

A Methanolic Extract of *Ocimum basilicum* Exhibits Antioxidant Effects and Prevents Selenite-induced Cataract Formation in Cultured Lenses of Wistar Rats

Thiraviyam Anand¹, Mahalingam Sundararajan¹, Muniyandi Anbukkarasi¹, Philip Aloysius Thomas², Pitchairaj Geraldine^{1,*}

ABSTRACT

Objective: An extract (Methanolic) of the *Ocimum basilicum* leaf was analysed for potential to abrogate experimental formation of cataract *in-vitro*. **Methods:** Phytoconstituents were first detected in *O. basilicum* extracts (Aqueous or methanolic) by gas chromatographic-mass spectrometric analysis. The putative antioxidant activity of these extracts was then assessed by measuring *in-vitro* radical-scavenging activity, ion-chelating potential and reducing potency. Potential cytotoxicity of the extract on Human lenticular epithelial B3 (HLE-B3) cells was also sought. Finally, possible prevention of cataract formation by the methanolic extract was gauged in selenite-exposed lenses obtained from Wistar rats. There were 3 groups (8 Lenses in each): Group I (Lenses incubated in Dulbecco's modified Eagle's medium [DMEM] alone); Group II (Lenses incubated in DMEM with sodium selenite [100 µM/ml]); Group III (Lenses incubated in DMEM with selenite [100 µM/ml] and the *O. basilicum* methanolic extract (200 µg/ml DMEM). Gross lenticular morphology was assessed. Levels of lenticular malondialdehyde (MDA) and reduced glutathione (GSH) were also measured. **Results:** A higher intensity of antioxidative activity was noted in the methanolic extract than in the aqueous extract. The methanolic extract exhibited negligible cytotoxicity. On morphological examination, marked opacification was seen in all 8 Group II lenses whereas there was no opacification in 7 of 8 Group III lenses. Near normal mean levels of reduced glutathione and malondialdehyde, were noted within Group III lenses. **Conclusion:** The methanolic extract of the *O. basilicum* leaf appears to prevent selenite-induced cataract formation *in-vitro*.

Key words: *Ocimum basilicum*, Phytoconstituents, Oxidative stress, Antioxidants, Cataract, Crystallins.

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INTRODUCTION

Cataract formation, in which the ocular lens becomes opacified, is the principal aetiology of blindness worldwide; 51% of preventable blindness possibly involves cataract.¹ In India, cataract reportedly accounts for 50-80% of the bilaterally-blind.² Currently, removal of the cataractous lens (By various surgical techniques) followed by intraocular placement of a synthetic lens constitutes the universally-accepted method to manage this condition.³

Lenticular opacification is postulated as a consequence of oxidative stress.⁴ Following photochemical formation of Reactive Oxygen Species (ROS) within the lens and aqueous humour, there is oxidative damage to lenticular tissue.⁵ Oxidative stress possibly induces cataractogenesis by bringing about several changes, such as massive oxidation of thiol to both protein and mixed disulfides,⁶ decreasing density of lenticular epithelial cells and levels of Soluble Sulfhydryls (S-SH) and depleting cellular antioxidants.⁷ Further, the induction of a pro-oxidant environment of the lens leads to abnormal

accumulation of lenticular calcium, resulting in proteolysis of lenticular crystallins by activation of calpain isoforms.⁸

Supplementing a diet with antioxidant-rich biocompounds reportedly maintains lenticular antioxidant status at normal levels while shielding cells from oxidative damage.⁹ Antioxidants from natural sources, rather than synthetic antioxidants, are preferred for use.¹⁰ Antioxidant-replete plant extracts which reportedly exhibit anticataractogenic potential include *Pleurotus ostreatus* (Oyster mushroom),¹¹ *Ocimum sanctum*,¹² *Vitex negundo*,¹³ *Cineraria maritima*,¹⁴ *Aegle marmelos*,¹⁵ *Tabernaemontana divaricata*¹⁶ and *Leucas aspera*.¹⁷ Crude extracts of medicinal herbs are possibly more biologically-active than are purified compounds.¹⁸ Mazandarani *et al.* have reported that solvent polarity in an extract possibly enhances efficacy of its polyphenol constituents, which represent a copious reserve of antioxidants.¹⁹ The solvent system and extraction method

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used also influence the antioxidant yield.²⁰ Aqueous, ethanol, acetone and methanol are extensively used as solvents to recover plant constituents.²¹

Ocimum basilicum (*O. basilicum*), a member of family Lamiaceae, is a kitchen herb. It also serves as a traditional Indian remedy²² for certain disorders, including cough, inflammation, dyspepsia, aches, pains and eye diseases.^{23,24} *O. basilicum* reportedly possesses hypolipidemic,²⁵ anti-inflammatory²⁶ and anti-hyperglycemic²⁷ activity. *O. basilicum* is also documented to be a copious source of antioxidants.^{28,29}

Using Gas Chromatographic-Mass Spectrometric (GC-MS) analysis, we sought phytoconstituents within a methanolic extract and an aqueous extract of the leaf of *O. basilicum*. Following this, we assessed possible *in-vitro* antioxidative activity of these extracts. Based on its perceived superior efficacy, the methanolic extract was chosen to undergo assessment as a potential anticataractogenic agent in an experimental (*in-vitro*) setting of selenite-induced formation of cataract in lenses surgically removed from the eyes of Wistar rats.

MATERIALS AND METHODS

Chemicals and solvents

Sodium selenite was purchased from LOBA Chemie (Mumbai, India). Dulbecco's modified Eagle's medium (DMEM), the antibiotics streptomycin and penicillin and different chemicals and analytical grade solvents were procured from HiMedia (Mumbai, India).

Collection and authentication of identification of *Ocimum basilicum* plant

The *O. basilicum* plant used in this investigation was cultivated in the garden area of Bharathidasan University, Tiruchirappalli, Tamil Nadu state, India. Following identification and authentication of the plant (Authentication No. TA001) by Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics (St. Joseph's College [Campus], Tiruchirappalli, Tamil Nadu, India), fresh leaves were obtained, shade-dried and powdered. These powdered leaves were extracted independently with aqueous or methanol, followed by final processing.

Preparation of the methanolic and aqueous extracts of *O. basilicum* leaves

The extraction procedure followed was that described by Kadan *et al.*³⁰ wherein 30G of powdered leaves and the solvent (150 ml) were loaded within a Soxhlet apparatus. The resulting solvent underwent evaporation under lowered pressure at 55-60°C and then was dried in a vacuum. The residue underwent filtration and concentration to a dry state by vacuum distillation. Both aqueous and methanolic extracts then underwent GC-MS analysis.

Analysis of extracts by gas chromatography-mass spectrometry

GC-MS analysis (GC/MS-series QP2010, Shimadzu, Tokyo, Japan) was performed using Thermal Desorption (TD) system 20 under defined experimental conditions: RTX-5MS capillary column (30 m x 0.25 mm and a film thickness of 0.25 mm), helium gas (Serving as carrier) at 1.5 ml/min, a gun temperature of 250°C, a detector temperature of 290°C and a column temperature of 60°C-180°C at a rate of 5°C/min, followed by 180°-280°C at 10°C/min (10 min), a scanning speed of 0.5 scan/sec of m/z 40 and 350 and a split ratio of 1:200. The volume injected was 1 µl. The samples (Dissolved in chloroform) underwent a full run within a range of 50-650 mass-to-charge ratios (m/z). The mass spectrometer was operated with 70 eV ionization energy. Individual components were identified by mass spectral fragmentation using the NIST Mass 08 spectral library, in addition to retention indices and compared with published data.

Elucidation of possible *in vitro* antioxidative mechanisms of the *Ocimum basilicum* extracts

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay

DPPH radical-scavenging by the extracts was quantified by a modification of a frequently-used procedure (Details provided in Supplementary Files).³¹ The DPPH radical-scavenging potential for each extract was separately calculated thus:

$$\text{DPPH scavenging activity (\%)} = [(A_{\text{cont}} - A_{\text{test}}) / (A_{\text{cont}})] \times 100,$$

A_{cont} being absorbance of the control reaction and A_{test} being absorbance of the mixture with extract or that of a defined reference solution. The assay was done thrice and DPPH radical-scavenging activity (%) was plotted against different dilutions of each extract.

Metal chelating assay

Ferrous ion chelation by each extract was quantified using a modified method of Mathew and Abraham (details in Supplementary Files).³² Inhibition (%) of the ferrozine-Fe²⁺ complex that was formed was calculated per the above-stated formula (DPPH scavenging assay).

Reducing power assay

This was carried out essentially as stated by Oyaizu (Details in Supplementary Files).³³ A greater absorbance of the reaction mixture was taken to indicate an increased reducing power.

Superoxide anion radical-scavenging assay

The superoxide anion radical (O₂⁻)-scavenging assay was done per a modified procedure proposed by Rajeshwar *et al.*³⁴ details of which are in the Supplementary Files. Superoxide anion radical-scavenging (%) was calculated per the above formula (DPPH scavenging assay).

Hydroxyl radical-scavenging assay

This was done per a previously-described procedure by Halliwell *et al.*³⁵ details of which are in Supplementary Files. The intensity of scavenging activity was determined per the above formula (DPPH scavenging assay).

Culture of Lenticular Epithelial Cells

Human lenticular epithelial B3 (HLE-B3) cells were a kind gift from Aravind Eye Research Foundation, Madurai, Tamil Nadu, India. HLE-B3 cells were cultured in DMEM supplemented with 20% Fetal bovine serum (FBS), 45 IU/mL penicillin and 45 IU/mL streptomycin at 37°C in a humidified 5% CO₂ atmosphere.

Cytotoxicity assay

Potential cytotoxicity of the *O. basilicum* extract was determined by using 3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT) (Sigma-Aldrich, St. Louis, MO, USA) as the indicator of cytotoxicity. In brief, HLE-B3 cells were cultured as described earlier, except that these cells were seeded (Density of 5 × 10³ cells/well) in 96-well tissue culture plates. To each well, 200 µl of the extract were added, yielding concentrations ranging from 10 to 300 µg/ml of medium; the cells were then incubated for 24 h. Dimethyl Sulfoxide (DMSO) was used as a negative control. The cells were treated with 0.5 mg/mL of the MTT- labelling reagent and incubated at 37°C for 1 h. A dark blue formazan product of MTT was extracted by the MTT lysing solution (40 mM HCl in isopropyl alcohol) and measured (Absorbance of 570 nm) by an automatic ELISA reader (Spectra Max 250; Molecular Devices, Sunnyvale, CA, USA).

Assessment of the potential of *O. basilicum* leaf methanolic extract to prevent cataract formation *in vitro* in lenses surgically removed from eyes of Wistar rats

Removal of lenses from eyes of Wistar rats and culture in DMEM

All experiments and animal handling in this investigation conformed to Institutional guidelines (Institutional Ethical Committee approval Reference No.: BDU/IAEC/2017/NE/ 30/21.03.2017) and to the principles of the *Association for Research in Vision and Ophthalmology Statement for the use of Animals in Research*. Wistar rats (*Rattus norvegicus*; 75-90 g) were first anesthetized using diethyl ether and then underwent sacrifice by cervical dislocation. The lens of each eye was dissected out carefully and then incubated at 37°C in 3 ml of DMEM (supplemented with fetal bovine serum [10%] and sodium bicarbonate [0.9 g/L]) in a 12-well Falcon plastic culture plate placed within an incubator with an atmosphere of 95% air and 5% CO₂. Streptomycin (60 µg/ml) and penicillin (60 µg/ml) were also added to avoid microbial contamination. After incubation for 2 h, any opaque lenses were removed from the set-up; only lenses exhibiting complete transparency were chosen for subsequent experimental studies. The selected lenses were placed in DMEM and were assigned to 3 groups:

1. In Group I (n = 8), lenses were kept in DMEM alone (Normal control).
2. In Group II (n = 8), lenses were kept in DMEM that contained only sodium selenite (100 µM selenite/ml DMEM) (Selenite-exposed but untreated).
3. In Group III (n = 8), lenses were kept in DMEM that contained sodium selenite (100 µM selenite/ml DMEM) as well as the (methanolic) extract of *O. basilicum* leaves (200 µg/ml DMEM) (Selenite-exposed and *O. basilicum* extract-treated).

Morphological evaluation of lenticular opacification

All lenses underwent gross examination, with dark grid lines serving as a backdrop. A dissecting microscope was used for magnification. The grades of opacification were:

- a) 0: no opacity seen (Grid lines were distinctly visible).
- b) +: slight opacity seen (Minimal haziness obscuring grid lines, which were still visible).
- c) ++: diffuse opacification of almost the full lens (Moderate haziness obscuring grid lines, which were faintly visible).
- d) +++: marked opacification of the full lens (Total haziness obscuring grid lines, which were not visualized at all).

Preparation of lenticular homogenate

Lenses underwent homogenization in 10-fold their mass of phosphate buffer (50 mM, pH 7.2), followed by centrifugation (12,000 × g) for 15 min at 4°C. The supernatant yielded was separated and processed for biochemical analysis. The concentration of protein in each sample was measured per the procedure formulated by Lowry *et al.* with bovine serum albumin serving as a standard.³⁶

Quantitative measurement of concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) GSH

GSH content (Micromoles/g tissue) in lenticular homogenate was measured by a procedure described by Moron *et al.* (Procedure in Supplementary Files).³⁷

Lipid peroxidation

The lenticular concentration of MDA, a measure of lipid peroxidation was assayed following the method described by Ohkawa *et al.* (Procedure in Supplementary Files).³⁸

Statistical analysis

The values are documented as mean ± the standard deviation of multiple readings. The statistical significance of differences between groups was calculated by One-Way Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) software package for Windows, version 21, IBM Corporation, New York, USA. Where one-way ANOVA yielded significant results, post-hoc testing was performed for inter-group comparisons using the least significant difference test. The chi-square test with Yates' correction (GraphPad Prism [Version 6], GraphPad Software Inc., San Diego, California, USA) was used wherever appropriate. Values of *P* < 0.05 were deemed statistically significant (Highlighted by distinct symbols in tables and figures).

RESULTS

Phytoconstituents present in the methanolic extract and the aqueous extract of leaves of the *O. basilicum* plant were located and analysed. The antioxidative potency of the extracts was also assayed.

GC-MS analysis for phytochemicals in aqueous and methanolic extracts of *O. basilicum* leaves

Several phytochemicals with bioactivity (Percentage peaks expressed with their retention indices shown in Figures 1a and 1b), which probably

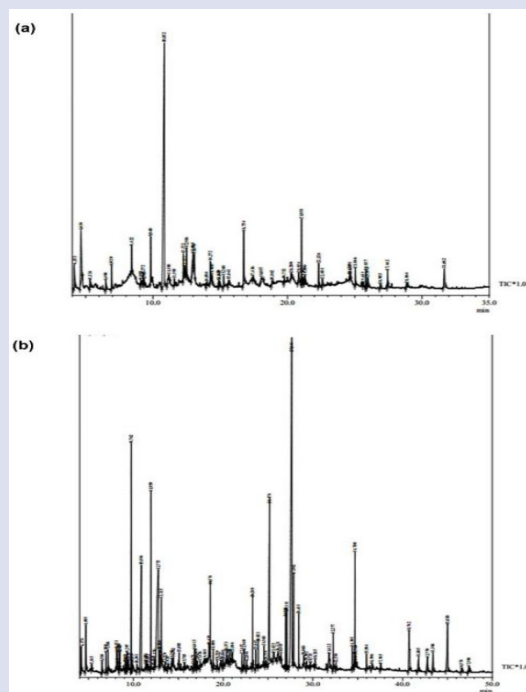


Figure 1: Gas chromatography-mass spectrometry chromatogram. (a) Gas chromatography-mass spectrometry chromatogram of the aqueous extract of leaves of *Ocimum basilicum*. (b) Gas chromatography-mass spectrometry chromatogram of the methanolic extract of leaves of *Ocimum basilicum*.

*Peaks indicate the area percentage of identified phytoconstituents in the extracts of *Ocimum basilicum*.

rendered the extracts bioactive, were located and compared with those reported from different medicinal plants (Supplementary data- Tables 1 and 2). By GC-MS analysis, the aqueous extract revealed 47 constituents, of which 19 are documented as biologically-active compounds and 5 are reportedly antioxidants (Supplementary data- Table 1); in the methanolic extract, 89 constituents were present, of which 40 are documented as biologically-active compounds and 19 are reportedly antioxidants (Supplementary data- Table 2). n-hexadecanoic acid (8.10 %), eugenol (4.89 %) and estragole (3.68 %) in the methanolic extract and 2-furan-methanol (2.31 %) in aqueous extract have been designated as major antioxidant components.

In vitro antioxidative potency of extracts of *O. basilicum* leaves

Scavenging of DPPH radicals by the extracts

Potential free radical-scavenging by the extracts was assayed by the DPPH method and data generated were compared to results with ascorbic acid standard (Figure 2a). Both extracts and also ascorbic acid, showed significant dose-dependent inhibition of DPPH radicals. However, radical-scavenging by these extracts was inferior to that by ascorbic acid (Figure 2a). Interestingly, 100 µg/ml concentrations of the aqueous and methanolic extracts exhibited radical-scavenging of 77.85 % and 80.43 %, respectively ($P < 0.05$) (Figure 2a).

Metal chelating activity of the extract

The ferrous ion-chelating potential of both extracts was compared with that of ascorbic acid (Figure 3a); the methanolic extract, in a concentration of 100 µg/ml, showed a significantly higher chelating percentage (82.84 %) than that (72.33 %) shown by 100 µg/ml of the aqueous extract, irrespective of the ferrous ion concentration (Figure 3a).

Reducing power of the extracts

The reducing power of the *O. basilicum* extracts was contrasted with that of ascorbic acid (Figure 3b); the methanolic extract displayed a reducing power that was superior to that of the aqueous extract and this was found to rise with increasing extract concentrations.

Potential of the extracts to scavenge superoxide anion-radicals

Even low concentrations (50 µg/ml) of the *O. basilicum* extracts, showed marked scavenging of superoxide anion radicals (Figure 2b), with the intensity of scavenging by the methanolic extract (81.25 %) exceeding that shown by the aqueous extract (71.52 %) (Figure 2b).

Scavenging of hydroxyl radicals by extracts

Inhibition of hydroxyl radical-mediated deoxyribose damage by the *O. basilicum* extracts was evaluated utilizing the iron (II)-dependent DNA damage assay (Figure 2c). Hydroxyl radical-scavenging activities of 65.5 % (Methanolic extract 50 µg/ml) and 76.35 % (aqueous extract 50 µg/ml) were noted (Figure 2c); ascorbic acid, which effectively inhibits hydroxyl radicals, showed 81.39 % scavenging effect (Significantly superior to both extracts) (Figure 2c).

In contrast to the aqueous extract, the methanolic extract possessed a greater number of phytochemicals that have been reported to show antioxidant activity (Supplementary data- Tables 1 and 2) and exhibited superior scavenging of various radicals (DPPH, superoxide anion, hydroxyl) and a greater intensity of ferrous ion-chelating activity. Hence, the methanolic extract alone underwent further assessment for its *in-vitro* anticataractogenic potency in rat lenses.

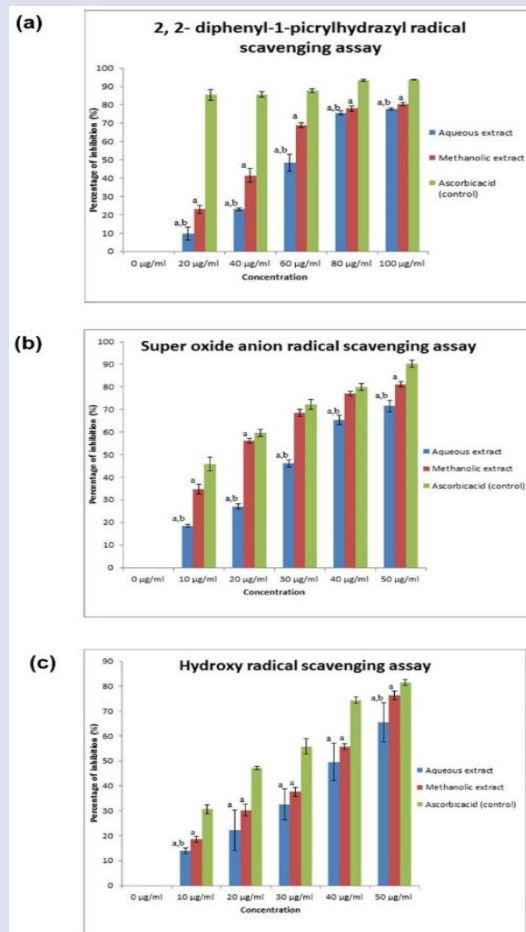


Figure 2: Differential scavenging effects of ascorbic acid (Control) and of an aqueous extract and a methanolic extract of leaves of *Ocimum basilicum* on radicals.

a) Differential scavenging effects on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals; b) Differential scavenging effects on superoxide anion radicals; c) Differential scavenging effects on hydroxyl-anion radicals.

Statistical Analysis

Each value is expressed as mean \pm standard deviation of six determinations. One-way analysis of variance [ANOVA] with post hoc testing (Least significant difference).

^aStatistically significant difference ($P < 0.05$) when compared with ascorbic acid (Control) values.

^bStatistically significant difference ($P < 0.05$) when compared with methanolic extract values.

Cytotoxicity of the methanolic extract of *O. basilicum*

In the present investigation, the potential cytotoxicity of the methanolic extract of *O. basilicum* was tested on HLE-B3 cells (Figure 4a), employing MTT assay. As illustrated in Figure 4b, HLE-B3 cells were exposed to varying concentrations of the extract (10 – 300 µg/mL) for 24 h. The methanolic extract exhibited negligible cytotoxicity, with an IC_{50} value of 127 µg/mL at the end of the 24 h exposure period. There was a decrease in the viability of cells only when exposed to concentrations greater than 300 µg/mL of the extract.

The potential of the *O. basilicum* leaf methanolic extract to prevent cataract formation in cultured lenses surgically removed from Wistar rat eyes

Gross morphology of the cultured lenses

Even after incubation in DMEM alone for 24 h, all 8 (100%) control lenses did not reveal any opacity (Grade 0 opacification). In contrast, after 24 h incubation, all eight (100%) Group II lenses (incubated in DMEM containing sodium selenite) showed marked opacity formation (Grade +++). Interestingly, after 24 h incubation, only one (12.5 %) of eight Group III lenses (which were incubated in DMEM containing sodium selenite and the methanolic extract added simultaneously), showed Grade + opacification, while the other seven (87.5%) showed Grade 0 (no) opacification (Table 1, Figure 5).

Quantitation of mean levels of GSH and MDA

The mean lenticular level of GSH in the group of lenses exposed to sodium selenite but not treated, was significantly less than the mean lenticular GSH level in control (Normal) lenses (Table 2). The mean lenticular level of GSH in the group of lenses exposed to selenite and treated with the extract was significantly higher than the mean lenticular GSH level in the untreated group but did not differ significantly compared to mean lenticular level of GSH in the normal control group (Table 2).

Although the mean lenticular MDA concentration in the selenite-exposed, untreated group was significantly greater than the mean lenticular MDA concentrations in the normal control lenses and the selenite-exposed, extract-treated lenses (Table 2), the mean MDA level in extract-treated lenses did not differ significantly from the mean MDA level in control (Normal) group of lenses (Table 2).

DISCUSSION

Exposure to excessive oxidative stress reportedly leads to lenticular opacification.³⁹ Antioxidants may prevent or impede evolution of diseases arising from oxidative stress by raising levels of endogenous antioxidant defences.⁴⁰ Possibly, antioxidative treatment may effectively prevent cataracts and other oxidative stress-related complications. However, different clinical studies have yielded inconsistent results. *Ocimum basilicum*, commonly called the basil plant, finds application in Unani and Ayurveda medications. Although *O. basilicum* possesses antioxidant potential, this plant has yet to be screened to detect phytochemicals with antioxidative potency. A plant's medicinal property depends on its phytoconstituents and associated secondary metabolites.⁴¹ We tried to detect phytoconstituents in aqueous (Figure 1a) and methanolic (Figure 1b) extracts of leaves of *O. basilicum*. While the two extracts exhibited many phytoconstituents, GC-MS analysis suggested that the methanolic extract possessed a greater number of antioxidant compounds than that possessed by the aqueous extract (Supplementary data- Table 1 and 2).

The major constituent detected in the extract, namely n-hexadecanoic acid (Area percentage 8.1 %) (Supplementary data-Table 2), has previously been observed in an extract of *Pistia stratiotes* leaf⁴² and in *Neolamarckia cadamba*⁴³ leaves is reportedly endowed with antioxidant and anti-inflammatory properties.⁴⁴ Eugenol (4.89%), an additional major constituent (Supplementary data-Table 2), is documented to exhibit antioxidant properties and to reduce free-radical generation within an *in-vitro* experimental system.⁴⁵ Devendran and Balasubramanian have also reported that an extract of *Ocimum sanctum* had eugenol as a major constituent.⁴⁶ Yet another major constituent, estragole (3.68 %) (Supplementary data- Table 2), which was found in an extract of *Artemisia dracunculus*⁴⁷ is reportedly endowed with antioxidant potential.

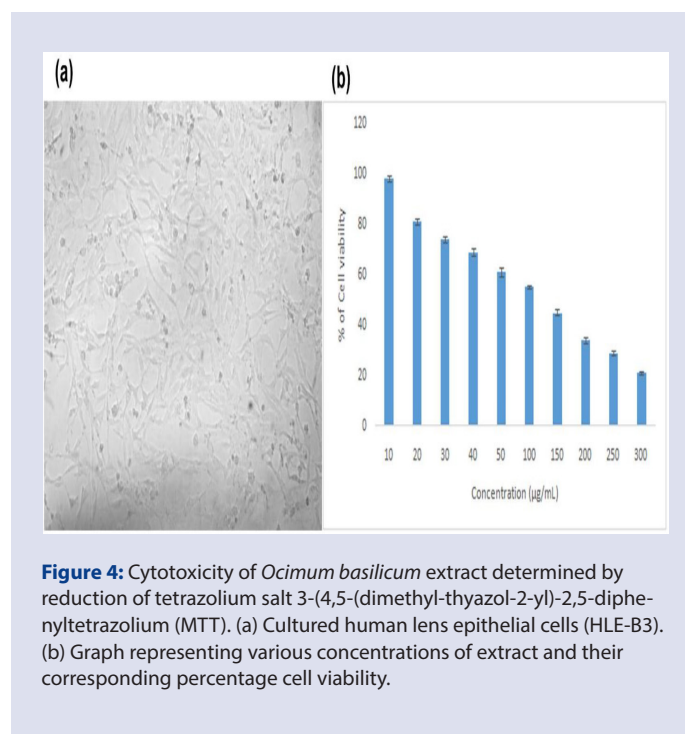


Figure 4: Cytotoxicity of *Ocimum basilicum* extract determined by reduction of tetrazolium salt 3-(4,5-(dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium (MTT). (a) Cultured human lens epithelial cells (HLE-B3). (b) Graph representing various concentrations of extract and their corresponding percentage cell viability.

Table 1: Grading of lenticular opacification in Wistar rat lenses incubated for 24 h in Dulbecco's modified Eagle's medium (DMEM).

Groups of lenses (Incubated in DMEM)*	Total number of lenses	Grades of opacification**	Number of lenses with different grades of lenticular opacification
Group I	8	0	all 8
Group II	8	+++	all 8
Group III	8	0 +	7 of 8 1 of 8

*Groups of Lenses

- (1) Group I (n = 8) comprising normal lenses incubated in DMEM alone (Control).
- (2) Group II (n = 8) comprising lenses incubated in DMEM containing sodium selenite only (100 µM selenite/ml DMEM) (Selenite-exposed, untreated).
- (3) Group III (n = 8) comprising lenses incubated in DMEM containing sodium selenite (100 µM selenite/ml DMEM) and the methanolic extract of *Ocimum basilicum* leaves (200 µg/ml DMEM) (Selenite-exposed, *O. basilicum* extract-treated).

**Grades of Opacification

- 0 = absence of opacification (Gridlines clearly visible);
 + = presence of a slight degree of opacification (Minimal clouding of gridlines [Gridlines still visible]).
 ++ = presence of diffuse opacification involving almost the entire lens (Moderate clouding of gridlines [gridlines faintly visible]).
 +++ = presence of extensive thick opacification involving the entire lens (Total clouding of gridlines [Gridlines not seen at all]).

Statistical Analysis

Lenticular opacification in Group II vs. that in Group I and in Group III; Yates' chi-square (χ^2) test [Degree of freedom = 2] = 9.54; $p < 0.05$.

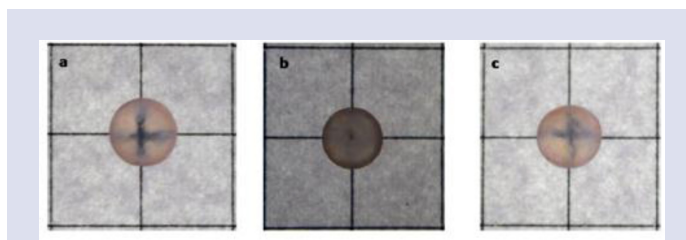


Figure 5: Morphological assessment of lenticular opacification in Wistar rat lenses incubated for 24 h in Dulbecco's modified Eagle's medium.

- a) **Group I:** Lenses incubated in Dulbecco's modified Eagle's medium (DMEM) alone (Normal control). Grade 0 opacification present (No opacification).
- b) **Group II:** Lenses incubated in DMEM containing sodium selenite only (100 μ M selenite/ml of DMEM) (Selenite-exposed, untreated). Grade 3 opacification present.
- c) **Group III:** Lenses incubated in DMEM containing sodium selenite (100 μ M selenite/ml DMEM) and the methanolic extract of *Ocimum basilicum* leaves (200 μ g/ml DMEM) (Selenite- challenged, *O. basilicum* extract-treated). Grade 1 opacification present.

Table 2: Mean levels of reduced glutathione and of malondialdehyde in Wistar rat lenses incubated for 24 h in Dulbecco's modified Eagle's medium (DMEM).

Component analysed (unit)	Mean values in lenses *		
	Group I	Group II	Group III
Reduced glutathione (μ mol/gm tissue)	7.09 \pm 0.18	5.07 \pm 0.29 ^a	6.61 \pm 0.44 ^b
Malondialdehyde (nmol/gm tissue)	14.98 \pm 4.92	23.87 \pm 1.89 ^a	17.21 \pm 1.95 ^b

*Groups of Lenses

- (1) Group I (n = 8) comprising normal lenses incubated in DMEM alone (Control).
- (2) Group II (n = 8) comprising lenses incubated in DMEM containing sodium selenite only (100 μ M selenite/ml DMEM) (Selenite-exposed, untreated).
- (3) Group III (n = 8) comprising lenses incubated in DMEM containing sodium selenite (100 μ M selenite/ml DMEM) and the methanolic extract of *Ocimum basilicum* leaves (200 μ g/ml DMEM) (Selenite-exposed *O. basilicum* extract-treated).
Statistical Analysis

Values represent mean \pm standard deviation of six determinations; One-way analysis of variance with post-hoc test [Least significant difference].

^aStatistically significant difference ($P < 0.05$) when compared with group I or group III values.

^bStatistically significant difference ($P < 0.05$) when compared with group II values.

The biological activities of phytoconstituents are possibly linked to their antioxidant characteristics.⁴⁸ The DPPH radical is not affected by metals and enzyme inhibition, since it has an unpaired electron, which exhibits a potent absorption maximum at 517 nm in visible spectroscopy.^{31,49} We noted good DPPH radical-scavenging potency in both extracts (Figure 3), although the methanolic extract appeared to be more effective than the aqueous extract in this characteristic (Figure 2a).

In the metal-chelating assay, an antioxidant reduces a Fe (III) salt through an electron transfer mechanism. Transition metals, including copper and iron, heighten oxidative stress. The chelation of metal ions can decrease their activity, thereby reducing generation of ROS.⁵⁰ In our

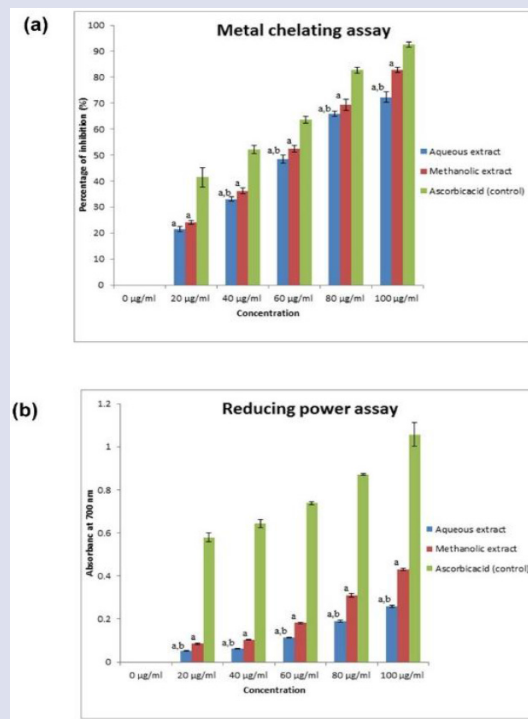


Figure 3: Differential Chelating Effects and Reducing Power of Ascorbic Acid (Control) and of an Aqueous Extract and a Methanolic Extract of Leaves of *Ocimum basilicum*.

- a) Differential chelating effects on ferrous ions; b) Differential reducing power of *Ocimum basilicum*.

Statistical Analysis

Each value is expressed as mean \pm standard deviation of six determinations.

One-way analysis of variance [ANOVA] with post hoc testing (Least significant difference).

^aStatistically significant difference ($P < 0.05$) when compared with ascorbic acid (Control) values.

^bStatistically significant difference ($P < 0.05$) when compared with methanolic extract values.

experiments, the *O. basilicum* methanolic extract appeared to be more potent than the aqueous extract in chelating iron (Figure 3a). The reducing power of a compound may point to its antioxidative potential.⁵¹ We noted that the reducing power exhibited by both extracts progressively rose proportionate to increasing concentrations of the extracts (Figure 3b). The higher reducing power of the *O. basilicum* methanolic extract, compared to the aqueous extract, possibly arose from its greater hydrogen-donating capacity.

Superoxide anion ($O_2^{\cdot-}$), which represents the end product of a one-electron reduction of oxygen, exhibits toxicity to cellular components, as a consequence of being a precursor of most ROS as well as a mediator in oxidative chain reactions.⁵² Our observations point to the methanolic extract being superior to the aqueous extract in scavenging superoxide anions (Figure 2b). The hydroxyl radical is the most reactive oxygen-centered species and may severely damage adjacent biomolecules.⁵³ The hydroxyl radical-scavenging assay was used to demonstrate the potency of the extracts versus that of an ascorbic acid standard in inhibiting

hydroxyl radical-mediated deoxyribose degradation in a Fe³⁺-EDTA ascorbic acid and H₂O₂ reaction mixture (Figure 2c). Our data suggest that the methanolic extract is more potent than the aqueous extract in scavenging hydroxyl radicals (Figure 2c).

Similar antioxidative potency has been noted in other plants, including *Leucas indica*,⁵⁴ *Phyllanthus acidus*⁵⁵ and *Azima tetracantha*.⁵⁶ Previous reports point to positive associations between supplementation with antioxidative compounds and decreased frequency of occurrence and progression of senile cataracts in human⁵⁷ and in selenite cataracts in experimental animals.⁵⁸ This finding prompted us to test the possible efficacy of the methanolic extract in preventing lenticular opacity in selenite challenged rat lenses.

HLE-B3 cells were exposed to varying concentrations of the methanolic extract (10 – 300 µg/mL) for 24 h (Figure 4b). The methanolic extract exhibited negligible cytotoxicity, with an IC₅₀ value of 127 µg/mL at the end of the 24 h exposure period. There was a decrease in the viability of cells only when exposed to concentrations greater than 300 µg/mL of the extract. The result suggests that the methanolic extract of *O. basilicum*, is not toxic to HLE-B3 cells, thereby ensuring potential application for preventing/retarding cataractogenesis in humans.

As GC-MS analysis yielded a greater number of antioxidant constituents in the methanolic extract, this extract alone was tested for anticataractogenic potential. Gross morphological examination of cultured lenses revealed intense (Grade +++) opacification in all eight (100%) lenses that had been exposed to sodium selenite but not treated. In contrast, seven (87.5%) of the eight selenite-exposed, *O. basilicum* methanolic extract-treated lenses (Group III) showed no opacity at all (Grade 0 opacification) after 24 h incubation, whereas one single lens (12.5%) manifested minimal opacification (Grade +; Table 1, Figure 5). Thus, the methanolic extract of *O. basilicum* apparently prevented cataract formation in lenses that had been exposed to sodium selenite. Several antioxidant-rich herbal extracts, such as those of *Aralia elata*,⁵⁹ *Emblica officinalis*,⁶⁰ and of *Vaccinium corymbosum*,⁶¹ also exhibit anticataractogenic potential.

In order to gauge the intensity of oxidative stress among the experimental groups of rat lenses, the level of GSH, that serves as a significant first line of defence versus oxidative stress, was assayed. The concentration of MDA, which is often used as a marker of oxidative damage to lipids, was also determined. GSH, an essential tripeptide thiol, is a crucial intracellular and extracellular antioxidant that shields cells against oxidative stress-induced damage.⁶² A decreased cellular GSH concentration may affect GSH-dependent enzymes, including glutathione peroxidase, glutathione reductase, glutathione S-transferase and glucose-6-phosphate dehydrogenase, leading to further oxidative damage.⁶³ Treatment of the selenite- challenged lenses with the *O. basilicum* extract apparently prevented reduction of lenticular GSH levels, therein possibly contributing to maintenance of lenticular transparency. MDA is, an important aldehyde resulting from membrane lipid peroxidation.³⁸ In the present investigation, disruption of membrane lipids was a possible reason for the observed increase in MDA levels in the lenses exposed to selenite but untreated (Group II), in contrast to MDA levels in control (normal) lenses (Group I); this elevated MDA level possibly contributed to severe oxidative damage and lenticular opacification. The observed lowering of the MDA level in *O. basilicum* extract-treated group of lenses (Group III) suggests that the extract nullified disruption of membrane lipids, thereby preventing opacification of lens.

CONCLUSION

A methanolic extract of *O. basilicum* has potent antioxidant activity by virtue of its scavenging actions on DPPH, hydroxyl and superoxide radicals, its reducing power on ferric ions and its chelation of ferrous ions. *O. basilicum* also exhibits great potential to prevent cataract formation in

Wistar rat lenses by reducing the intensity of lipid peroxidation and by enhancing the level of GSH, possibly due to its antioxidant activity. The antioxidant principles identified within the plant extract, such as n-hexadecanoic acid, eugenol and estragole and 2-furanmethanol, all possibly contributed to the observed effects. These experimental results suggest that a methanolic extract of *O. basilicum* can prevent selenite-induced cataract formation *in-vitro*; further studies are needed to evaluate possible application in the clinical setting.

APPENDIX A

Supplementary data.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ABBREVIATIONS

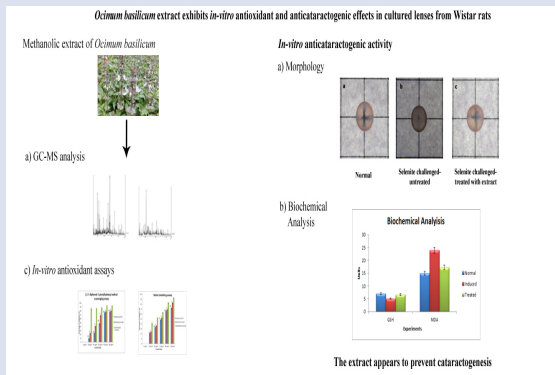
DMEM: Dulbecco's modified Eagle's medium; **MDA:** Malondialdehyde; **GSH:** Reduced glutathione; **ROS:** Reactive oxygen species; **GC-MS:** Gas chromatographic-mass spectrometry; **DPPH:** 2,2-Diphenyl-1-picrylhydrazyl; **ANOVA:** One-way analysis of variance; **SPSS:** Statistical Package for Social Sciences.

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GRAPHICAL ABSTRACT



SUMMARY

- The current study sought to identify bioactive constituents present in the aqueous and methanolic extracts of *O. basilicum* leaves by using gas chromatography-mass spectrometry analysis and to elucidate various antioxidant characteristics.
- The results suggest that the methanolic extract of *O. basilicum* leaf could serve as a better source of antioxidants than the aqueous extract.
- The results suggest that the *O. basilicum* leaf methanolic extract retards selenite-induced cataractogenesis by preventing or dampening selenite-induced free radical formation in lenticular cells.

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