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EVALUATION OF ANTIOXIDANT, ANTI-INFLAMMATORY AND CYTOPROTECTIVE PROPERTIES OF *KANDELIA CANDEL* L. DRUCE

SHITAL MANOHAR PUNEKAR[a], SUMREEN SULTANA[a], NIVEDIKA M.B.[a], TEKUPALLI RAVIKIRAN [a*]

[a] Department of Microbiology and Biotechnology, Bangalore University, Jnana Bharathi Campus, Bengaluru 560056, India

***Corresponding author**

Tekupalli Ravikiran

Email- ravikiran@bub.ernet.in

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Abstract

Oxidative stress is a contributing factor in the development of numerous diseases, including cancer, diabetes, cardiovascular disease and neurodegenerative disorders. Plant-derived bioactive compounds are known for their biological properties. The present study investigated the antioxidant, anti-inflammatory, lipid peroxidation inhibition and cytoprotective activities of *Kandelia candel* leaf and stem extracts. Antioxidant potential was analysed using DPPH, ABTS, reducing power and total antioxidant activity assays. The anti-inflammatory and inhibition of lipid peroxidation properties were assessed using the protein denaturation assay and the TBARS assay. Methanolic leaf extract exhibited antioxidant activity as displayed by its phenol and flavonoid content. Similarly, anti-inflammatory, lipid peroxidation inhibition activities were demonstrated by methanolic extracts, as revealed by lower IC_{50} values as compared to other extracts. Further, the cytoprotective effect of the extracts was investigated against H_2O_2-induced oxidative stress in SH-SY5Y using the MTT assay. The methanolic extracts showed better cytoprotective effect compared to the aqueous extract. These results indicate that the *K. candel* methanolic extract exerts antioxidant, anti-inflammatory, and cytoprotective effects, and highlight its therapeutic potential in neurodegenerative disorders and oxidative stress-associated diseases.

INTRODUCTION

Oxidative stress (OS) is an imbalance between pro-oxidants and antioxidants, which leads to the overproduction of reactive oxygen species (ROS). Excessive ROS adversely affects cellular components and membranes, ultimately leading to apoptosis and necrosis (Gautam *et al.,* 2022). Thus, OS has been a triggering element for numerous chronic diseases, including cardiovascular, neurodegenerative, age-related diseases, cancer, diabetes mellitus, autoimmune disorders, and inflammation (Naik *et al*., 2025; Zhou *et al.,* 2025; Sirin *et al.,* 2023). Conversely, antioxidants are natural compounds that can mitigate OS by neutralizing free radicals, controlling pro-oxidants, and obstructing lipid peroxidation (Razghandi *et al.,* 2024; Ayoub & Mehta, 2018). They can donate electrons or hydrogen and act as stable radical intermediates (Chaves *et al.,* 2020). These compounds can suppress signaling pathways involved in inflammatory response and protect macromolecules. Synthetic antioxidants are commonly used as an alternative, but are coupled with health risks due to their possible toxicity. Hence, there is an increased interest in the application of plant-derived antioxidants

such as phytochemical compounds (Gulcin, 2025).

The traditional medicine systems, such as Chinese, Unani, and Ayurveda, used plant products to treat various disease conditions. Phytochemicals are bioactive compounds synthesized in different parts of the plant, including leaves, shoots, roots, flowers, and fruits and protect the plant from biotic and abiotic environmental stresses. They include primary and secondary metabolites like flavonoids, terpenoids, alkaloids, phenols, tannins, and glycosides, and have a broad range of pharmacological activities (Hussen & Endalew, 2023).

Mangrove plants are inhabitants of tropical and subtropical areas and are categorized into two subgroups, true and associate mangroves. Mangrove plants consist of 84 species, out of which 70 are true, which include 16 genera and 11 families (Manohar, 2024). India has about 5% of the world's mangrove coverage with an area of about 4827 km^2 (Pranav *et al.,* 2022). According to ISFR (2023), three coastal districts of Karnataka have 1420 ha of mangrove forests (Ramteke *et al.,* 2026). Mangrove plays a promising role in the ecosystems and possesses diverse bioactive compounds. They are always under harsh environments like high salinity, moisture, hypoxia, and low nutrition (Khadeeja *et al.,* 2022; Wang *et al*., 2014). To endure such harsh conditions, they synthesize secondary metabolites like phenols and flavonoids (Tekupalii *et al.,* 2020). Mangroves have been used for various medicinal applications because of their antioxidant, antibacterial, anticancer, neuroprotective, and antimalarial properties (Kumari *et al.,* 2026; Cerri *et al.,* 2024; Chowdhury, 2024; Thao *et al.,* 2015).

Kandelia candel L. Druce (*K. candel*), a true viviparous mangrove, belongs to the *Rhizophoraceae* family, commonly distributed along the western coast of India and known to have pharmacological properties (Xu *et al.,* 2023; Shettar *et al.,* 2018). The leaf extract of *K. candel* was reported to have antioxidant, anti-inflammatory, and anticancer activities (Shettar et al., 2018; Minakawa *et al.,* 2012), and bark for its antidiabetic properties (Habeebulla & Velraj, 2022). Limited studies are reported on the antioxidant and anti-inflammatory properties of *K. candel leaf extracts.* To the best of our knowledge, no studies have been reported on lipid peroxidation and cytoprotective activities of these extracts.

Hence, the present study emphasized the phytochemical profile, antioxidant, anti-inflammatory, lipid peroxidation, and cytoprotective potential of the leaves and stem of *K. candel*.

MATERIALS AND METHODS

Chemicals and reagents

2, 2 -Azinobis -3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2, 2 - Di (4-tert-octyl phenyl)-1-Picryl Hydrazyl (DPPH), Thiobarbituric acid, were purchased from Sigma Aldrich, USA. Streptomycin, penicillin, Dulbecco's Modified Eagle Medium/F12 (DMEM/F12), trypsin-EDTA, Fetal bovine serum (FBS), 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide (MTT), antibiotic and antimycotic solution, and phosphate-buffered saline (PBS) were procured from Himedia. H_2O_2, Dimethyl sulfoxide (DMSO), and other chemicals were from SRL.

Collection of plant material

Fresh leaves and stems of *Kandelia candel* (L.) were collected from Kemmannu, Udupi, Karnataka (13° 23' 57.3468'' N Latitude and 74° 42' 31.7952'' E Longitude). Plants were identified and authenticated by Dr. V. Rama Rao, Research Officer, Botany, CARI, Bengaluru, Karnataka, India. (RRCBI mus-365).

Preparation of extract

The extracts of the leaf and stem of *K. candel* were prepared using aqueous and methanol solvents by following the protocol of Suganthy and Devi (2016). Briefly, leaf and stem samples were washed thoroughly in running water, shade-dried, and finely powdered using an electric blender. 10 gm powder was dissolved in 100 mL of solvents, allowing it to stand at a magnetic stirrer for 24 h at room temperature, and filtered through the Whatman no. 1 paper. Finally, the filtrate is lyophilized and stored (4 °C) for further studies. The percentage of yield was determined by using the given formula.

$$\% \text{ Yield of extract (g/100g)} = \frac{W1}{W2} \times 100$$

Where W1- weight of the dried extract, and W2- weight of dried plant powder.

Phytochemical analysis

The various phytochemical constituents, such as phenols, tannins, carbohydrates, saponins, flavonoids, alkaloids, glycosides, terpenoids, and steroids, were analysed by standard methods of Yadav et al. (2014) and Bankole et al. (2019).

Measurement of total phenolic content (TPC)

To measure the TPC of leaf and stem extracts, the protocol of Siddiqui et al. (2017) was followed. Briefly, the extract was mixed with Na_2CO_3 (75 mg/mL) and

Folin-Ciocalteu reagent (5 mL). After 30 min of incubation, the solution mixture was read at 765 nm and expressed as gallic acid equivalents (mg/g).

Measurement of total flavonoid content (TFC)

According to the protocol of Meda et al. (2005), TFC was determined. Briefly, 2% aluminium trichloride ($AlCl_3$) and quercetin or extract were mixed (1:1) together. The absorption was recorded after 10 min at 415 nm against a blank with quercetin or extract and expressed as mg of quercetin equivalents (mg/g).

Measurement of Antioxidant Activities

DPPH radical scavenging assay

To estimate DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenging activity, the methodology of Shon et al. (2003) was employed. Briefly, the extract and DPPH solution (0.001M in 95% ethanol) were incubated in the dark (20 min), and read at 517 nm. The given equation was used to calculate the scavenging activity.

$$\text{Scavenging (\%)} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

ABTS radical assay (2, 2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid))

The Lee et al. (2006) protocol was utilized for the ABTS assay to measure radical-scavenging activity. Briefly, the $ABTS^+$ was generated by mixing ABTS (7 mM) and 2.4 mM potassium persulfate (2.4 mM) (1:1) and then keeping it for 12 h**.** The solution absorbance was maintained at 0.700 at 734 nm by adding ethanol. The diluted ABTS solution (900 µL) was mixed with the extract (100 µL) and read at 734 nm. The scavenging activity quantified using the equation stated above.

Reducing power assay

The methodology of Soni and Sosa (2013) was utilized to measure the reducing power. Briefly, the extract, 1% potassium ferricyanide (2.5 mL) and phosphate buffer were combined and kept at 50 °C (20 min). Further, 10 % of trichloroacetic acid was added and centrifuged (1036 x g, 10 min). The distilled water and upper layer (1:1) were mixed with 0.1 % $FeCl_3$ (0.5 mL). The absorbance was noted at 700 nm.

Total antioxidant capacity assay (TAC)

The TAC of the extracts was estimated by the Prieto et al. (1999) protocol. Briefly, the extract was incubated (95°C, 90 min) with a reagent solution consisting of 28 mM sodium phosphate, 0.6M sulfuric acid, and 4 mM ammonium molybdate and measured at 695 nm.

***In vitro* anti-inflammatory activity**

The Chandra et al. (2012) protocol was followed to assess the anti-inflammatory activity. Briefly, egg albumin (0.2 mL), 2.8 mL of phosphate-buffered saline (pH 6.4) and 2 mL of extract were mixed. After 15 min, heated at 70 ºC for 5 min, cooled, and recorded at 660 nm. Diclofenac sodium and prednisolone drugs were used as standards, and the inhibition was obtained using the equation below:

$$\text{Percentage of Inhibition} = \frac{(\text{A control} - \text{A sample})}{\text{A control}} \times 100$$

Lipid peroxidation inhibition

Lipid peroxidation was measured by thiobarbituric acid-reactive species (TBARS) using egg yolk as a lipid source (Shashank *et al.,* 2021). Briefly, 10% egg yolk (0.5 mL) and the extract (0.1 mL) were mixed and made up with distilled water (1 mL). Further, 0.07 M $FeSO_4$ (0.005 mL) was added, incubated (30 min), and combined with 20% acetic acid (1.5 mL, 3.5 pH), 0.8% TBA prepared in 1.1% sodium dodecyl sulfate, and 20% TCA (0.5 mL). The mixture was heated at 95 ºC for 60 min. After cooling, the supernatant was read at 532 nm, and the inhibition percentage was determined by the equation given above.

***In vitro* studies**

Cell culture and maintenance

The SH-SY5Y cells were purchased from the National Centre for Cell Sciences (NCCS), Pune, Maharashtra. The cells were cultured in DMEM/F-12 HAMs medium (1:1), with 10% FBS, L-glutamine, and 1% antibiotic and antimycotic solution at 37 ºC in 5% CO_2-humidified incubator. The medium was changed and cells sub-cultured after attending 80% confluency.

MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was employed to evaluate the cytotoxicity of *K. candel* leaf and stem extracts on the SH-SY5Y cells (Mosmann 1983). The cells were seeded (10×10^3 cells/well) in the 96-well plate and treated with leaf and stem extracts at various concentrations for 24 h; further nontoxic doses of the extracts were determined. To assess the neuroprotective effect of leaf and stem extracts, cells were pretreated with selected nontoxic doses for 2 h before the H_2O_2-exposure for 24 h. Thereafter, 5mg/mL MTT was added for 4 h at 37°C, followed by 100 µL DMSO, and absorbance was read at 570 nm in a Synergy LX (Agilent Technologies).

Statistical analysis

All the data were acquired in triplicate and expressed as mean ± SE. The

data was then analysed by ANOVA followed by Tukey's test.

RESULTS AND DISCUSSION

Extraction yield

Extraction yield assesses the solvent's efficiency in extracting the bioactive component from the plant sample. It depends on the water-to-raw material ratio and the polarity of the solvent used for extraction (Mannoubi, 2023). The solvent selection is based on the plant samples and the phytocompounds being extracted (Haminiuk *et al.,* 2014). In the given study, we employed methanol and water as solvents to extract the phytocomponents from the leaf and stem of *K. candel.* We observed higher methanol extraction efficiency than with an aqueous solvent, as shown in Table 1.

Table 1. Effects of extraction solvent on the percentage yield of *Kandelia candel* plant extracts

Solvent	Plant parts	%Yield
Methanol	Leaf	9.60
	Stem	2.51
Water	Leaf	1.33
	Stem	0.96

Phytochemical analysis

The concurrent evaluation of phytochemicals using different solvents can provide a comprehensive comparative analysis of the compounds available in different plant parts. In the current study, preliminary phytochemical analysis of aqueous and methanolic extracts yielded the presence of phenols, flavonoids, tannins, saponins, and carbohydrates (Table 2). In contrast, no alkaloids and steroids were present in any of the extracts, whereas glycosides and terpenoids were present only in the methanolic extract. Our results are in association with the study of Jasna & Khaleel (2020), who reported the presence of phytocompounds in the bark of *K. candel* collected from Valapattanam in Kannur district of Kerala.

Table 2. Phytochemical constituents in the *Kandelia candel* leaf and stem extracts

Phytoconstituents	Leaf extract		Stem extract	
	Aqueous	Methanol	Aqueous	Methanol
Phenols	+	+	+	+
Tannin (Braymer's test)	+	+	+	+
Saponin (Foam test)	+	+	+	+
Flavonoids	+	+	+	+
Glycosides (Liebermann's Test)	-	+	-	-
Steroids (Salkowski test)	-	-	-	-
Carbohydrates (Molisch's Test)	+	+	+	+
Alkaloids (Hager's Test)	-	-	-	-
Terpenoids	-	+	-	+

+ indicates presence, -indicates absence

Total phenol content (TPC)

Plants synthesize phenolic compounds in response to oxidative stress as a defensive mechanism, which exhibit significant antioxidant potential because of their ability to quench free radicals (Yosefiyan *et al*., 2024). Hence, the TPC of the sample is directly correlated to its antioxidant activity (Rozirwan *et al.,* 2023). TPC was measured quantitatively by using Folin-Ciocalteu reagent and expressed in equivalent value of gallic acid (Table 3). The maximum content of phenol was obtained in the methanolic leaf extract (75.05±0.38mg GAE/g) followed by the methanolic stem extract (55.09±1.23mg GAE/g), aqueous leaf extract (16.88±0.71mg GAE/g), and aqueous stem extract of *K. candel* (13.96±0.81mg GAE/g). Thus, the TFC varied with the solvent used, and methanol was a more efficient solvent than aqueous, stating the importance of solvent selection for the better extraction of phytochemicals (Mehmood *et al.,* 2022). This is ascribed to the high solubility of the phenolic, flavonoids, and terpenoids in polar methanol solvent (Lee *et al*., 2024; Mohammed *et al*., 2022). Our results are supported by previous investigations of Shettar et al. (2018) and Lin et al. (2006), who reported the higher phenolic content in the leaf extract of *K. candel.*

Table 3. The total phenolic content in *Kandelia candel* plant extracts

Plant Parts	Total phenolics mg GAE/g	
	Aqueous extract	Methanol extract
Leaves	16.88±0.71[a]	75.05±0.38[c]
Stem	13.96±0.81[b]	55.09±1.23[d]

The values were represented as mean ± SE (n=3) and analyzed through two-way ANOVA followed by Tukey's test. Different superscript letters indicate significant differences between the samples (p <0.05).

Total flavonoid content (TFC)

Flavonoids are low-molecular-weight secondary metabolites synthesized by plants. They include classes such as flavone, flavonone, isoflavone, flavonol, anthocyanin, and catechin (Akullo *et al.,* 2023). Flavonoids play a considerable role in alleviating abiotic and biotic stresses by their antioxidant attribute, which helps to manage OS and maintain membrane integrity (Patil *et al.,* 2024). The TFC was higher in the methanolic leaf (26.0±0.10 mg Qu/g) and stem (17.2±0.06 mg Qu/g) extract of *K. candel*. However, relatively less content was found in the aqueous extract of the leaf (11.5±0.02 mg Qu/g) and stem (8.9±0.12 mg Qu/g) (Table 4). Our results imply that the leaf of *K. candel* is a richer source of antioxidants than the stem, and the total flavonoid content was governed by the extraction solvent used. Similar results were reported in a previous study by Zhang et al. (2010), stating the presence of epicatechin and epigallocatechin in the methanolic leaf extract.

Table 4. The total flavonoid content in *Kandelia candel* plant extracts

Parts	Total flavonoids mg Quercetin/g	
	Aqueous extract	Methanol extract

Leaves	11.5±0.02[a]	26.0±0.10[c]
Stem	8.9±0.12[b]	17.2±0.06[d]

The values were represented as mean ± SE (n=3) and analyzed through two-way ANOVA followed by Tukey's test. Different superscript letters indicate significant differences between the samples (p <0.05).

Antioxidant activity

DPPH free radical scavenging assay

Antioxidants function as free-radical scavengers, reducing agents, and metal ion chelators (Rafiee *et al*., 2025; Karam & Abdulrahman, 2022). The scavenging activity of antioxidants can be estimated by using the DPPH assay. The assay is driven by the reduction of DPPH by an antioxidant, which is detected as a change from purple to yellow colour at 517nm (Chaves *et al.,* 2020). The percentage radical-scavenging activity was quantified using IC_{50} values, which denote the concentration of the extract required to achieve 50% scavenging activity (Rafiee *et al*., 2025). The IC_{50} values for the methanolic and aqueous extracts of the leaf (45±0.33 µg/mL and 91±0.17 µg/mL) and the stem (85±2.70 µg/mL and 137±5.84 µg/mL) indicate higher scavenging activity in the methanolic extract (Table 5). These results were associated with the total phenol and flavonoid contents. Our findings are compatible with previous studies by Wei et al. (2010), who highlighted that the hypocotyls of *K. candel* possess potent antioxidant activity. Shettar et al. (2018) also documented dose-dependent DPPH reduction by the methanolic leaf extract.

ABTS radical assay

The ABTS assay is another important assay to evaluate the antioxidant capacity. The antioxidant present in the extract donates an electron to the preformed blue-green coloured $ABTS^+$ radical (Agustini *et al.,* 2022). Upon reduction, $ABTS^+$ radical changes its colour intensity and can be read at 734 nm (Rohmah, 2022). The ABTS radical-scavenging activity showed a dose-dependent inhibitory effect across all extracts. Table 5 shows the IC_{50} values of the methanol and aqueous extracts of the leaf and stem of *K. candel.* Among all extracts, the methanolic leaf extract exhibited the strongest ABTS radical-scavenging activity (54±1.29 µg/mL) as revealed by lower IC_{50} values, which was

relatively lower than aqueous stem extract (105±1.54 µg/mL), suggesting the significantly higher antioxidant potential of the methanolic extract than the aqueous.

Reducing power assay

Reducing power assay measures the Fe^{3+} to Fe^{2+} reduction by an antioxidant (Gulcin & Alwasel, 2025). Figure 1 represents the dose-dependent reducing power of extracts relative to ascorbic acid. The higher reducing ability was exhibited by the methanolic extracts compared to the aqueous extracts. The significantly reduced ability of leaf extract over stem extract may be ascribed to the greater extraction capacity of the methanol solvent for antioxidant compounds like terpenoids, phenols, and flavonoids (Ghada *et al*., 2025).

Total antioxidant capacity (TAC)

The phosphomolybdenum assay was used to estimate the TAC of the *K. candel* extracts. Antioxidants from the extract act as reducing agents that reduce molybdenum (VI) to molybdenum (V), followed by the green-coloured phosphate-molybdenum (V) complex formation under acidic conditions, and measured at 765 nm (Sarma & Datta, 2025). Figure 1 compares the TAC of methanolic and aqueous extracts of the leaf and stem of *K. candel*. The results depict a concentration-dependent increase in activity. Similar to the reducing power assay, the methanolic leaf extract displayed the highest reducing capacity with respect to other extracts, attributed to the better reducing and electron-donating ability of the bioactive compounds (Siddeeg *et al.,* 2021). Our findings are in line with earlier studies by Shettar et al. (2018).

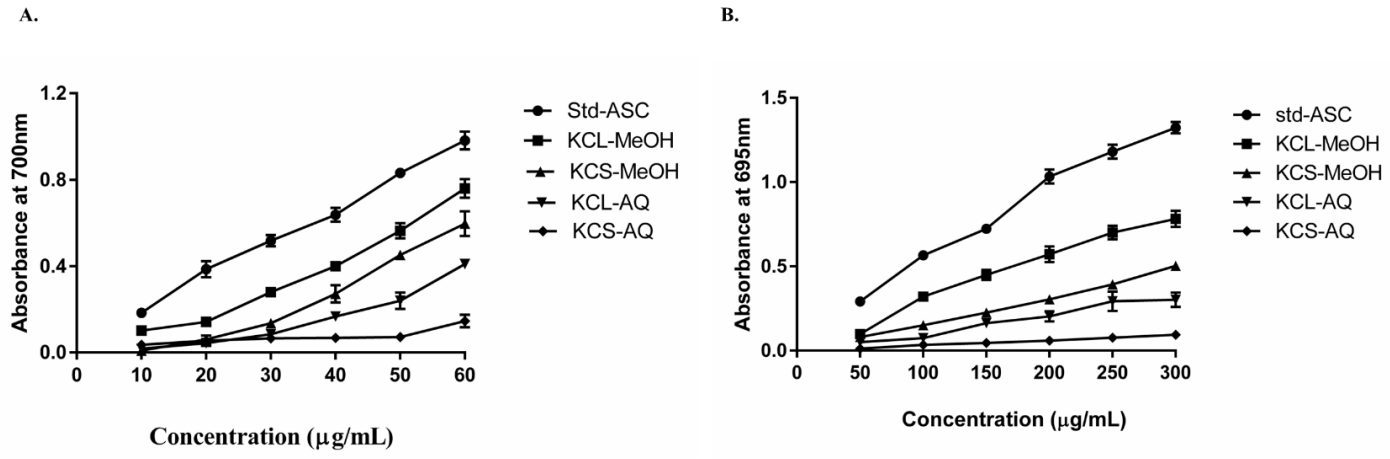

Fig. 1. A) Reducing power assay, and B) Total antioxidant capacity of *Kandelia candel* extracts and standard. The data is expressed as mean ± SE (n = 3). (Where Std-ASC-Ascorbic acid, KCL-MeOH-*Kandelia candel* leaf methanolic extract, KCL-AQ-*Kandelia candel* leaf aqueous extract, KCS-MeOH-*Kandelia candel* stem methanolic extract, and KCS-MeOH-*Kandelia candel* stem aqueous extract).

The anti-inflammatory activity

The anti-inflammatory activity was measured by heat-induced denaturation of egg albumin. Protein denaturation is one of the well-known mechanisms associated with inflammation (Adetutu & Olukorede, 2021). The denaturation process disrupts the secondary, tertiary and quaternary structures of protein via alteration in various covalent and noncovalent interactions, leading to loss of function and eventually cell damage (Manubolu *et al.,* 2023; Adetutu & Olukorede, 2021). In the present study, the extracts of *K. candel* exhibited dose-dependent inhibition and were compared with standard drugs prednisolone and diclofenac sodium. As shown in Table 5, the methanolic extract of the leaf demonstrated higher inhibition with an IC_{50} value (1430±0.12 µg/mL), followed by the methanolic stem extract (2601±0.98 µg/mL), aqueous leaf extract (3621±1.5 µg/mL), and the aqueous stem extract (5213±1.78 µg/mL). However, the inhibitory effect of standard prednisolone (229±0.199 µg/mL) and diclofenac sodium (597±0.30 µg/mL) was more than that of all the extracts. The higher anti-inflammatory activity of methanolic extracts may be due to the presence of potent phytocompounds such as terpenoids and flavones that regulate critical inflammatory signaling pathways (An *et al.,* 2025; Khanam *et al.,* 2025).

Lipid peroxidation inhibition

The TBARS (Thiobarbituric acid reactive substances) assay was employed to investigate lipid peroxidation using egg yolk. Free radicals initiate peroxidation by oxidising membrane lipids and low-density lipoprotein (LDL) to peroxides, which, upon auto-oxidation, convert to TBARS, including aldehydes and ketones (Kaczmarek & Muzolf-Panek, 2022). *K. candel* extracts exhibited dose-dependent inhibition of lipid peroxidation. The methanolic extracts (Leaf- 141±0.27 µg/mL and stem-214±0.43 µg/mL) depicted the lowest IC_{50} value over aqueous extracts (Leaf- 352±0.61 µg/mL and stem-763±0.66 µg/mL), respectively (Table 5). The higher inhibition of lipid peroxidation

by methanolic leaf extract might be attributed to phenolic compounds, which inhibited oxidation through antioxidant scavenging activity (Bernardo *et al*., 2025; Kokkosi *et al.,* 2024).

Table 5. The IC_{50} values for DPPH, ABTS, in vitro anti-inflammatory and lipid peroxidation inhibition assay of *Kandelia candel* plant extracts and standard.

Samples		DPPH	ABTS	Anti-inflammatory activity	Lipid peroxidation inhibition
Leaf	Methanol	45 ± 0.33^{a}	54 ± 1.29^{a}	1430 ± 0.12^{e}	141 ± 0.27^{c}
	Aqueous	91 ± 0.17^{b}	95 ± 1.54^{b}	2601 ± 0.98^{f}	352 ± 0.61^{k}
Stem	Methanol	85 ± 2.70^{b}	77 ± 5.84^{b}	3621 ± 1.5^{g}	214 ± 0.43^{i}
	Aqueous	137 ± 5.84^{c}	105 ± 1.54^{b}	5213 ± 1.78^{h}	763 ± 0.66^{l}
Ascorbic acid	-	10 ± 1.20^{d}	25 ± 0.06^{ad}	-	35.231 ± 1.44^{a}
Prednisolone	-	-	-	229 ± 0.199^{i}	-
Diclofenac sodium	-	-	-	597 ± 0.30^{j}	-

The values were represented as mean ± SE (n=3) and analyzed through two-way ANOVA followed by Tukey's test. Different superscript letters indicate significant differences between the samples (p <0.05).

Effect of *K. candel* on cell viability of SH-SY5Y cells

The cytotoxicity of *K. candel* leaf and stem extracts was assessed by the MTT assay. The SH-SY5Y cells were treated with increasing doses of each extract for 24 h. As shown in Fig. 2, methanolic leaf extract was found to be non-toxic up to a dosage of 150 µg/mL. Up to 7o% calls were viable at 200 µg/mL in the stem extract. However, in aqueous extracts, higher concentrations up to 400 µg/mL were found to be nontoxic. Hence, these non-toxic doses were selected to determine the cytoprotective effect of the extracts. Various studies have used H_2O_2 as an inducer of OS to investigate the

neuroprotective activity in SH-SY5Y cells as an in vitro model (Sayuti *et al.,* 2023; Jaafaru *et al.,* 2018). The H_2O_2 concentration (IC_{50} of 135 µM) was selected based on our lab's previous study (Mamatha *et al*., 2024) to induce oxidative stress in SH-SY5Y cells. The cytoprotective effect of selected doses of *K. candel* leaf and stem extracts was evaluated on H_2O_2-exposed cytotoxicity in SH-SY5Y cells. As shown in Fig. 3, H_2O_2-induced cells displayed a lower cell density (50%) when compared to control cells. The cells pretreated with the methanolic leaf extract exhibited greater neuroprotection against H_2O_2-induced cytotoxicity than those pretreated with other extracts. The higher cytoprotective effect of methanolic extracts may be due to the synergistic effect of bioactive compounds. Morphological changes further validated these results.

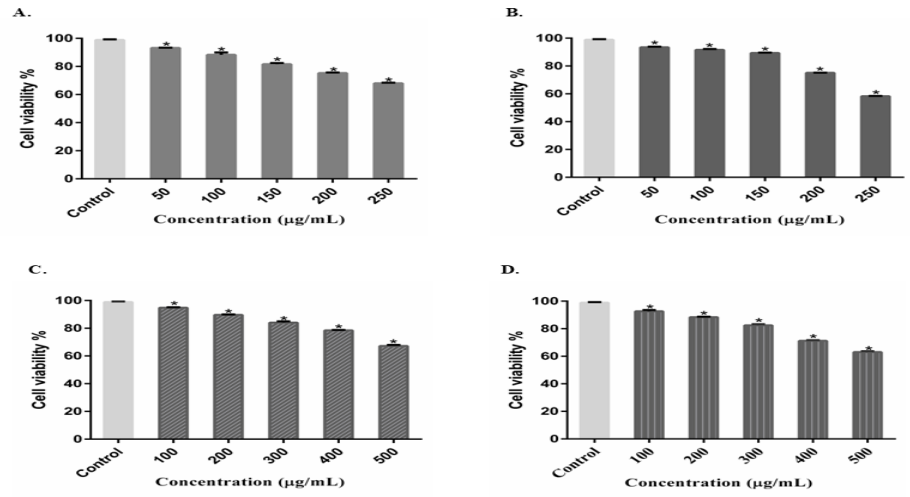

Fig. 2. Effect of *Kandelia candel* a) Leaf methanolic extract, b) stem methanolic extract, c) leaf aqueous extract, and d) stem aqueous extract on SH-SY5Y cells. The data are expressed as mean ± SE (n=3) and analysed by one-way ANOVA followed by Tukey's test. * Indicates p <0.05 significance compared to the control.

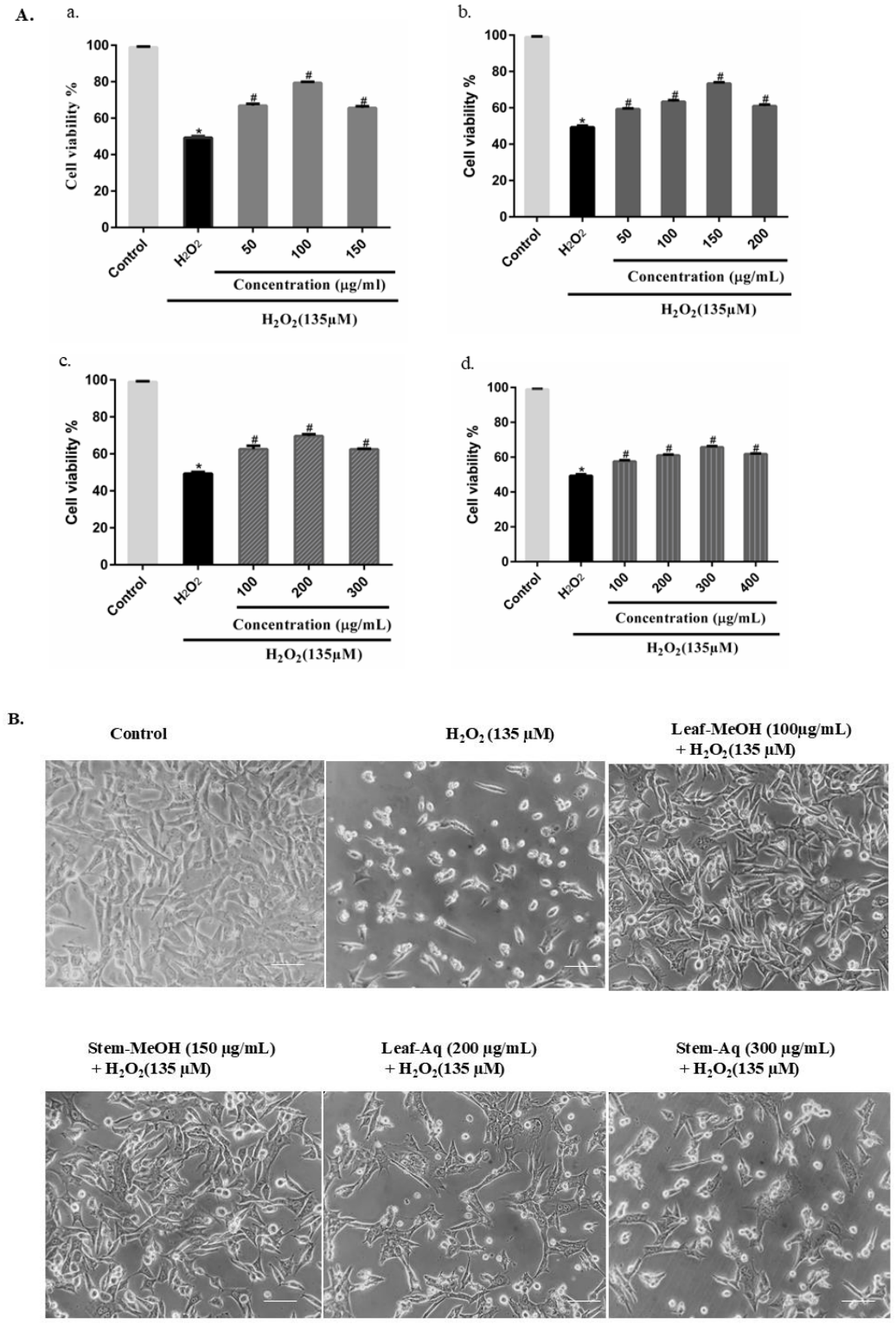

Fig. 3. Neuroprotective effect of A) *Kandelia candel* a) Leaf methanolic extract b) stem methanolic extract c) leaf aqueous extract and d) stem aqueous extract, on H_2O_2-induced cytotoxicity in SH-SY5Y cells. B) Morphological images of H_2O_2-induced SH-SY5Y cells. The data are expressed as mean ± SE (n=3) and analysed by one-way ANOVA followed by

Tukey's test. *Indicates p <0.05 significance compared to the control. # indicates p <0.05 significance compared to the H_2O_2. (magnification-20X).

CONCLUSION

The current study investigated the phytochemical composition, antioxidant, anti-inflammatory lipid peroxidation inhibition and cytoprotective activities of *K. candel* leaf and stem extracts. The preliminary phytochemical analysis detected various bioactive compounds in methanolic extracts. The antioxidant, anti-inflammatory and lipid peroxidation inhibition activities were most notable in the methanolic extracts compared to the aqueous extracts. Further, the methanolic extract was more potent in attenuating H_2O_2-induced oxidative stress in SH-SY5Y cells. Nevertheless, further studies to identify the bioactive compounds and conduct detailed in vitro investigations are needed to understand their therapeutic potential.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest regarding this paper.

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