

DNA Damage Protecting Activity and Antioxidant Potential of *Launaea taraxacifolia* Leaves Extract

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Abstract

Background: The leaf extract of *Launaea taraxacifolia* commonly known as African Lettuce is used locally to treat dyslipidemia and liver diseases, which are associated with oxidative stress. Methanol extract from *L. taraxacifolia* leaves was tested for its antioxidant activity and its ability to protect DNA from oxidative damage. **Materials and Methods:** *In vitro* antioxidant potential of the leaf extract was evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH), nitric oxide (NO), and hydroxyl (OH) radical scavenging assays. Ferric reducing power, total antioxidant capacity (TAC), metal chelating, and anti-lipid peroxidation ability of the extract were also examined using gallic acid, ascorbic acid, citric acid, and ethylenediaminetetraacetic acid as standards. **Results:** *L. taraxacifolia* leaves extract showed antioxidant activity with IC₅₀ values of 16.18 µg/ml (DPPH), 123.3 µg/ml (NO), 128.2 µg/ml (OH radical), 97.94 µg/ml (metal chelating), 80.28 µg/ml (TAC), and 23 µg/ml (anti-lipid peroxidation activity). *L. taraxacifolia* leaves extract exhibited a strong capability for DNA damage protection at 20 mg/ml concentration. **Conclusion:** These findings suggest that the methanolic leaf extract of *L. taraxacifolia* could be used as a natural antioxidant and also as a preventive therapy against diseases such as arteriosclerosis associated with DNA damage.

Keywords: Antioxidant, DNA damage, *Launaea taraxacifolia*, oxidative stress, scavenging

INTRODUCTION

Reactive oxygen species (ROS) such as superoxide anion, hydroxyl (OH) radicals, and hydrogen peroxide radicals are generated spontaneously in living organisms during metabolism. An imbalance between the generation of ROS and cellular antioxidant capacity can lead to oxidative stress.^[1,2] ROS are implicated in the etiology of several degenerative diseases such as stroke, rheumatoid arthritis, diabetes mellitus, peptic ulcer, and cancer.^[3,4] Phytomedicine plays a very important role in the management of most of these diseases. Studies have shown that the consumption of polyphenolic compounds found in tea, fruits, and vegetables is associated with low risk of these diseases.^[5] Consequently, there is a growing research interest in edible plants that contain antioxidants and health-promoting phytoconstituents as potential therapeutic agents.

One such plant is African Lettuce (*Launaea taraxacifolia*), a tropical herb belonging to the *Asteraceae* family. It is a perennial plant which can grow up to 150 cm tall. The plant which is held in the soil by a creeping root system has an erect stem with leaves at the base in a rosette form capped by golden yellow flowers.^[6] The leaves of *L. taraxacifolia* plant have been used traditionally in Senegal, Sierra Leone, Nigeria, and Ghana for centuries as vegetables in salads and sauces as well as remedy for several ailments including dyslipidemia, liver diseases, and heartburns.^[7] The plant has been shown

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to possess anticancer properties.^[8] Previous studies on the leaves revealed the presence of flavonoids, tannins, terpenoids, saponins, steroids, and bioactivities such as hypolipidemic, renoprotective, hepatoprotective.^[9-12]

In recent years, research in phytomedicine has increased with interest in natural products that possess antioxidant properties and also have the ability to protect against DNA damage. Synthetic antioxidants such as butylated hydroxyanisole, butylated hydroxytoluene, rutin, and tert-butylhydroquinone are widely used.^[13] However, there are reports that most of these synthetic drugs have adverse effects including enzymatic and lipid alterations, high toxicity, cell damage, inflammation, and atherosclerosis along with moderate antioxidant activity.^[14,15] Therefore, a search for natural antioxidants is important as they are perceived to have minimal health risks to consumers. In spite of the wide use of the leaves of *L. taraxacifolia* as food and medicine, little is known about its antioxidant and DNA-protecting abilities. The present study sought to evaluate the antioxidant potential of methanolic extract obtained from the leaves of *L. taraxacifolia* plant and further assess its potential in protecting against DNA oxidative damage.

MATERIALS AND METHODS

Chemicals and reagents

The followings are the chemicals used in the study: 1,1-diphenyl-2-picrylhydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], gallic acid (GA), ascorbic acid (AS), and $FeCl_3$, were purchased from Sigma-Aldrich Chemical Co., (St. Louis, MO, USA), and ammonium molybdate, Folin-Ciocalteu's phenol reagent, and sodium carbonate were from Merck Chemical Supplies (Darmstadt, Germany). All the other chemicals used, including the solvents, were of analytical grade.

Preparation of crude plant extracts

L. taraxacifolia (UCC/BS/4401) was obtained and authenticated by botanists at the University of Cape Coast, Ghana. Voucher specimen is deposited at the University of Cape Coast herbarium.

The plant material was thoroughly washed under running tap water and air-dried for 2 weeks. It was pulverized into powder, and a portion (70 g) was extracted in 700 mL of 70% methanol in aqueous medium for 24 h. The extract was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated to dryness at 40°C using a rotary evaporator. It was labeled as *L. taraxacifolia* extract (LTE) and stored at -20°C until use. The yield obtained was 5.45%.

Antioxidant assays

Determination of ferric reducing power of the extracts

The reducing power of the LTE was evaluated according to the method described by Oyaizu.^[16] A mixture containing 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 mL of various concentrations (20–200 µg/ml) of extract and standards

prepared in distilled water. The resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 mL of trichloroacetic acid (TCA) (10% w/v), which was then centrifuged at 3000 rpm for 10 min. About 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1% w/v). The absorbance was then measured at 700 nm against a blank sample containing a phosphate buffer. Increased absorbance of the reaction mixture indicated higher reducing power of the plant extract.

Total antioxidant assay

The antioxidant power of LTE and standards was evaluated by the phosphomolybdenum reduction assay according to the procedure described by Prieto *et al.*^[17] A reagent solution prepared contained ammonium molybdate (4 mM), sodium phosphate (28 mM), and sulfuric acid (0.6 M), mixed in 1:1:1 ratio, respectively. Accurately, 0.3 mL of the various concentrations of the extracts (20–200 µg/ml) was mixed with 3 mL of the reagent solution. The mixtures were incubated for 1 h at 95°C after which the absorbance of the green phosphomolybdenum complex formed was measured at 695 nm against a blank. For standards (AS and GA), concentrations of 20–200 µg/ml were used. Each concentration was prepared in triplicates. A mixture containing 0.3 mL methanol and 3 mL reagent solution was used as a blank.

Nitric oxide radical scavenging activity

The nitric oxide (NO) radical scavenging activity was determined according to the method described by Green *et al.*^[18] with slight modifications. A volume of 2 mL of 10 mM sodium nitroprusside prepared in 0.025 M of phosphate buffer (pH 7.4) was mixed with 0.5 mL of plant extracts and standards (GA and AS) individually at concentrations of 20–200 µg/ml. The mixture was incubated at 25°C for 150 min. About 0.5 mL of the incubated solution was mixed with 0.5 mL of Griess reagent (1% sulfanilamide reagent and 0.1% naphthylenediamine dichloride dissolved in 5% phosphoric acid). The mixture was further incubated at room temperature for 30 min, after which the absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and its subsequent coupling with naphthylenediamine dichloride was measured at 540 nm against a phosphate buffer blank. The reaction mixture without the test sample was used as a control. All experiments were conducted in triplicates. The percentage inhibition of NO was calculated using the formula: % NO radical scavenging activity = (Abs control - Abs sample) / (Abs control) × 100.

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The method of Blois^[19] was used in determining LTE scavenging activity of DPPH-free radical. DPPH (1.0 mL, 0.135 mM) prepared in methanol was mixed with 1.0 mL of dissolved extracts and different concentrations (20–200 µg/ml) of standards (AS and GA). The reaction mixture was shaken vigorously and left in the dark at room temperature for 30 min. The absorbance was measured spectrophotometrically at

517 nm against a blank. All experiments were conducted in triplicates. Methanol was used as the blank. Control experiment contained only methanol and DPPH-free radical. The percentage scavenging activity of DPPH radical was calculated using the formula: % scavenging activity of DPPH radical = (Abs control – Abs sample or standard)/(Abs control) × 100.

Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activities (HRSA) of LTE and standards, citric acid, and ethylenediaminetetraacetic acid (EDTA) were determined according to the method described by Klein *et al.*^[20] with slight modifications. Accurately, 0.5 mL of various concentrations (20–200 µg/ml) of extract and standard (citric and EDTA) was each mixed with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA all mixed in a ratio of 1:1). Then, 0.5 mL of EDTA solution (0.018%) and 1.0 mL of dimethyl sulfoxide (0.85% v/v in 0.1 M phosphate buffer, pH 7.4) were added. The reaction was initiated by adding 0.5 mL of AS (0.22%) and incubated at 80°C–90°C for 15 min. After incubation, 1.0 mL of ice-cold TCA (17.5% w/v) was added to terminate the reaction. A 3 mL Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid and 2.0 mL of acetylacetone mixed, and the volume raised to 1 L with distilled water) was added and left at room temperature for 15 min. All experiments were conducted in triplicates. The reaction mixture without the test sample was used as a control. The intensity of the chromophore formed was measured spectroscopically at 412 nm against phosphate buffer blank.

The percentage HRSA was calculated using the formula:

% HRSA = $([A_0 - A_1]/A_0) \times 100$, where A_0 is the absorbance of the control and A_1 is the absorbance of the extract/standard.

Metal ion chelating activity

The ferrous ion chelating potential of the plant extract was measured according to the method of Yamaguchi *et al.*^[21] About 1 mL of FeSO₄ solution was mixed with extract and standards (EDTA and citric acid) of different concentration (20–200 µg/ml). About 1 mL of Tris-HCl buffer (pH 7.4) and 2, 2'-bipyridyl solution were added together with hydroxylamine – HCl and ethanol, respectively. The reaction mixture was adjusted to the final volume of 6 mL with double-deionized water, shaken well, and incubated for 10 min at room temperature. Absorbance was determined at 522 nm and percentage chelation was calculated using the following equation:

Metal ion chelating activity = $([A_0 - A_1]/A_0) \times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample or standard.

Lipid peroxidation inhibition assay

A modified thiobarbituric acid (TBA)-reactive species assay described by Dasgupta and De^[22] was used to measure the lipid peroxide formed using egg-yolk homogenates as lipid-rich media. About 0.5 mL of egg-yolk homogenate (10% v/v in distilled water) and 0.1 mL of the extract and standard

(20–200 µg/ml) were mixed separately in a test tube, and the volume was made up to 1 mL with distilled water. Finally, 0.05 mL Fe₂SO₄ (0.07 M) was added to the mixture and incubated for 30 min to induce lipid peroxidation. Thereafter, 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% TBA (w/v) (prepared in 1.1% sodium dodecyl sulfate) and 0.05 mL 20% TCA were added, vortexed, and heated in a boiling water bath at 100°C for 60 min. To eliminate nonmalondialdehyde (MDA) interference, another set of samples was treated in the same way, but incubating without TBA, so as to subtract the absorbance of the non-MDA interference from the test and standard absorbance. After cooling, the colored TBA-MDA complex was extracted with 5 mL butanol by vigorously shaking and centrifuging at 3000 × g for 10 min. The absorbance of the organic upper layer was measured at 532 nm. For control, 0.1 mL of distilled water was used in place of the extract or standard. Percentage anti-lipid peroxidation activity = $([A_0 - A_1]/A_0) \times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample or standard. Where A_0 is the absorbance value of the fully oxidized control and A_1 is $([A_{532} + TBA] - [A_{532} - TBA])$.

Genomic DNA extraction

Cowpea seeds (*Vigna unguiculata*) purchased from Kotokuraba market, Cape Coast, in the Central region of Ghana, were sowed and allowed to germinate. After 1 week, the leaves of the germinated seeds were collected. Genomic DNA was extracted adopting a modified cetyltrimethylammonium bromide protocol described by Devi *et al.*^[23]

The integrity of the extracted DNA was tested by running a 5 µl DNA sample on gel electrophoresis at 100 V for 1 h. The purity was also determined by measuring the absorbance of each DNA sample at 260 nm and 280 nm and the ratio computed. A ratio $\geq 1.8 \leq 2.0$ implied a pure nucleic acid sample; however, a ratio < 1.8 indicates impurity depicting the presence of protein. Meanwhile, a ratio > 2.0 indicates that the samples were contaminated with phenol or chloroform. To standardize the samples, the concentration of the DNA was also determined using the formula:

$$\text{DNA concentration } (\mu\text{g / ml}) = \frac{A_{260} \times D \times 50 \mu\text{g / ml}}{1000},$$

Where A_{260} represents the absorbance at 260 nm and D is the dilution factor.

The determination of the concentrations enabled the standardization to 100 ng/µl. Samples that were highly concentrated were diluted with sterile Tris-EDTA (TE) buffer, whereas those with lower concentrations, the extraction process was repeated.

DNA damage protection assay

DNA damage inhibition potential of *L. taraxacifolia* leaves extracts was evaluated using cowpea (*V. unguiculata*) genomic DNA. Oxidative damage to DNA was induced using OH

radical generated from ultraviolet (UV)/H₂O₂-radical system and checked on a 1% agarose gel as described by Russo *et al.*^[24] The experiments were performed in a volume of 30 µl in a microfuge tube containing 10 µl aliquot of cowpea DNA, 10 µl of different concentrations of extracts (5 and 20 mg/ml), and 10 µl of 30% H₂O₂. The control contained only untreated DNA as internal control while the negative control contained DNA and H₂O₂ without treatment with extract or standard. The tubes were UV irradiated at a wavelength of 230 nm using UV transilluminator (UVP Upland, CA 91786, USA) for 12 h at room temperature. After irradiation, 5 µl of X6 bromophenol blue was added to each tube for visibility during gel electrophoresis. All samples were analyzed by gel electrophoresis run on 1% agarose gel containing ethidium bromide in TE buffer (pH 8) and photographed.

Statistical analysis

IC₅₀ was calculated by interpolation from linear regression analysis using Graph Pad Prism software version 6. The experimental results were expressed where appropriate as mean ± standard error of three replicates. The data were subjected to one-way analysis of variance, and differences between samples were determined by Bonferroni's *post hoc* test. Values of $P \leq 0.05$ were regarded as significant.

RESULTS

1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

The scavenging activity of LTE compared to GA and AS for DPPH is shown in Figure 1. The results indicate a concentration-dependent scavenging activity of the extract against DPPH radical. The scavenging ability of GA, AS, and the extract almost peaked at 200 µg/ml. Though the antioxidant activity of LTE was lower ($P < 0.05$) compared to GA and AS, LTE showed prominent antioxidant activity. The IC₅₀ values calculated were 16.18, 15.80, and 22.69 µg/ml for LTE, GA, and AS, respectively [Table 1].

Metal ion chelating activity

The iron (II) chelating ability of LTE is shown in Figure 2. All the results showed chelating activity in dose-dependent manner at concentration 20–200 µg/ml. The chelating potential of LTE (200 µg/ml) was significantly ($P < 0.05$) higher than the standards (citric acid and EDTA). The IC₅₀ values calculated were 81.13, 88.91, and 97.94 for citric acid, EDTA, and LTE, respectively [Table 1].

Nitric oxide scavenging activity

NO scavenging activities of LTE and the standards are presented in Figure 3. The NO scavenging activity was found in the following order LTE > AS > GA at 200 µg/ml. The % inhibition of NO by the LTE differed significantly ($P < 0.05$) from those of AS and GA at concentration of 200 µg/ml. However, the calculated IC₅₀ values of 94.96 µg/mL, 123.3 µg/ml, and 53.6 µg/ml for AS, LTE, and GA, respectively [Table 1] indicate that though LTE had the highest scavenging activity at maximum concentration of 200 µg/ml, its potency is lower than that of standards (AS and GA).

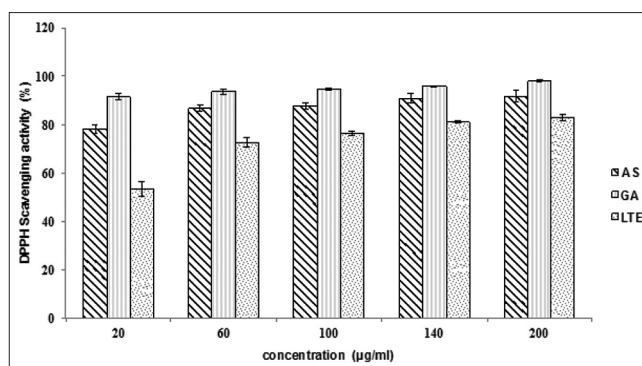


Figure 1: 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity of methanolic extract of *Launaea taraxacifolia* extract and standards (gallic acid and ascorbic acid) at different concentrations. Each value represents mean ± standard error of mean ($n = 3$)

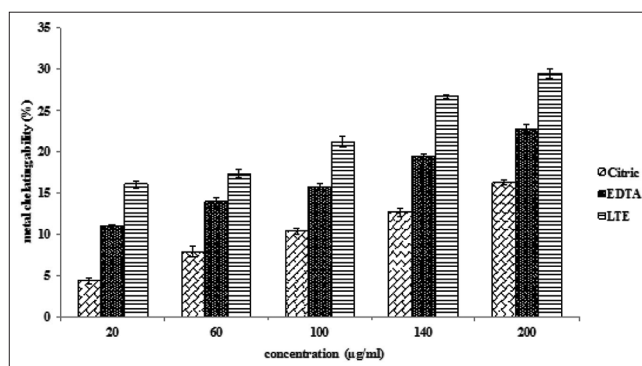


Figure 2: Metal ion chelating activity of methanolic extract of *Launaea taraxacifolia* and standards (citric acid and ethylenediaminetetraacetic acid) at different concentrations. Each value represents mean ± standard error of mean ($n = 3$)

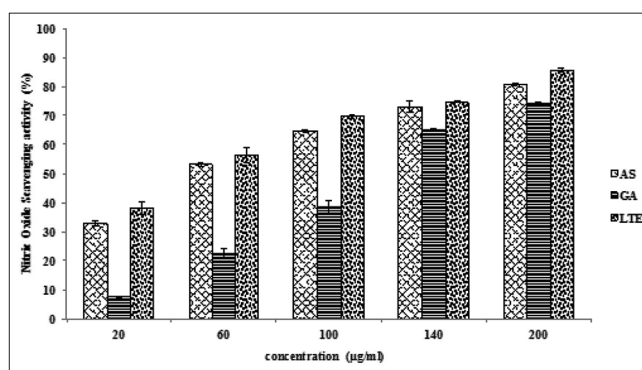


Figure 3: Nitric oxide scavenging activity of methanolic extract of *Launaea taraxacifolia* leaves and standards at (gallic acid and ascorbic acid) different concentrations. Each value represents mean ± standard error of mean ($n = 3$)

Hydroxyl radical scavenging activity

OH radical scavenging potential of LTE was determined in the concentration range 20–200 µg/ml and results are shown in Figure 4. The OH scavenging ability of LTE, citric acid, and EDTA at 200 µg/ml were 36, 32.8, and 60.4%, respectively. The EC₅₀ value of HRSA for the LTE, citric acid,

Table 1: Summary of IC₅₀ values of antioxidant potentials of *Launaea taraxacifolia* leaf extract (µg/ml)

Sample	DPPH	OH	NO	MCC	RP	TAC	Anti-LPO
LT extract	16.18±0.34	128.2±1.50	123.3±1.30	97.94±1.83	87.85±1.46	80.28±2.30	79±1.87
Gallic acid	15.8±0.02	-	53.36±0.54	-	93.35±0.23	63.50±1.20	95±2.30
Ascorbic acid	22.69±0.04	-	94.69±0.42	-	21.5±0.28	104.6±0.76	150±2.52
Citric acid	-	143.3±0.67	-	81.13±0.72	-	-	-
EDTA	-	143.3±0.32	-	88.91±0.68	-	-	-

TAC: Total antioxidant capacity, LT: *Launaea taraxacifolia*, NO: Nitric oxide, MCC: Metal chelating capacity, Anti-LPO: Anti-lipid peroxidation, RP: Reducing power, DPPH: 1,1-diphenyl-2-picrylhydrazyl, OH: Hydroxyl, EDTA: Ethylenediaminetetraacetic acid

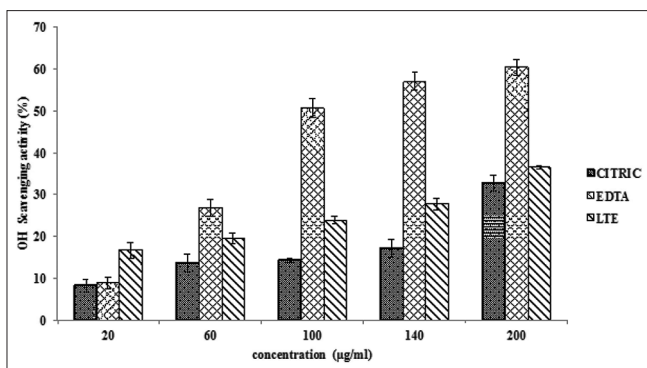


Figure 4: Hydroxyl scavenging activity of methanolic extract of *Launaea taraxacifolia* leaves and standards at different concentrations. Each value represents mean ± standard error of mean ($n = 3$)

and EDTA was found to be 128.2, 143.3, and 143.3 µg/ml, respectively [Table 1].

Ferric reducing capacity

The reducing power of the extract and standards, which is directly proportional to the absorbance, increased with increasing concentration [Figure 5]. At maximum concentration of 200 µg/ml, GA exhibited the highest reducing ability, followed by AS, while the lowest reducing activity was found in LTE. However, LTE exhibits the highest IC₅₀ value of 87.85 µg/ml [Table 1], implying the less potent reductant among the test substances.

Total antioxidant capacity

The study revealed that the total antioxidant activity, which is directly proportional to the absorbance, increases with increasing concentration of the plant extract and standards [Figure 6]. Though AS had the highest absorbance of 1.78 at the maximum concentration of 200 µg/ml, the IC₅₀ values showed that GA is the most potent with a value of 63.5 µg/ml [Table 1]. The IC₅₀ were in the order GA > LTE > AS.

Lipid peroxidation activity

The anti-lipid peroxidation activity of the extract and standard drugs in Fe²⁺-ascorbate system by inhibiting the formation of MDA is illustrated in Figure 7. At a concentration of 200 µg/ml, the inhibitory effect on the formation of MDA by LTE, GA, and AS was 52%, 66%, and 72%, respectively. The IC₅₀ values of extract, GA, and AS were 79, 95, and 150 µg/ml, respectively. The results

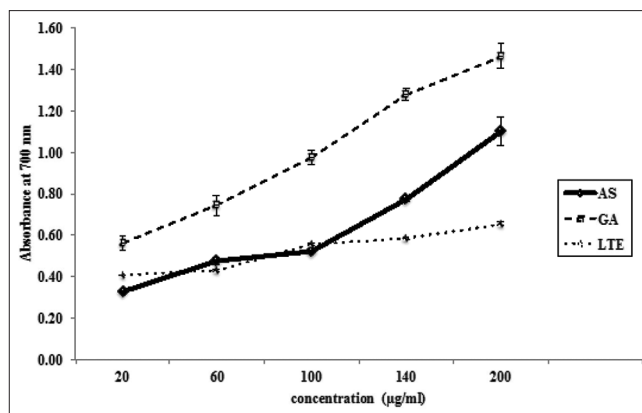


Figure 5: Ferric reducing power of methanolic extract of *Launaea taraxacifolia* leaves and standards at different concentrations. Each value represents mean ± standard error of mean ($n = 3$)

suggest that the extract is a more potent scavenger of MDA than GA and AS.

DNA damage protection

The electrophoretic pattern of DNA after UV-photolysis of H₂O₂ in the absence and presence of different concentrations of LTE is shown in Figure 8. The untreated cowpea genomic DNA showed a faster sharp bright band on agarose gel electrophoresis (lane 4), an indication of conservation of its native supercoiled circular nature. It was also observed that the UV irradiated DNA in the presence of H₂O₂ without extract resulted in the scission of supercoiled native DNA to give an open heavier slower DNA (lane 2). In lanes 1 and 3, where there was addition of LTE to DNA exposed to H₂O₂ in the presence of UV, considerable protection to damage of native supercoiled DNA was seen. This was depicted by the position of the bands (lanes 1 and 3), which is not different from the position of DNA that served as an internal control (lane 4). The results in this study confirm some level of protection from OH radical damage offered by LTE to DNA.

DISCUSSION

Free radicals have been implicated in a variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various ailments. Phytochemicals function as antioxidants either by quenching the ROS or by acting as a defense shield to protect the antioxidant defense mechanism.^[25] In this study, the antioxidant activity and DNA oxidative

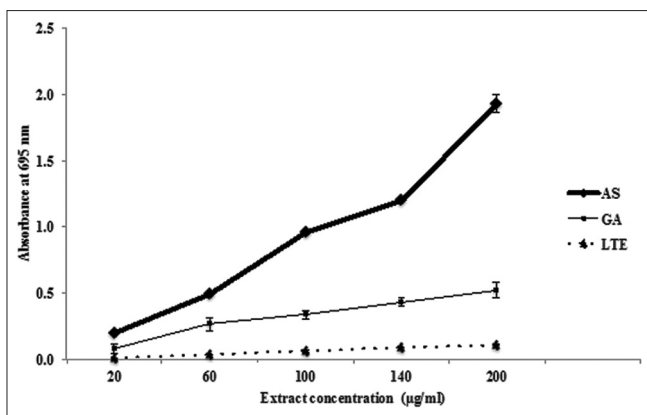


Figure 6: Total antioxidant capacity of methanolic extract of *Launaea taraxacifolia* leaves and standards at different concentrations. Each value represents mean \pm standard error of mean ($n = 3$)

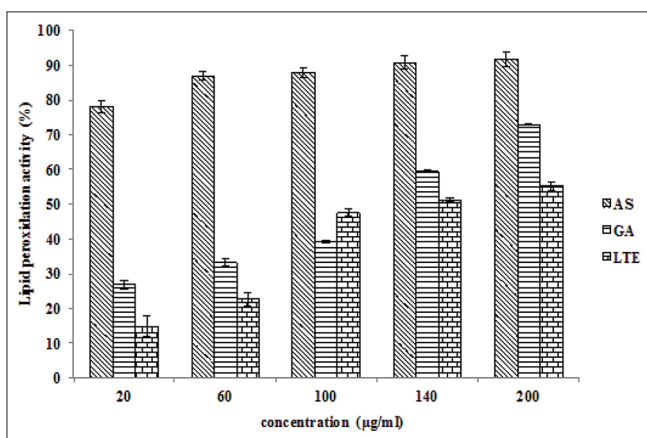


Figure 7: Anti-lipid peroxidation activity of methanolic extract of *Launaea taraxacifolia* leaves and standards at different concentrations. Each value represents mean \pm standard error of mean ($n = 3$)

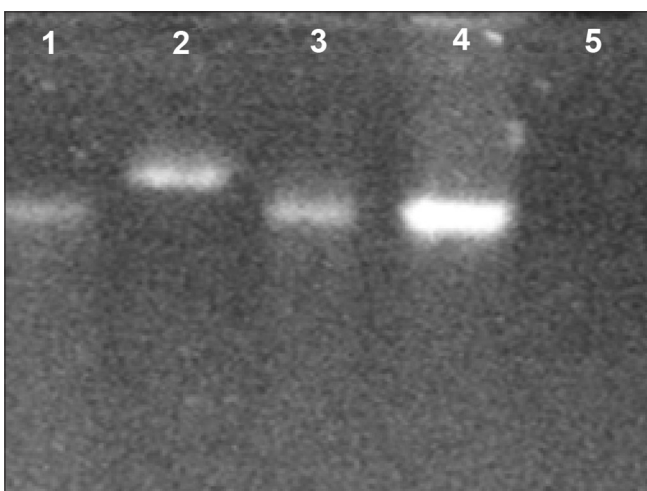


Figure 8: Electrophoregram of cowpea genomic DNA after ultraviolet-photolysis of H_2O_2 (30%) in the presence or absence of the extract. Lane 1: 5 mg/ml extract + UV + H_2O_2 + DNA, Lane 2: UV + H_2O_2 + DNA, lane 3: 20 mg/ml extract + UV + H_2O_2 + DNA, lane 4: untreated DNA, lane 5: double distilled water

damage protective potentials of LTE were investigated. DPPH, which is a good source of free radical, is frequently used to measure the electron-donating ability of molecules.^[26] The extent of color change is proportional to the strength and concentration of antioxidant. The LTE showed significant DPPH radical scavenging activity. The scavenging ability of the LTE is higher than some previously exploited natural sources of antioxidants. For instance, Megala and Geetha^[27] reported a 41.8% and 44.5% DPPH inhibition for aqueous and hydroalcoholic extract of *Pithecellobium dulce* fruit at 160 $\mu\text{g/ml}$ extract concentration. This is far lower than 81.3% DPPH scavenging activity obtained for LTE at concentration of 140 $\mu\text{g/ml}$. The LTE is more potent and efficacious than AS, and this may be due to its phytochemical constituents that are capable of donating hydrogen to free radical to scavenge it.

The ability to chelate/deactivate transition metals, which catalyze hydroperoxide decomposition and fenton-type reactions, is an important antioxidant mechanism. Metal chelating properties of certain organic compounds, such as EDTA and citric acid, have been reported.^[28] Report indicates that chelating agents, which form σ -bonds with a metal, are effective secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion.^[28] Although LTE has metal chelating potential, the standards (citric acid and EDTA) are more potent and efficacious.

NO reacts with oxygen to form highly reactive substances such as N_2O_4 , N_3O_4 , NO_3 , and NO_2 . Highly reactive NO compounds formed are responsible for altering the structure and functional behavior of many cellular components. Acute exposure to NOs is toxic to tissues and leads to vascular collapse with septic shock, whereas chronic exposure can lead to oxidative stress-related disorders such as diabetes mellitus, arthritis, and ulcerative colitis as reported by Taylor *et al.*^[29] NO also reacts with superoxide to form a highly reactive peroxynitrite anion (ONOO), which has a more damaging effect on tissues. The LTE effectively scavenged NO radical. This may be due to the antioxidant principles in the extract which compete with oxygen to react with NO, thereby inhibiting the generation of nitrite. Thus, LTE could be used to manage NO oxidative stress-induced diseases.

OH radical is a potent active oxygen species that stimulate lipid peroxidation and induces cellular damage.^[30] The OH radical scavenging assay shows the ability of a potential drug molecule to inhibit the generation of OH radical. The present study suggests that LTE has potent scavenging activity against OH radical, and this activity may be related to the flavonoid and phenolic contents of the plant. Implicitly, LTE could be used together with other known plants to manage OH radical-induced lipid peroxidation disorders.

The reducing property of a compound generally depends on the presence of reductants, which can inherently serve as an indicator of its antioxidant potential. This antioxidant property has been attributed to several mechanisms, among which are

the binding of transition metals, prevention of chain initiation, prevention of hydrogen abstraction, and decomposition of peroxides.^[31] The present investigations suggest that LTE is a reductant, and this reducing activity could be attributed to the presence of polyphenols,^[32] which may act in a similar fashion as reductones by donating the electrons and reacting with free radicals to convert them to more stable product.^[26,33] The total antioxidant activity of LTE determined by the reduction of Mo (VI) to Mo (V) and the subsequent formation of a green phosphate-Mo (V) complex revealed that LTE has a very good antioxidant potential for phosphomolybdate reduction. The total antioxidant capacity of LTE may be attributed to the collective contribution of phenolic and flavonoid contents in the extract.^[34,35]

Lipids of cellular membranes are predisposed to peroxidation. Lipid peroxidation by free radical results in the production of MDA which can induce cellular damage.^[36] In the present study, LTE significantly inhibited lipid peroxidation. This activity could be attributed to the cumulative contribution of its phenolics and flavonoids contents that play vital role in antioxidant manifestation.

OH-free radicals are well known to damage cellular DNA. UV-photolysis of H₂O₂ generates OH radicals, which is responsible for most protein and DNA oxidative damage. According to Guha *et al.*,^[37] OH radical binding to DNA leads to strand breakage and opening, deoxyribose sugar fragmentation, and nitrogenous base modification. The attack of DNA by OH radical opens up the circular structure and the strand becomes heavier. The oxidative damage of DNA is one of the most important mechanisms in the initiation of cancer and this damage is usually caused by OH radicals.^[38] In this study, the extract displayed considerable protective activity against free radical-mediated DNA damage and could, therefore, be used in cancer prevention. Other plants have been reported to protect against free radical-mediated DNA damage.^[37,39]

CONCLUSION

This study is the first to evaluate the antioxidant activity of *L. taraxacifolia* in a comprehensive manner employing a range of *in vitro* assays. The results of the present study show that the methanolic extract of *L. taraxacifolia* leaves possesses DNA protective properties and antioxidant activity evidenced by an excellent *in vitro* DPPH and NO radical scavenging activities, profound Fe²⁺-ascorbate lipid peroxidation inhibitory activity and reductive properties. The present findings suggest that the extract has a potential as a therapeutic agent in the prevention of diseases evoked by increased oxidative stress such as atherosclerosis and cancer. Further work is ongoing to identify the various active components contributing to the observed antioxidant activity.

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Conflicts of interest

There are no conflicts of interest.

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