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Protective role of lutein from *Methi* leaves against DNA damage

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Abstract
This study aimed to isolate high-purity lutein from *Trigonella foenum-graecum* (methi) leaves and evaluate its protective effects against oxidative DNA damage. Lutein was extracted via open-column chromatography on neutral alumina using hexane and dichloromethane:methanol (1:1, v/v), and its purity and structure were confirmed by UV-Vis spectroscopy, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and liquid chromatography-mass spectrometry (LC-MS). Bioefficacy was assessed by exposing human lymphocytes and calf thymus DNA to oxidative stress induced by hydrogen peroxide (H_2O_2), ultraviolet (UV) radiation, and Fe^{2+}/ascorbate (Fe:AA) systems, with DNA integrity analyzed using agarose gel electrophoresis and the diphenylamine assay. UV-Vis analysis showed a λmax at 445.5 nm, matching authentic lutein (446 nm), while TLC and HPLC confirmed ~99% purity, and LC-MS produced a major ion at m/z 551.48 ($[M-H_2O + H]^+$), consistent with lutein's molecular signature. Methi lutein (10-30 µM) markedly reduced DNA fragmentation, outperforming butylated hydroxyanisole (BHA) and α-tocopherol (each at 400 µM); at 30 µM, it completely inhibited H_2O_2-induced DNA degradation and preserved DNA integrity under UV exposure (345 nm) for up to 60 min at 20 µM. In the Fe:AA model, it achieved a 42% reduction in DNA fragmentation, exceeding the ~50% protection observed with BHA and α-tocopherol. This research provides a promising foundation for the development of natural antioxidant-based therapeutics, addressing the urgent need for effective strategies to mitigate oxidative genotoxicity with greater efficacy and safety.

Keywords: Lutein purification, *Trigonella foenum-graecum*, oxidative DNA damage, UV radiation, antioxidant activity

1. Introduction
Oxidative stress, resulting from the imbalance between reactive oxygen species (ROS) and antioxidant defenses, is a critical factor in the pathogenesis of numerous chronic and degenerative diseases, including cancer, neurodegeneration, and cardiovascular disorders [1, 2]. Among the major targets of ROS are nucleic acids, particularly DNA, which, when oxidatively damaged, can lead to mutagenesis, genomic instability, and cell death [3, 4]. Hydrogen peroxide (H_2O_2), a well-known ROS, is capable of inducing strand breaks in DNA via the Fenton reaction, particularly in the presence of transition metals like iron [5]. Similarly, ultraviolet (UV) radiation is a potent environmental factor that contributes to DNA oxidation through the formation of ROS and photoproducts such as cyclobutane pyrimidine dimers [6]. Therefore, identifying compounds that can effectively prevent oxidative DNA damage is a crucial area of biomedical research.
Dietary antioxidants have garnered significant attention for their ability to counteract oxidative DNA damage. Synthetic antioxidants like butylated hydroxyanisole (BHA) and naturally occurring compounds such as α-tocopherol (vitamin E) are known for their radical-scavenging properties [7, 8]. However, recent studies have focused on plant-derived phytochemicals that possess superior antioxidant efficacy with fewer side effects [9]. Lutein, a xanthophyll carotenoid predominantly found in green leafy vegetables, has shown promising antioxidant and photoprotective properties [10, 11]. Its ability to quench singlet oxygen and scavenge free radicals makes it a potential candidate for protecting cellular biomolecules

from oxidative stress [12]. Lutein also localizes in cell membranes and may interact directly with DNA and chromatin, thereby shielding them from oxidative insults [13]. *Trigonella foenum-graecum*, commonly known as fenugreek or "methi," is a medicinal plant widely used in traditional medicine systems. Fenugreek leaves are rich in carotenoids, especially lutein, and have been reported to exhibit multiple pharmacological properties, including antioxidant, anti-inflammatory, and hypoglycemic effects [14, 15]. Despite its traditional uses, the protective potential of lutein extracted specifically from fenugreek leaves (referred to here as Methi lutein) against genotoxic agents has not been thoroughly explored. Preliminary investigations indicate that Methi lutein may provide robust DNA protection, even at relatively low concentrations, compared to conventional antioxidants.

The present study investigates the efficacy of Methi lutein in mitigating DNA damage induced by H_2O_2, UV radiation, and Fe^{2+}/ascorbate in human lymphocytes and calf thymus DNA. Comparative analyses with standard antioxidants BHA and α-tocopherol were performed to evaluate the relative effectiveness of Methi lutein in preserving genomic integrity. Results indicate that Methi lutein not only significantly reduced oxidative DNA fragmentation but also maintained protection over prolonged exposure to oxidative stressors, highlighting its potential as a natural therapeutic agent for genomic protection and anti-mutagenic applications.

2. Materials and methods

Reagents

Ethidium bromide, BHA, Calf thymus DNA (CT DNA), SDS, Agarose, t-BOOH, Thiobarbutaric acid, Diphenylamine (DPA) was from sigma chemicals Co., USA. H_2O_2, EDTA, Ascorbic acid, BHA, Dimethylsulfoxide (DMSO), Ferrous sulphate, sodium acetate, Deoxyribose, Thiobarbuteric acid were purchased from Himedia Pvt.. Ltd., Bombay, India. All the other reagents were of analar grade.

2.1 Purification of lutein from Hexane extract of Methi leaves

The crude hexane extract of methi leaves was subjected to open column chromatography for the purification of lutein as per the described method [16]. A 1 mL aliquot of the concentrated hexane extract was loaded onto a neutral alumina column (25 × 2.0 cm) packed with 70-230 mesh neutral alumina. The column was initially eluted with hexane to separate β-carotene, followed by elution with a 1:1 (v/v) mixture of dichloromethane and methanol to obtain the lutein fraction (fig. 1).

The concentrated hexane fraction obtained from the neutral alumina column was carefully collected and subjected to spectral analysis using a double-beam Shimadzu UV-1601 spectrophotometer. The absorbance spectrum was recorded over the range of 200 to 600 nm to capture the characteristic absorption peaks of lutein. The spectral profile of the eluted methi lutein closely corresponded with that of the authentic standard lutein, exhibiting comparable absorption maxima and spectral shape, thereby confirming the identity of the purified compound as lutein.

To further validate the purity of the isolated lutein, thin layer chromatography (TLC) was concurrently performed. The TLC analysis was conducted using pre-coated silica gel plates with an appropriate solvent system, which effectively separated pigments based on their polarity. The methi lutein fraction exhibited a single, well-defined band under the detection conditions, consistent with the retention factor (Rf) of the standard lutein. The singularity of this band provides strong evidence of the high purity of the purified lutein, which was estimated to be approximately 99%.

To reinforce these findings, the purity of the methi-derived lutein was further validated by High-Performance Liquid Chromatography (HPLC) analysis [17]. The HPLC chromatogram of the column-purified lutein displayed a single sharp peak at 4.7 minutes, which corresponded precisely with the retention time of the standard lutein. The presence of a single peak without any detectable impurities confirms the high degree of purity and further validates the successful isolation of lutein from methi leaves.

The presence of a single peak in the HPLC chromatogram further confirmed the chemical purity and homogeneity of the lutein isolated from methi leaves, aligning well with the previous UV-Vis and TLC findings. To further characterize the molecular identity and confirm the structural integrity of the purified compound, Matrix-Assisted Laser Desorption/ Ionization Mass Spectrometry (MALDI-MS) analysis was performed. As shown in, the MALDI-MS profile of the purified lutein exhibited a molecular ion peak corresponding to a molecular weight of 551.48 Da, which is consistent with the theoretical molecular mass of lutein [18]. A slight shift in the molecular weight to 567.807 Da observed in the spectrum is attributed to the loss of two oxygen atoms during the ionization process, a commonly reported phenomenon in MALDI-MS analysis of carotenoids. This spectral data further substantiates the identity of the isolated compound as lutein and illustrates the high sensitivity of MALDI-MS in detecting molecular-level changes during analysis.

2.2 DNA damage by Hydrogen Peroxide & protection by Methi lutein

Sheared calf thymus DNA (10ug) was treated with H_2O_2 (144□M) with and without Methi lutein and standard antioxidants in 100ul of 20mM PBS pH-7.4 incubated at 37ºC for 30min. Protection offered by the Methi lutein in various concentrations & standard antioxidant on DNA was detected by 0.6% Agarose gel electrophoresis. In brief, DNA gel electrophoresis was carried out using 0.6% agarose prepared in TAE buffer containing 0.5ug\ml of Ethidium Bromide. Electrophoresis was carried out using Tris-TAE buffer (40mM Tris, 20mM Sodium acetate, 18mM Nacl, 2mM EDTA, pH 8.0). DNA bands were visualized under U.V transilluminator [19].

2.3 UV-Induced DNA Damage Protection by Lutein-Spectrofluorimetric Assay

The DNA protective efficacy of lutein isolated from *Trigonella foenum-graecum* (methi) leaves against UV-induced damage was evaluated using a spectrofluorimetric assay, based on the fluorescence interaction of ethidium bromide (EtBr) with intact and fragmented DNA, as described by Azqueta *et al.* (2009), with slight modifications. Calf thymus DNA (200 µg/mL) was used as the substrate for DNA damage assessment. The reaction mixture consisted of DNA (50 µL), phosphate-buffered saline (PBS, pH 7.4), and various concentrations (10-

50µg/mL) of methi-lutein/A-tocopherol/BHA incubated for 30 minutes at 37°C [20].

After pre-incubation, the samples were exposed to UV-C radiation (254 nm, 15 W lamp) at a distance of 15 cm for 60 minutes. A parallel set of samples without UV exposure served as the control. Post-irradiation, 10 µL of ethidium bromide (10 µg/mL) was added to each tube, and the fluorescence intensity was measured at different intervals of time (for every 20 minutes) using a spectrofluorimeter (excitation: 520 nm; emission: 600 nm). A reduction in fluorescence intensity indicated DNA strand breakage due to reduced EtBr intercalation in fragmented DNA. The protective effect of lutein was expressed as the percentage of fluorescence retained compared to non-irradiated DNA.

All experiments were performed in triplicate, and results were statistically analyzed. The assay effectively demonstrated the protective effect of methi lutein in mitigating UV-induced structural DNA damage through stabilization or scavenging of reactive species.

2.4 Ferrous Sulphate/Ascorbate-Induced DNA Damage Protection by Lutein -Spectrofluorimetric Assay

The protective effect of lutein isolated from *Trigonella foenum-graecum* (methi) leaves against DNA damage induced by ferrous sulphate/ascorbate-mediated oxidative stress was evaluated using a spectrofluorimetric method, as previously described by Halliwell and Aruoma (1991) with modifications. Calf thymus DNA (200 µg/mL) was incubated with ferrous sulphate (100 µM) and ascorbic acid (1 mM) in phosphate-buffered saline (PBS, pH 7.4) to induce hydroxyl radical-mediated DNA strand breaks via the Fenton reaction [21].

The assay mixture (total volume: 500 µL) consisted of 50 µL DNA solution, 50 µL of $FeSO_4$, 50 µL of ascorbic acid, and varying concentrations (10-50 µg/mL) of lutein/A-tocopherol/BHA. The reaction mixtures were incubated at 37°C for 60 minutes in the dark. A control group without $FeSO_4$/ascorbate served as the negative control, and a group without lutein served as the damage control. After incubation, 10 µL of ethidium bromide (EtBr, 10 µg/mL) was added to each sample, and the fluorescence intensity was measured using a spectrofluorimeter (excitation at 520 nm and emission at 600 nm). A decrease in fluorescence indicated DNA strand breakage due to loss of EtBr intercalation in damaged DNA. Protective efficacy of lutein was calculated as the percentage of fluorescence retained compared to undamaged DNA controls. All experiments were performed in triplicate.

2.5 Quantitative analysis of DNA fragmentation by diphenylamine method

The inhibitory effect of antioxidant on prooxidants induced DNA fragmentation in lymphocytes was studied by diphenylamine reaction method (Burton, 1956; Sellins and Cohen, 1987; Shabani & Rabbani, 2000). This method separated from chromosomic DNA upon centrifugal sedimentation. This includes lysis of cells to release the nuclear DNA, centrifugation of two fractions (corresponding to intact and fragmented DNA). The precipitation of DNA, hydrolysis and colorimetric quantitation upon staining with DPA that binds to deoxyribose [22].

100ul of cell suspension, corresponding to 1 x 10^6 cells were pretreated with or without Methi lutein or BHA & α-tocopherol at the concentrations ranging from 0-100ug in 0.5ml HBSS then added ferrous sulphate**:** ascorbate (2:20umole) in total volume of 1ml of HBSS and incubated at 37^{0}C for 60 minutes. Viability was tested and centrifuged at 1200-1500rpm, 20 min, 4^{0}C and processed for the estimation of DNA fragmentation by diphenylamine reaction method as follows.

For the assessment of DNA fragmentation, cells were lysed in 1 mL of TTE buffer (10 mM Tris-HCl, 1 mM EDTA, 0.2% Triton X-100, pH 7.4), facilitating chromatin release via membrane disruption by Triton X-100 and Mg^{2+} chelation by EDTA. Lysates were centrifuged at 1500 rpm for 10 min at 4°C to separate fragmented (supernatant) and intact (pellet) DNA. Both fractions were treated with 1 mL of 25% trichloroacetic acid (TCA), incubated overnight at 4°C, and centrifuged again. Pellets were hydrolyzed in 160 µL of 5% TCA at 90°C for 15 min, and DNA content was quantified using the diphenylamine (DPA) assay. For color development, 100 µL of freshly prepared DPA reagent (150 mg DPA in 10 mL glacial acetic acid, 150 µL concentrated H_2SO_4, and 50 µL of 16 mg/mL acetaldehyde) was added and incubated at room temperature for 24 h. Absorbance was measured at 600 nm, and DNA fragmentation and % inhibition were calculated relative to untreated controls.

$$\% \text{ DNA fragmentation} = \frac{\text{Absorbance of Test (T)}}{\text{Absorbance of Test (T)} + \text{Absorbance of Blank}}$$

% DNA fragmentation _ % DNA fragmentation Control

$$\% \text{ inhibition of DNA fragtion} = \frac{\text{Sample (test)}}{\% \text{ DNA fragmentation}} \text{X } 100$$

3. Results and Discussion

3.1 Purification of Lutein from Methi leaves

The successful purification of lutein from methi (fenugreek) leaves using open column chromatography highlights an efficient and selective method for isolating carotenoids from plant matrices. By employing a stepwise elution strategy—initially with non-polar hexane to separate β-carotene, followed by a 1:1 dichloromethane:methanol mixture to elute lutein—the method effectively exploited polarity differences between carotenoids. The use of neutral alumina minimized oxidative degradation, ensuring the structural integrity and purity of the isolated pigments. The clear separation of colored bands visually confirms the efficiency of the protocol, supporting its suitability for preparative applications in antioxidant research, nutraceutical development, and phytochemical studies.

The UV-Vis spectral analysis of the purified lutein fraction from methi leaves revealed a characteristic absorption profile with a prominent maximum at 445.5 nm, closely matching the absorption maximum of the authentic lutein standard at 446 nm. This close spectral congruence, including peak shape and intensity distribution across the visible region, provides strong evidence for the structural identity and purity of the isolated compound. Carotenoids such as lutein exhibit distinct absorption bands due to their conjugated double bond systems, and the reproducibility of these spectral features confirms the successful isolation of lutein with minimal contamination by other pigments. The use of a double-beam Shimadzu UV-Vis1601 spectrophotometer enabled high-resolution detection, supporting the methodological reliability of this non-

destructive purity assessment. Overall, the spectroscopic validation reinforces the effectiveness of the chromatographic protocol and highlights its suitability for obtaining analytically pure lutein for functional and bioactivity studies.

TLC analysis provided further validation of the purity and identity of the isolated lutein from methi leaves. As shown in, Lane 1 (crude hexane extract) displayed multiple pigment bands, while Lane 2 (column-purified lutein) exhibited a single, sharp band that co-migrated precisely with the band in Lane 3 (standard lutein), both loaded at equivalent concentrations (25 µg). The separation was achieved using a hexane: acetone (7:3, v/v) solvent system on a silica gel plate, enabling clear resolution based on pigment polarity. The absence of additional bands in the purified sample underscores its high purity (~99%) and successful separation from other carotenoids. This confirms the chromatographic efficiency and supports the analytical suitability of the isolated lutein for downstream applications.

The high-performance liquid chromatography (HPLC) analysis further confirmed the identity and purity of lutein isolated from methi leaves. As shown in, the purified methi lutein (B) exhibited a sharp peak at a retention time identical to that of the standard lutein (A), indicating co-elution and chemical equivalence. The use of a methanol:dichloromethane:acetonitrile (67:23:10, v/v) solvent system and detection at 450 nm enabled precise resolution of lutein, a carotenoid with strong absorbance in the visible range. The absence of major secondary peaks in the purified sample supports its high purity and minimal presence of co-extracted impurities. This analytical validation underscores the efficiency of the column chromatography method and establishes the suitability of the isolated lutein for advanced functional, nutritional, and therapeutic applications.

The LC-MS analysis provided definitive confirmation of the molecular identity of lutein purified from methi leaves. As shown in, the mass spectrum revealed a prominent peak at m/z 551.48, which corresponds to the $[M-H_2O+H]^+$ ion of lutein, consistent with its known molecular weight of 568.87 g/mol. This characteristic fragmentation pattern is in agreement with previously reported mass spectral data for lutein, further validating the structural integrity of the isolated compound. The analysis, performed using a Waters 2996 modular HPLC system coupled to a mass detector, confirms the high purity and molecular specificity of the methi-derived lutein. These findings reinforce the success of the chromatographic isolation method and demonstrate its effectiveness in yielding structurally intact, analytically verified lutein suitable for biofunctional studies.

The comprehensive analytical workflow employed in this study confirms the successful purification and structural validation of lutein from methi (fenugreek) leaves. Column chromatography using neutral alumina enabled efficient separation of lutein from co-existing pigments, as evidenced by the distinct elution profile. UV-Vis absorption spectroscopy revealed a characteristic peak at 445.5 nm, matching the standard lutein, while TLC analysis demonstrated a single, well-resolved band co-migrating with the authentic standard, indicating high purity (~99%). Further, HPLC profiling showed a sharp peak at a retention time identical to that of standard lutein, with minimal background interference. Most conclusively, LC-MS analysis identified a major ion at m/z 551.48, consistent with lutein's molecular weight and known fragmentation pattern. Collectively, these results validate the identity, purity, and structural integrity of the isolated lutein, establishing methi leaves as a viable natural source and the adopted methodology as a reliable and reproducible protocol for obtaining analytically pure lutein for functional and therapeutic applications.

3.2 Protective Effect of Methi Lutein against H_2O_2-Induced DNA Damage

The protective effect of Methi lutein against hydrogen peroxide (H_2O_2)-induced oxidative DNA damage was assessed in human lymphocyte DNA. Exposure to H_2O_2 (144 µM) resulted in significant DNA fragmentation, as indicated by the presence of low molecular weight DNA bands (Lane 2), consistent with hydroxyl radical-induced strand breaks through the Fenton reaction [23]. However, co-treatment with antioxidants such as butylated hydroxyanisole (BHA, 400 µM), α-tocopherol (400 µM), and Methi lutein at concentrations of 10, 20, and 30 µM significantly protected the DNA from oxidative degradation. Among these, Methi lutein at 30 µM (Lane 6) provided the highest level of protection, comparable to the intact DNA control (Lane 1), and notably more effective than both BHA and α-tocopherol, despite being used at a much lower concentration.

The superior protective effect of Methi lutein may be attributed to its dual antioxidant and DNA-binding properties. Lutein, a xanthophyll carotenoid found in fenugreek leaves, is known to localize in cellular membranes and interact with chromatin, potentially shielding DNA from radical-mediated cleavage [24]. Moreover, its ability to quench singlet oxygen and neutralize peroxyl radicals reinforces its role in preventing oxidative stress-induced genotoxicity [25]. These findings highlight Methi lutein's potential as a potent natural compound for safeguarding genomic integrity, outperforming standard antioxidants in both efficacy and required concentration.

The illustrates the comparative efficacy of lutein extracted from *Trigonella foenum-graecum* (Methi leaves) and BHA in protecting DNA from UV radiation-induced oxidative damage over a 60-minute exposure period. DNA samples exposed to UV radiation without any protective agents ("DNA alone") exhibited a progressive and significant increase in DNA damage, rising from 0% to nearly 70% over the course of 60 minutes. This trend reflects the well-documented impact of UV radiation in generating reactive oxygen species (ROS), which induce strand breaks and oxidative modifications in DNA, a process directly linked to mutagenesis and carcinogenesis [26].

In contrast, samples treated with lutein (20 µM) demonstrated remarkable protection, maintaining DNA damage below 15% even after 60 minutes of UV exposure. BHA, a commonly used synthetic antioxidant (400 µM), also conferred protection, limiting DNA damage to approximately 20% at 60 minutes. However, lutein showed superior performance, maintaining greater genomic integrity despite being used at a much lower concentration. This is consistent with lutein's known efficacy in quenching singlet oxygen and scavenging free radicals, particularly under photo-oxidative stress conditions [27]. Furthermore, its lipophilic nature may enable better integration into biological membranes and DNA complexes, thereby offering enhanced protection [28]. These results underscore the potential of Methi-derived lutein as a natural, cost-

effective photoprotective agent with applications in nutraceuticals and skin health.

The illustrates the comparative efficacy of methi lutein (ML) against Fe^{2+}/ascorbate (Fe:AA)-induced DNA fragmentation in human lymphocytes. As shown, exposure of lymphocytes to Fe:AA (10:20 µmol) markedly increased DNA fragmentation to approximately 88%, signifying extensive oxidative damage. In contrast, pre-treatment with methi lutein (20 µg/ml) significantly reduced the extent of DNA fragmentation to around 42%, demonstrating its potent antioxidant and DNA-protective properties.

Interestingly, methi lutein outperformed both BHA and α-tocopherol (each at 400 µM), which showed around 50% inhibition of DNA fragmentation. Despite being used at a much lower concentration, methi lutein was comparably or more effective, highlighting its superior antioxidative capacity. This enhanced protection could be attributed to lutein's structural ability to quench reactive oxygen species and possibly intercalate with DNA or membrane structures, preventing oxidative strand breaks [29, 30]. These findings support the growing body of evidence emphasizing the therapeutic potential of dietary carotenoids, especially lutein, in mitigating cellular oxidative stress31.

Conclusion

This study comprehensively demonstrates the potent protective effects of lutein extracted from *Trigonella foenum-graecum* (Methi leaves) against multiple forms of oxidative DNA damage, including those induced by hydrogen peroxide, UV radiation, and Fe^{2+}/ascorbate complexes. Methi lutein significantly mitigated DNA fragmentation in human lymphocytes and calf thymus DNA, outperforming standard antioxidants such as BHA and α-tocopherol even at substantially lower concentrations. Its superior efficacy is likely attributed to its dual function as a reactive oxygen species (ROS) scavenger and potential DNA-binding agent, which confers stability and integrity to genomic material under oxidative stress conditions.

Given its broad-spectrum protective capability and natural origin, methi lutein presents itself as a promising candidate for development as a bioactive nutraceutical or therapeutic agent aimed at reducing oxidative genotoxicity. These findings align with and extend existing literature on the protective roles of dietary carotenoids, emphasizing the value of plant-derived compounds in combating oxidative stress-related cellular damage and offering preventive strategies against age-related degenerative diseases and carcinogenesis. Further *in vivo* studies and clinical trials are warranted to validate these protective effects and explore potential applications in human health and skin photoprotection.

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Author Contributions

Shobith Rangappa supervised the overall study design, provided critical revisions, coordinated the manuscript development, and managed correspondence with the journal. Thammanna Gowda SS conceptualized the study, performed the experimental work, analyzed the data, and drafted the initial manuscript. Parimala Hanumesh contributed to experimental design, assisted in data interpretation, and reviewed the manuscript for intellectual content.

Conflicts of Interest

The authors declare that there are no conflicts of interest related to this study.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

References

1. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007;39(1):44-84.
2. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature. 2000;408(6809):239-247.
3. Cadet J, Wagner JR. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. Cold Spring Harb Perspect Biol. 2013;5(2):a012559.
4. Loft S, Poulsen HE. Cancer risk and oxidative DNA damage in man. J Mol Med. 1996;74(6):297-312.
5. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press; 2015.
6. Ravanat JL, Douki T, Cadet J. Direct and indirect effects of UV radiation on DNA and its components. J Photochem Photobiol B. 2001;63(1-3):88-102.
7. Williams GM, Iatropoulos MJ, Whysner J. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chem Toxicol. 1999;37(9-10):1027-1038.
8. Traber MG, Atkinson J. Vitamin E, antioxidant and nothing more. Free Radic Biol Med. 2007;43(1):4-15.
9. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. Am J Clin Nutr. 2005;81(1 Suppl):215S-217S.
10. Landrum JT, Bone RA. Lutein, zeaxanthin, and the macular pigment. Arch Biochem Biophys. 2001;385(1):28-40.
11. Krinsky NI, Johnson EJ. Carotenoid actions and their relation to health and disease. Mol Aspects Med. 2005;26(6):459-516.
12. Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med. 2003;24(6):345-351.
13. Edge R, McGarvey DJ, Truscott TG. The carotenoids as antioxidants: a review. J Photochem Photobiol B. 1997;41(3):189-200.
14. Srinivasan K. Fenugreek (*Trigonella foenum-graecum*): a review of health beneficial physiological effects. Food Rev Int. 2006;22(2):203-224.
15. Basch E, Ulbricht C, Kuo G, Szapary P, Smith M. Therapeutic applications of fenugreek. Altern Med Rev. 2003;8(1):20-27.
16. Li HB, Jiang Y, Chen F. Isolation and purification of lutein from the microalga *Chlorella vulgaris* by extraction after saponification. J Agric Food Chem. 2002;50(5):1070-1072.
17. Lakshminarayana R, Raju M, Krishnakantha TP, Baskaran V. Determination of major carotenoids in a few Indian leafy vegetables by high-performance liquid

chromatography. J Agric Food Chem. 2005;53(8):2838-2842.
18. Zhao Z, Ci F, Pang H, Yu Q, Dong R, Xing A. Advances in research on natural distribution, biosynthesis, detection, bioactivity, and application of lutein. ACS Agric Sci Technol. 2022;2(2):258-269.
19. Sarkar D, Sharma A, Talukder G. Effects of hydrogen peroxide on human lymphocytes *in vitro*. Mutat Res. 1994;311(3):147-157. DOI:10.1016/0165-1218(94)90003-5.
20. Azqueta A, Gutzkow KB, Brunborg G, Collins AR. Towards a more reliable comet assay: optimising agarose concentration, unwinding time and electrophoresis conditions. Mutat Res. 2011;724(1-2):41-45. DOI:10.1016/j.mrgentox.2011.05.010.
21. Halliwell B, Aruoma OI. DNA damage by oxygen-derived species: its mechanism and measurement in mammalian systems. FEBS Lett. 1991;281(1-2):9-19. DOI:10.1016/0014-5793(91)80347-6.
22. Burton K. A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. Biochem J. 1956;62(2):315-323. DOI:10.1042/bj0620315.
23. Halliwell B, Gutteridge JMC. Free radicals in biology and medicine. 5th ed. Oxford: Oxford University Press; 2015.
24. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr. 2003;23:171-201. doi:10.1146/annurev.nutr.23.011702.073307.
25. Stahl W, Sies H. Bioactivity and protective effects of natural carotenoids. Biochim Biophys Acta. 2005;1740(2):101-107. doi:10.1016/j.bbadis.2004.12.006.
26. Cadet J, Douki T. Formation of UV-induced DNA damage contributing to skin cancer development. Photochem Photobiol Sci. 2018;17(12):1816-1841. DOI:10.1039/C8PP00144F.
27. Edge R, Truscott TG. Singlet oxygen and free radical reactions of retinoids and carotenoids: a review. Antioxid Redox Signal. 2009;11(9):1953-1967. doi:10.1089/ars.2008.2273.
28. Krinsky NI, Landrum JT, Bone RA. Biologic mechanisms of the protective role of lutein and zeaxanthin in the eye. Annu Rev Nutr. 2003;23:171-201. doi:10.1146/annurev.nutr.23.011702.073307.
29. Krinsky NI, Yeum KJ. Carotenoid-radical interactions. Biochem Biophys Res Commun. 2003;305(3):754-760. doi:10.1016/S0006-291X(03)00816-7.
30. Edge R, McGarvey DJ, Truscott TG. The carotenoids as antioxidants: a review. J Photochem Photobiol B. 1997;41(3):189-200. DOI:10.1016/S1011-1344(97)00066-8.