels of serum cholesterol, low-density lipoprotein (LDL), and triglycerides.[10] Moreover, studies on seeds of *Alpinia zerumbet* elevated high-density lipoprotein (HDL)-cholesterol levels because of its high contents of rutin, quercetin, and polyphenols.[100]

Previous studies have demonstrated various anti-obesity biomaterials having an inhibitory effect against pancreatic lipase. For instance, crude ethanol and water extract of *Illicium religiosum* (wood) and *Juniperus communis* (bark) exhibited the inhibitory activity of pancreatic lipase.[101] Besides, the chemical compound, proanthocyanidin of *Cassia mimosoides* inhibited the activity of pancreatic lipase in rats fed with high-fat diet for 8 weeks. This subsequently resulted in approximately 60% decrease in body weight gain.[102]

Previous studies on antiobesity herbs that promote energy expenditure indicated that oral administration of 400 mg/kg crude aqueous extract of *Pinellia ternate* in obese Zucker rats for 6 weeks, resulted in decreased bodyweight gain, increased UCP-1 expression in BAT as well as overexpression of PPARα in WAT.[103] Some studies have also demonstrated various antiobesity biomaterials having an inhibitory effect against adipocyte differentiation. For instance, genistein, the active component isolated from *Glycine max* exhibited a 60% inhibition of preadipocyte differentiation 48 hours following its administration.[104] Similarly, treatment with epigallocatechin gallate of *Camellia sinensis* (green tea) inhibited preadipocyte differentiation by 7-fold 48 hours after its administration.[105] Antiobesity biomaterials promoting lipid metabolism were also reported in some studies using animal models. For example, administration of 900 mg/kg aqueous extract of *Salacia oblonga* (root) in ZDF rats for 28 days caused 40% decrease

in liver/body weight ratio through hepatic PPARα activator.[106] Moreover, treatment with 1% crude ethanolic leaf extract of *Nelumbo nucifera* mice fed with high-fat diet for 12 weeks caused a 15% decrease in body weight gain through activation of the β-adrenergic receptor.[107]

Attempts to mitigate obesity and associated complications could provide an avenue to enhancing life-longevity. Obesity-induced diseases often result in premature death and/or reduced life span.[6] Since obesity exacerbates oxidative damage, therapeutic application with herbal medicines is of supreme option in situations of infectivity of synthetic drugs.[10] The chemical compounds contained in these natural products confer their antioxidant activities through neutralization of free radicals into less active stable products, blockage of the initiators of free radical attack,[27] regulation of electron transport chain, repair of oxidized proteins, termination of chain reaction effect, and salvage of the oxidized antioxidants thereby restoring the cells' functional capacity.[27,92]

The antioxidant activity exhibited by DCM leaf extract of *G glauca* can be attributed to the presence of various phytochemicals that are thought to function interactively and synergistically to neutralize ROS and RNS.[27] The GC-MS analysis of *G glauca* revealed the presence of bioactive compounds among which are phenolic compounds (flavonoids, stilbenes, chalcones, tannins), lipids (fatty acid esters and phytosterols), terpenoids (monoterpene, diterpenes, and triterpenes) as well as vitamin E. These bioactive compounds have been shown to maintain the redox homeostasis through multiple-step processes of antioxidant reactions which involves initiation, propagation, branching and termination of free radicals.[27]

Flavonoids are bioactive phenols with antioxidant, anti-inflammatory, antiobesity, and antidiabetic properties.[108] Flavonoids exert their antioxidant activities through quenching or scavenging of free radicals, chelating of metal ions, donation of an electron and hydrogen ion, and inhibition of enzymatic systems responsible for the generation of free radicals.[109] Flavonoids bind with transition metal elements such as iron and copper and plays a key role in inhibiting metal-catalyzed free radical formation.[110] The chelation effect of flavonoids inhibits lipid peroxidation, Fe^{2+} catalyzed the oxidation of glutamine synthase and oxidation of linoleic acid through the removal of metal ions from catalytic sites and scavenging of free radicals.[111] The reducing power (ability to donate electron and hydrogen ions) of flavonoids contributes to the termination of lipid peroxidation chain reaction.[112] Flavonoids interact with various signaling pathways that regulate the cell cycle, differentiation, and apoptosis.[113]

Stilbenes such as pinosylvin and resveratrol have been implicated in scavenging of most oxidizing molecules such as singlet oxygen, and other free radicals.[114] They suppress the formation of reactive oxygen species, scavenge reactive species, chelate metal ions involved in the production of free radicals as well as protects and up-regulate antioxidant defenses.[114]

Quercetin exhibits its antioxidant activities through its ability to scavenge superoxide radicals, hydroxyl radicals, and lipid peroxyl radicals.[115] Previous studies in mice showed that quercetin supplementation normalized the concentration of nitric oxide, glutathione, and glutathione peroxidase thereby protecting the liver from oxidative damage.[116] Another study reported that quercetin conferred neuroprotection against neurotoxicity of amyloid β-peptide via its acetylcholinesterase inhibitory property and free radical scavenging effects.[117] Quercetin modulates its anti-inflammatory effects via antioxidant responsive elements (ARE), nuclear factor-kappa B (NF-κB), and xenobiotic responsive elements.[118] The anti-adipogenesis effects of quercetin are mediated by the adenosine monophosphate-activated protein kinase (AMPK) in preadipocytes and mitogen-activated protein kinases signaling pathways (MAPK) in mature adipocytes.[119]

Studies have shown that vitamin E (tocopherols and tocotrienols) results in a significant reduction in lipid peroxides, nitrogen dioxide, singlet oxygen, and superoxide anion in plasma.[120] The mechanism of their enhanced efficiency of scavenging hydroxyl, peroxyl, and alkoxyl radicals is based on the ease with which the hydrogen on the hydroxyl group of their chroman ring can be donated to neutralize the free radicals. This, in turn, creates a more stable tocopheroxyl radical.[121] Vitamin E increases the levels of serum glutathione and stimulates the catalytic activity of glutathione peroxidase and catalase.[122] Tocotrienols are also reported to inhibit LDL oxidation.[123] Oxidized LDL is a potent chemokine that induces an influx and adhesion of monocytes as well as facilitates the activation and recruitment of macrophages.[124] The presence of monocytes and activated macrophages stimulates the production of proinflammatory cytokines such as TNF-α and IL-6.[121] The ability of vitamin E to inhibit the oxidation of LDL prevents the buildup of plaques in arteries thereby conferring protection against cardiovascular diseases such as atherosclerosis, heart attack and stroke.[124] Besides, it signifies its anti-inflammatory effects.[121]

Terpenoids and sterols quench free radicals, acts as reducing agents and are involved in termination of the free radical chain reaction.[125] Sterol esters reduce the concentration of LDL cholesterol in plasma.[125] The α-amyrin acetate isolated from the fruits of *Ficus racemose* showed hypoglycemic effects in the streptozotocin-induced diabetic rat model and prevented oxidation of LDL.[125]

Squalene is implicated in protection from obesity-mediated inflammation through its antioxidant activity.[126] Squalene inhibits gene expression of proinflammatory mediators by enhancing histone deacetylase activity and activates the transcription factors that antagonize chronic inflammation.[127]

Naringenin chalcone showed neuroprotective activity by reducing inflammatory load and prevention from oxidative damage thereby increasing neurogenesis and intraneuronal signaling.[128] It also showed its anticancer activity by downregulating the gene expression of cyclooxygenase 2 (COX-2).[129] Gallocatechin-catechin flavan reduces the levels of inflammatory cytokines such as TNF-α and IL-6 in plasma.[130]

Curcumin has been shown to inhibit the generation of ROS in macrophages and red blood cells.[131] Curcumin effectively scavenges different classes of free radicals such as superoxide

and hydroxyl anions. The metal-chelating activity of curcumin and the metal complexes of curcumin are reported to be effective radical scavengers.[132] In addition, curcumin inhibits nitric oxide synthase enzymes resulting in decreased levels of nitric oxide.[133] Curcumin was found to upregulate the expression levels and the catalytic activity of different antioxidant enzymes such as catalase, superoxide dismutase, activated protein-1, heme oxygenase-1 and glutathione peroxidase.[134] Studies demonstrated that treatment with curcumin efficiently mitigated lipid peroxidation.[135] Curcumin regulates the antioxidant response by inhibiting the phosphorylation of Akt and ERK.[136] Moreover, curcumin modulates cell death by reducing the expression levels of TNF-α and endogenous Bcl-xL and Bcl-2.[137]

Catechins exert their antioxidant and anti-inflammatory effects through enhancing the activity of antioxidant enzymes that are regulated by nuclear factor erythroid 2p45 (NF-E2)-related factor 2 (Nrf2) bound to the AREs.[138,139] Catechins reduce inflammation via inhibition of prostaglandin production and NF-κB activity.[140,141] Studies have demonstrated that catechins decrease uptake of LDL by macrophages, inhibit LDL oxidation, and lower LDL aggregation.[135]

Gallic acid is a strong antioxidant compound that has been found to inhibit lipid peroxidation through the reduction in levels of oxidized plasma MDA.[142] Gallic acid hinders the oxidation of LDL and reduces levels of plasma TBA reactive substance (TBARS).[143] Another compound, neryl acetate, increases the bioavailability of reduced glutathione and stimulates synthesis and catalytic activity of glutathione peroxidase.[77]

Ferulic acids reduce levels of cytokines and C-reactive proteins in plasma.[144] The increased circulating levels of pro-inflammatory cytokines and C-reactive proteins are definitive of low-grade systemic inflammation, a hallmark of metabolic syndromes. Ferulic acids inhibit transcription factor Nfκβ and increase the threshold for an inflammatory response by enhancing the binding of short-chain fatty acids to G-protein coupled receptors.[145]

Luteolin reduces levels of lipopolysaccharides in obesity-related inflammatory liver diseases.[146] Diet supplementation of luteolin decreased markers of inflammation in adipose tissue by restricting the translocation of lipopolysaccharides from the large bowel in the high-fat diet-fed mice models.[145]

Conclusion

Based on the outcomes of this research, it is concluded that DCM leaf extract of *G. glauca* exhibited significant reducing power abilities, DPPH radical scavenging activities, nitric oxide radical scavenging activities, hydrogen peroxide scavenging potential, superoxide radical scavenging activities, hydroxyl radical ($^{\cdot}OH$) scavenging activities, inhibition of lipid peroxidation as well as iron (Fe^{2+}) chelating activities. These activities were comparable to those of the standard compounds used. Interestingly, the plant extract recorded a lower IC_{50} value than those of standards, an indication of high free radical scavenging activities at low extracts concentrations.

Obesity is associated with increased circulating levels of free fatty acids and systemic pro-inflammatory cytokines, prostaglandins, and nitric oxide, which in turn precipitates oxidative stress. Obesity-induced oxidative damage is a prerequisite of many degenerative diseases such as cognitive impairment, anxiety, diabetes mellitus, arteriosclerosis, panic attacks, some cancers, and aging. Amelioration of mediators of an oxidative attack under obesogenic states is key in protecting from metabolic and symptomatic complications of obesity. The antioxidant and free radical-scavenging activities of *G glauca* could be attributed to the presence of phytochemicals that have been associated with antioxidant effects. Therefore, under obesogenic states, the synergistic effects of these bioactive compounds increase their bioavailability and action on multiple molecular targets thereby correcting imbalance-mediated oxidative stress. Overall, findings of this research indicate that *G glauca* can be a useful therapeutic agent in the prevention of obesity-induced oxidative damage. However, there is a need to conduct further studies to isolated individual biologically active compound from *G glauca* in order to establish their precise mode of action.

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Authors Contributions

AW carried out the study and wrote the manuscript. PMN designed the research and co-directed the research work. KCM and MJN and contributed to conception of the review and supervised the manuscript writing. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical Approval

The experimental protocols and procedures used in this study were approved by the Ethics Committee of Kenyatta University, Kenya.

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