DOI: https://doi.org/10.52756/ ijerr.2024.v39spl.016 *Int. J. Exp. Res. Rev.*, Vol. 39: 200-212 (2024)

Original Article | Peer Reviewed | Open Access

International Journal of Experimental Research and Review (IJERR)
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ISSN: 2455-4855 (Online)
www.iaph.in

Methanol extract of *Isodon ternifolius* (D. Don) KUDO leaves has antimicrobial and antioxidant activities but no neuroprotective activity

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Article History:
Received: 23rd Jan, 2024
Accepted: 25th May, 2024
Published: 30th May, 2024

Keywords:
Bactericidal, free radicals' quencher, fungicidal, neurotoxicity, phytochemicals

How to cite this Article:
Aruna Chanu Hijam, Arunkumar Singh Koijam and Reena Haobam (2024). Methanol extract of *Isodon ternifolius* (D. Don) KUDO leaves has antimicrobial and antioxidant activities but no neuroprotective activity. *International Journal of Experimental Research and Review*, *39*(spl.) 200-212.
DOI: https://doi.org/10.52756/ijerr.2024.v39spl.016

Abstract: *Isodon ternifolius* (D. Don) KUDO is a medicinal plant traditionally used by the indigenous people of Manipur, India, as a room fumigant and a means to ward off evil spirits. The current study was designed to assess the antimicrobial, antioxidant, and neuroprotective properties of *I. ternifolius*. The dried leaves of *I. ternifolius* were heat extracted with methanol (ME) and screened for phytochemical constituents. The antimicrobial activity was investigated against pathogenic microbes. *In-vitro* antioxidant activity was assessed by 2-deoxyribose, 2, 2´-Azinobis-3-ethylbenzo-Thiozoline-6-sulphonate (ABTS), and 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay methods. The endogenous reactive oxygen species (ROS) level on SH-SY5Y cells was detected using 2′,7′-Dichlorofluorescein diacetate (DCF-DA). The neuroprotective activity of ME was studied in rotenone-treated SH-SY5Y cells. ME was observed to have substantial antimicrobial potential against four pathogenic bacterial strains - *B. subtilis*, *Streptococcus*, *K. pneumoniae* and *E. aerogenes* and also revealed antifungal activity against *C. albicans*. ME also showed potent free radical quenching activity in chemical and cell-culture-based DCF-DA assays and possesses high phenolic and flavonoid contents. However, ME did not show neuroprotective activity in rotenone-treated SH-SY5Y cells.

Introduction

Isodon ternifolius (D. Don) Kudo, locally known as "Khoiju" in Manipuri, belongs to the family of Lamiaceae. The genus *Isodon* is an ethnobotanical important group of plants comprising 153 species (Liu et al., 2017). In Manipur (India), the aerial parts and leaves of *I. ternifolius* have been used to treat fungal and bacterial diseases. Further, the plant's leaves are also used to fumigate maternity rooms and others as a tradition for disinfection. Naturally derived antimicrobial compounds from medicinal plants have mild side effects on the host organisms and can effectively inhibit the growth of pathogens (Saeed et al., 2019). Hence, they are considered excellent candidates for developing new antimicrobial drugs that may combat drug-resistant microbes.

Oxidative stress is a critical contributor to the pathogenesis of different chronic diseases (Kiran et al., 2023; Husain et al., 2023). The exogenous intake of antioxidants as a dietary supplement can decrease oxidative damage (Bouayed and Bohn, 2010; Zhu et al., 2023). Besides plants' flavonoids, phenolics and other phytoconstituents have been reported to exert numerous biological activities, including anti-tumour, anti-inflammatory, and antioxidant actions (Gauri et al., 2016; Ullah et al., 2020; Bagchi, 2021; Ghosh et al., 2022; Acharya et al., 2022a, b; Rudrapal et al., 2022; Bee et al., 2023; Jakkana and Yamala, 2024). Hence, there is a

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growing exploration for natural, economic and more effective antioxidant compounds derived from medicinal plants, such as phenolic acids, polyphenols, and flavonoids, than synthetic drugs (Sacchetti et al., 2005; *Maiti* et al., 2010, 2013; Biswas et al., 2016; Nasim et al., 2022; Hore and Bhaben, 2023; Dey-Ray et al., 2024).

A large number of terpenoids have been isolated and identified from *I. ternifolius*. Some of the diterpenoids have been found to have anti-tumour (Zou et al., 2012; Zou et al., 2013; Liao et al., 2014; Gou et al., 2019; Zhang et al., 2020; Elshamy et al., 2023), anti-stemness (Yuan et al., 2017), protein tyrosine phosphatase 1B (PTP1B) inhibitory (Pham et al., 2020) and anti-fibrotic (Deng et al., 2023) activities. The scientific statements about the activities of this plant against microbial infections and free radical-induced oxidative damages still need to be made. Therefore, the antimicrobial properties and the antioxidant activities of the crude extract of this plant growing in Manipur, India, have been intended to be explored in the study. Furthermore, since many neurodegenerative diseases are related to oxidative stress and plants with free radical scavenging activities are good claimants for neuroprotective studies, the neuroprotective action of this plant could also be explored. Our results revealed the antimicrobial and antioxidant properties of the methanol crude extract of *I. ternifolius* leaves in various gram-positive and negative bacterial strains and a fungal strain. However, the plant extract did not show protection against rotenone-induced neurotoxicity in SH-SY5Y neuroblastoma cells.

Materials and Methods

Plant materials collection and extract preparation

I. ternifolius' leaves were collected from Manipur, India. The plant was identified at the Department of Life Science (Botany), Manipur University, Canchipur, India, where a voucher specimen was deposited with species accession number 001296. About 45 gm of finely powdered plant materials were hot extracted using methanol in a Soxhlet assembly at 55°C for 24 hours. The methanol extract (ME) was then concentrated using a vacuum evaporator under reduced pressure and kept at 4°C for future utilization (Putra et al., 2022).

Antimicrobial Assays

Microbial culture and maintenance

In vitro studies for antimicrobial were carried out against four Gram-positive bacterial strains - *Bacillus subtilis* (MTCC121), *Micrococcus luteus* (MTCC106), *Staphylococcus aureus* (MTCC96), and *Streptococcus*; four Gram-negative bacterial strains - *Escherichia coli* (DH5α), *Pseudomonas aeruginosa* (DN1), *Enterobacter aerogenes* (MTCC111), *Klebsiella pneumoniae* (MTCC432); and one fungal strain – *Candida albicans* (MTCC227). All the isolates were kept at -20°C. Inocula were ready from each culture's isolate in a sterilized nutrient broth. The culture was permitted to be nurtured for 24 hours in a shaker incubator at 37°C and checked until the visible turbidity was equal (Mohamed et al., 2020). The culture was then used for further analysis.

Agar disc diffusion method

The antimicrobial actions of ME were screened via the agar disc diffusion method with minor modification (Turkoglu et al., 2010; Bhalodia and Shukla, 2011). Briefly, the microbial and fungal strains were inoculated on nutrient agar at 10^6 microorganisms/mL concentrations for bacteria and 10^4 microorganisms/mL for fungus. Antiseptic discs (6 mm in diameter) were saturated with 20 µL of ME (corresponding to 5 mg/mL of the extracts) and positioned on the inoculated agar dishes at a uniform distance. After 24 hours of incubation for bacterial strains at 37ºC and 72 hours at 28ºC for fungal strains, the antimicrobial actions of the extract were determined. A clear zone of inhibitions around the discs indicated the prospective of antimicrobial activity. The diameters of the clear areas of inhibition shaped by ME were measured in millimetres (mm). Methanol only was used as a negative control for their respective extracts.

In-vitro Anti-oxidant Assays

Hydroxyl radical (˙OH) quenching assay

Radical scavenging capacity against ˙OH was determined by a 2-deoxyribose assay (Tomic et al., 2009). In brief, 20 µL of ME in four different concentrations (8 µg/mL, 40 µg/mL, 200 µg/mL and 1 mg/mL) was assorted with 100 µL of 4 mM $FeCl_3$, 100 µL of 4 mM EDTA, 200 µL of 50 mM 2-deoxyribose, 20 µL of 1.5% hydrogen peroxide (H_2O_2) and 100 µL of 4 mM L-Ascorbic acid in phosphate buffer (pH 7.4) and made up the final volume to 2 mL. Following 1 hour incubation at 37ºC, 1 mL of 1% (w/v) TBA solution in 50 mM NaOH was added, followed by 1 mL of 2.8% (w/v) solution of TCA. The solutions were cooled at 100ºC for 15 minutes, and the absorbance was taken at 532 nm via a UV-spectrophotometer. Methanol was used as a control, and Quercetin was used as a standard antioxidant. The capacity of percentage Inhibition was determined by using the succeeding formula:

$$\% \text{ of Inhibition} = [A_{(control)} - A_{(sample)} / A_{(control)}] \times 100$$

DPPH radical scavenging assay

The radical scavenging activity of ME was further confirmed by the 1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) assay method (Shimada et al., 1992). About 0.5 mL of

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0.1 mM DPPH methanolic solution is supplemented into 1.5 mL of ME solution to give final concentrations of 100 µg/mL, 10 µg/mL, 1 µg/mL and 0.1 µg/mL as test solutions. The test solutions were kept at 37°C in darkness for 30 minutes. The optical density was read at 517 nm and transformed into a percentage of antioxidant action. Ascorbic acid was considered as standard antioxidant. The proportion of DPPH reduction (%) was calculated as follows:

$$\% \text{ of Inhibition} = [A_{(control)} - A_{(sample)} / A_{(control)}] \times 100$$

ABTS˙+ radical quenching assay

2, 2´-Azinobis-3-ethylbenzo-Thiozoline-6-sulphonate (ABTS) assay was determined using the procedure of Re et al. (1999) with a few modifications. Briefly, ABTS cation radicals (ABTS˙+) were released by reacting 0.007 M ABTS solution in water with 2.45 mM APS at a 1:1 ratio. The reaction mixture was permitted to rest in darkness for 16 hours at RT to generate the ABTS˙+ radicals. Later, the ABTS + solution was thinned in water until the absorbance of the ABTS˙+ solution reached 0.75 ± 0.025A at 734 nm. About 0.5 mL each of ME was added into ABTS˙+ solution of 1.5 mL to make an eventual concentration of 0. 1 µg/mL, 1 µg/mL, 10 µg/mL, and 100 µg/mL as test solutions and incubated at RT for 7 minutes in darkness. The absorbance of the solutions was taken at 734 nm. Ascorbic acid was considered a standard. The proportion of radical quenching property was estimated as the percentage of Inhibition by using the formula:

$$\% \text{ of Inhibition} = [A_{(control)} - A_{(sample)} / A_{(control)}] \times 100$$

Phytochemical examination

Estimation of total phenolic content (TPC)

The TPC of ME was assessed by Folin-Ciocalteu's reagent method following Singleton et al. (1999) with minor changes using gallic acid as standard. In brief, 0.2 mL of gallic acid solutions at different concentrations was assorted with 1 mL of 10 times thinned Folin-Ciocalteau's phenol reagent. After 2 minutes of incubation at RT, 0.8 mL of 700 mM Na_2CO_3 was added, following further incubation of the solution for 1 hour. A 1 mg/ml ME concentration was used for the test sample. The transmission density was measured at 765 nm. TPC was determined from the gallic acid's standard curve and quantified as a microgram of Gallic Acid Equivalent (GAE) per gram dried mass of the test sample. The TPC amount was calculated using the succeeding formula:

$$T = (C \times V) / M$$

where T = total phenolic content (mg of GAE/gm dried mass of the test sample), C = concentration of gallic acid calculated from the calibration curve (mg/mL), V = volume of extract (mL) and M = weight of the methanol crude extract (gram).

Assessment of total flavonoid content (TFC)

The TFC of ME was measured using the aluminium chloride colourimetric method of Chang et al. (2002), which used quercetin as the standard. About 0.2 mL of quercetin of distinct concentrations were assorted with 0.04 mL of 10% (v/v) $AlCl_3$ followed by 0.04 mL of 1 M (w/v) CH_3COOK. About 1.12 mL of water was added. After that, the solution was incubated for 30 minutes at RT. A test sample with a 1 mg/mL concentration was used. The absorbance of the solutions was quantified at 415 nm. The amount of flavonoid was estimated from the standard curve and expressed as a microgram of Quercetin Equivalent (QE) per gram dried weight of the test sample. The TFC was estimated as follows:

$$T = (C \times V) / M$$

where T = total flavonoid content (mg of QE/gm dried weight of the test sample), C = concentration of quercetin calculated from the calibration curve (mg/mL), V = volume of the crude extract (mL) and M = mass of the methanol crude extract (gram).

Measurement of intracellular ROS levels in SH-SY5Y neuroblastoma

Intracellular ROS formed in 100 mM H_2O_2 treated cells were assessed with fluorescent probe 2′,7′-Dichlorofluorescein diacetate (DCF-DA) in a fluorescence microscope (Park et al., 2015). In brief, SH-SY5Y neuroblastoma was seeded on a poly-L-lysine encrusted coverslip in a culture plate and maintained in high glucose Dulbecco's Modified Eagle Medium (DMEM) complemented with 100 µg/mL Streptomycin, 100 U/mL Penicillin solutions, and 10% Fetal Bovine Serum (FBS). Cells were primarily treated with 10 µM DCF-DA in the dark for 30 minutes at 37ºC. After pre-treatment of cultured cells with or without ME for 1 hour, the cells were experienced to 100 mM H_2O_2 for another 30 minutes. Then, the cells were rinsed with 100 mM PBS, and the fluorescence of 2´,7´-dichlorofluorescein (DCF) was observed with a fluorescence microscope (LEICA DM2500).

Determination of the neuroprotective activity of ME

SH-SY5Y cells were seeded on the 96-well plate. Forty-eight hours later, cells were nurtured with 10 µM retinoic acid (RA) in 3% DMEM for 3 days to differentiate into neuronal cells (Shipley et al., 2016). On the 4th day, cells were pre-treated with different dosages of ME (5 µg/mL, 1 µg/mL, 200 ng/mL, 40 ng/mL, and 8 ng/mL) for 1 hour and then incubated with rotenone (750 nM) for 24 hours. Appropriate control groups were also included in the study. Cells were then encouraged with 3-

Table 1. Antimicrobial activity of ME compared with commercial standard antibiotics.

Micro-organisms	ME (200mg/mL)	Kanamycin (20µg/mL)	Nystatin (100units/mL)
Gram-Positive Bacteria			
Bacillus subtilis (MTCC121)	19.20 ± 1.20	18.00 ± 0.74	NT
Micrococcus luteus (MTCC106)	17.43 ± 2.13	17.60 ± 1.91	NT
Staphylococcus aureus (MTCC96)	NA	20.50 ± 0.50	NT
Streptococcus	21.00 ± 0.32	20.00 ± 0.82	NT
Gram-Negative Bacteria *Pseudomonas aeruginosa* (DN1)	NA	10.67 ± 0.21	NT
Escherichia coli (DH5α)	19.4 ± 1.77	23.00 ± 1.34	NT
Klebsiella pneumoniae (MTCC432)	24.00 ± 0.91	23.00 ± 3.01	NT
Enterobacter aerogenes (MTCC111)	21.75 ± 0.72	18.75 ± 1.18	NT
Yeast *Candida albicans* (MTCC227)	13.00 ± 1.00	NT	22.50 ± 2.50

Results are presented as diameter of the zone of inhibition (mm) and the values are expressed as mean±SEM, (SEM = Standard Error) for *E. coli, B. subtilis and P. aeruginosa* (n = 5); *M. luteus and Streptococcus* (n = 7); *S. aureus* and *K. pneumoniae* (n = 4); *E. aerogenes* (n = 8); *C. albicans* (n = 3); NT = Not Tested; NA = No Activity.

(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT, 0.005 g/mL] in serum-starving Roswell Park Memorial Institute Medium (RPMI) medium for 4 hours at 37°C. After incubation, media were discarded, and formazan crystals were solubilized in dimethyl sulfoxide (DMSO). The absorbance of formazan products was evaluated using a microplate reader (ELx 800, Biotek) at 570 nm (Dhanalakshmi et al., 2015). Results were quantified as the MTT reduction percentage compared to the absorbance of negative controls. The negative control was the 100% viability of cells treated with DMEM alone.

Statistical analysis

Each sample in triplicates was used for statistical analysis, and the values were represented as means ± standard error (SEM). IC_{50} of the sample for antioxidant assays was calculated from the linear regression plots. The significant differences between means were verified by student T-test. Statistical variances at $p < 0.05$ were considered as significant. The fluorescence intensity of DCF was analysed using ImageJ software.

Results

Anti-microbial activity

ME showed vigorous activity against three gram-positive bacteria – *B. subtilis* (19.2 ± 1.20), *M. luteus* (17.43 ± 2.13), and *Streptococcus* (21.0 ± 0.32) and against three gram-negative bacteria – *E. coli* (19.4 ± 1.77), *E. aerogenes* (21.75 ± 0.72) and *K. pneumoniae* (24.00 ± 0.91). ME also showed antifungal activity against the yeast – *C. albicans* (13.0 ± 1.00). However, ME had no activity against *S. aureus* and *P. aeruginosa*. Methanol alone was also used as a negative control. Data are shown in Table 1.

Anti-oxidant activity

ME was revealed to have $^{\cdot}OH$ radical scavenging action at 20.39 µg/mL as its IC_{50} value in a 2-deoxyribose assay. However, ME showed a lower amount of $^{\cdot}OH$ radical quenching activity than the standard quercetin (IC_{50} = 3.316 ng/mL), as represented in Figure 1 (A). In the DPPH assay, ME tended to quench DPPH radicals in a concentration-dependent mode. The result revealed that ME had intense DPPH radical quenching activity at 8.84 µg/mL as its IC_{50} value. The activity was compared to ascorbic acid (62.89 µg/mL as its IC_{50} value), used as the standard (Fig. 1, B).

Further, ME was noticed to be an effective quencher of ABTS radicals with an IC_{50} value of 55.05 µg/mL, and this radical quenching activity was similar to that of ascorbic acid (44.13 µg/mL as its IC_{50} value). ME was found to have equivalent efficacy in removing free radicals in the technique with the standard ascorbic acid at all concentrations. The ABTS free radicals quenching activity of ME was almost parallel to that of the DPPH free radicals quenching activity, as presented in Figure 1A-C.

Phytochemical analysis

The TPC in ME evaluated with the Folin-Ciocalteu's reagent was expressed as gallic acid equivalent (GAE). It is described by the equation of the standard curve: $y = 4.6187x - 0.0722$ with $R^2 = 0.9981$. The TPC of ME was found to be 74.42 ± 0.69 mg GAE/gm dried weight, as shown in Table 2. The total flavonoid content of ME was established to be 14.18 ± 0.04 mg QE/gm dried weight,

which described an equation of y = 1.4607x - 0.174 with $R^2 = 0.8902$ (Table 2).

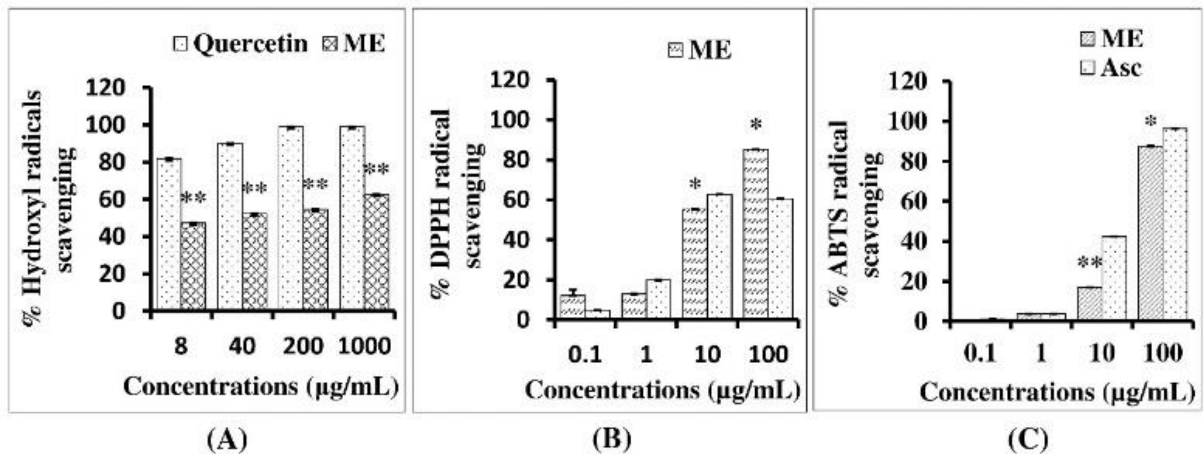

Figure 1. Free radicals scavenging activity of ME. (A) Hydroxyl radicals (·OH) scavenging activity (IC_{50} = 20.39 µg/mL) of ME. (B) DPPH radicals quenching activity (IC_{50} = 41.21 µg/mL) of ME. (C) ABTS radicals scavenging activity (IC_{50} = 55.05 µg/mL) of ME. ME = methanol extract of *Isodon ternifolius*; Asc = Ascorbic acid. * $p < 0.05$ & ** $p < 0.01$.

Table 2. Total phenolic and flavonoid content of ME

Extract	Phenolic content (mg GAE/gm extract)	Flavonoid content (mg QE/gm extract)
ME	74.42 ± 0.69	14.18 ± 0.04
Each value represents three repeated studies' mean±SEM (SEM = Standard Error).		

In-vitro antioxidant activity

The level of intracellular ROS accumulation was examined in SH-SY5Y cells using DCF-DA dye in a fluorescence microscope. ROS generation induced by H_2O_2 was determined in cells pre-treated with or without ME. The green fluorescence of DCF in 100 mM H_2O_2 exposed cells was noticeably more robust than that in the control cells. However, pre-treatment of the SH-SY5Y cells with ME (Fig. 2) revealed a significant dose-dependent decrease in the DCF fluorescence intensity of the ME-treated group, up to a 27% reduction compared to the H_2O_2 group.

Neuroprotective analysis

The neuroprotective potential of ME was tested on SH-SY5Y cells after contact with the neurotoxin rotenone. The exposure of SH-SY5Y cells to rotenone (750 nM) led to a decline in cell viability to about 45% after 24-hour incubation. Pre-treatment of ME could not revert the neurotoxic effects of rotenone on the feasibility of SH-SY5Y cells (Fig. 3).

Discussion

The significant findings in the study are as follows: (1) *I. ternifolius* shows potent antimicrobial properties against pathogenic organisms and validates its traditional use. (ii) *I. ternifolius* possesses high antioxidant activities comparable to the standard Vitamin C. The existing study screened the antimicrobial action of *I. ternifolius* against a fungus strain and eight disparate bacterial strains. It demonstrated that ME shows antimicrobial activity against the six tested bacteria (*B. subtilis* MTCC121, *M. luteus* MTCC106, *Streptococcus*, *E. coli* DH5α, *E. aerogenes* MTCC111, and *K. pneumoniae* MTCC432) and a fungal pathogen, namely - *C. albicans* (MTCC227). The study shows that the extract possessed a robust inhibitory effect against the growth of the tested micro-organisms and it justified the conventional use of this plant as an antibacterial and antifungal agent to disinfect the room of maternity and others. Consequently, *I. ternifolius* can be lucratively exploited as an antimicrobial agent.

Diverse methods were employed to analyze ME's antioxidant activity. Hydroxyl radicals are extremely reactive free radicals that can injure DNA (strand break), proteins, and other essential bio-molecules, leading to cancer and ageing (Collin., 2019). It was observed in the study that ME has hydroxyl radical scavenging activities. In the DPPH assay, the results exhibited that ME scavenges DPPH free radicals in a concentration-

dependent manner. Further, the IC_{50} values for ME and ascorbic acid in the DPPH assay are 41.21 µg/mL and 62.89 µg/mL, revealing that ME demonstrated higher antioxidant activity than ascorbic acid. ABTS assay is also an improved method for screening the antioxidant compounds present in the plant extract. A critical property of this method is its applicability for testing both hydrophilic and lipophilic compounds (Re et al., 1999; Munteanu and Apetrei, 2021). In the ABTS assay, the IC_{50} values of ME and ascorbic acid were 55.05 µg/mL and 44.13 µg/mL, respectively. Notably, the extract displayed a superior level of effectiveness, even though it is not as much as ascorbic acid. This fact indicates that other phytoconstituents in ME may obstruct ME's capability to scavenge ABTS radicals, as the ME was a crude extract. The DCF-DA assay in SH-SY5Y cells further validated the antioxidant potential of ME. The above outcomes demonstrate that ME has a great potential to quench free radicals.

The TPC value of the ME confirms that ME is abundant in phenolic compounds. Phenolics have eminent scientific interest recently because of their broad presence in the plant kingdom as secondary metabolites, and they are tremendous sources of potential natural antioxidants. ME showed lesser total flavonoid content than TPC quantitatively. However, phenolic and flavonoid compounds' free radical quenching activities have already been known in biological systems (Mathew et al., 2015; Sarker and Oba, 2020). Again, polyphenols are well familiar for their excellent antioxidant properties because of their oxidoreduction potential (Gebicki and

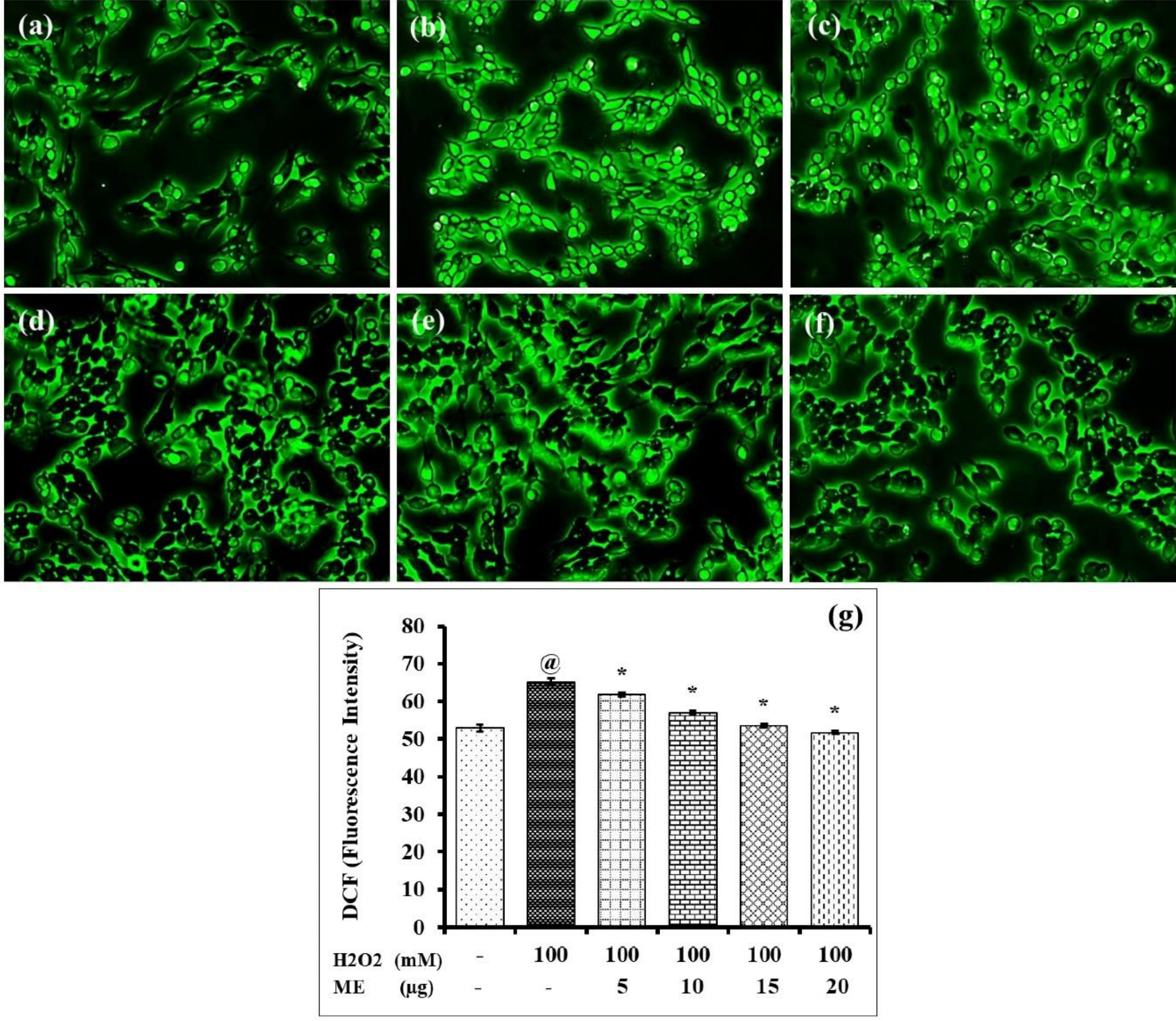

Figure 2. ME reduces H_2O_2-induced ROS accumulation in SH-SY5Y. (a) 10 µM DCF-DA only as control; (b) 100 mM H_2O_2 only; (c) 100 mM H_2O_2 plus 5 µg ME; (d) 100 mM H_2O_2 plus 10 µg ME; (e) 100 mM H_2O_2 plus 15 µg ME; (f) 100 mM H_2O_2 plus 20 µg ME; (g) Quantification of DCF fluorescence intensity of the cells stained with DCF-DA dye. *$p<0.05$ = H_2O_2 treatment vs. ME; @$p<0.05$ = Control vs. H2O2 treatment; DCF-DA = 2′,7′-Dichlorofluorescein diacetate; DCF = 2´,7´-Dichlorofluorescein; H_2O_2 = hydrogen peroxide.

DOI: https://doi.org/10.52756/ ijerr.2024.v39spl.016

Nauser, 2021; Rudrapal et al., 2022; Rami et al., 2023; Bhatta et al., 2023; Verma et al., 2024).

The presence of antioxidants in *I. ternifolius* could be because of the existence of flavonoid and phenolic

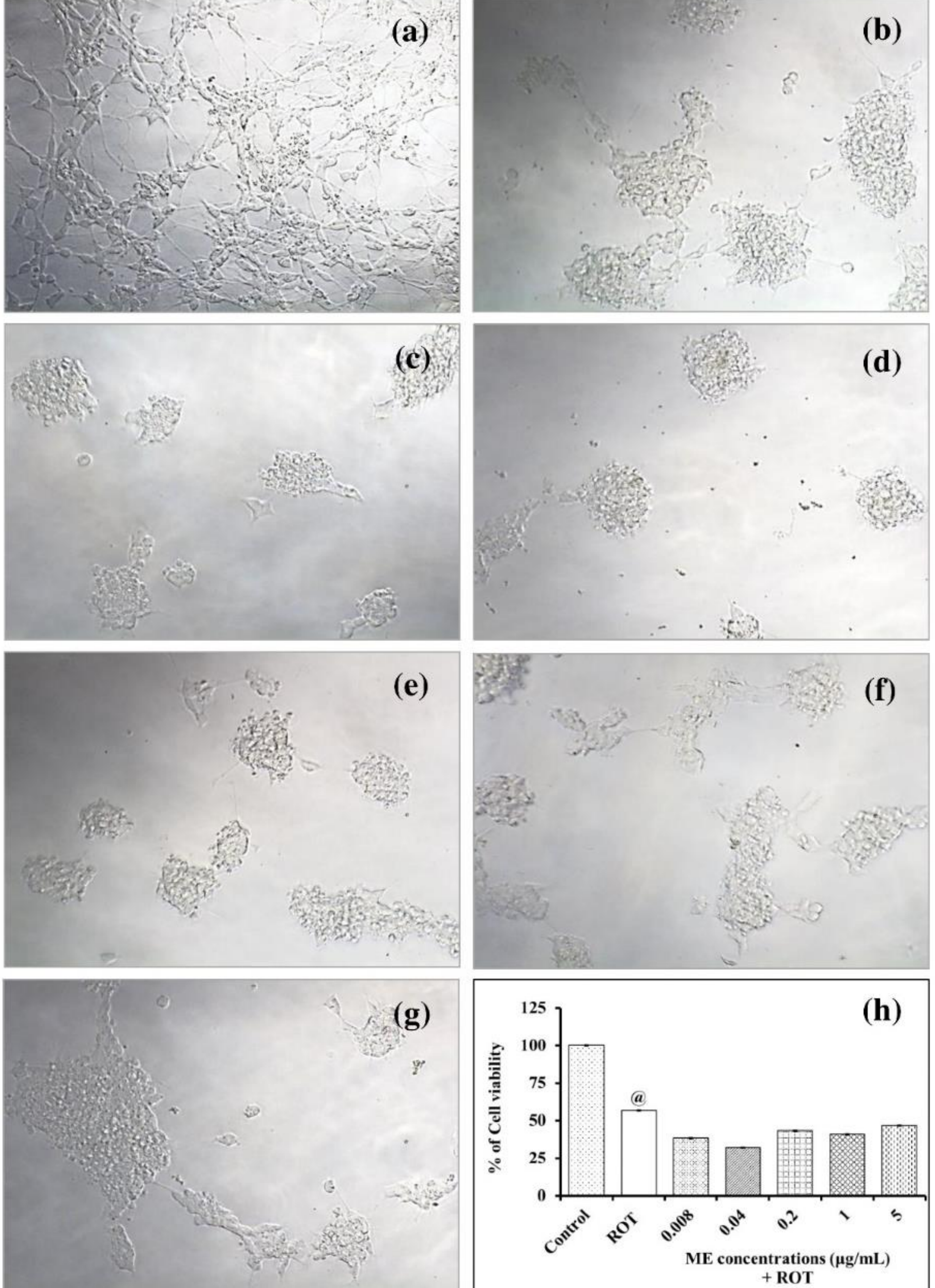

Figure 3. Cell viability of RA-differentiated SH-SY5Y cells exposed to rotenone for 24-hour with different concentrations of ME by MTT assay. (a) Control; (b) ROT; (c) 0.008 µg/mL ME plus ROT; (d) 0.04 µg/mL ME plus ROT; (e) 0.2 µg/mL ME plus ROT; (f) 1 µg/mL ME Plus ROT; (g) 5 µg/mL plus ROT; and (h) Graphical representation of cell viability at different treatment conditions. * = p<0.05 vs. control. ROT = rotenone. ME = *Isodon ternifolius* methanol extract. RA = retinoic acid.

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compounds. Despite its immense antioxidant properties, the plant did not demonstrate neuroprotective activity against rotenone-induced neurotoxicity in human SH-SY5Y neuroblastoma cells. It might be due to many phytochemicals, such as *ent*-kaurene diterpenoid, that are toxic to the cells (Li et al., 2006; Thirugnanasampandan and Jayakumar, 2009; Sun et al., 2021). There are reports on the chemical isolation and purification of many terpenoids from the species *I. ternifolius* (Zou et al., 2012; Zhang et al., 2020). The terpenoids, especially the diterpenoids such as ent-kaurene, are described to encompass biological activities, for instance, anti-inflammatory, antioxidant (El-hawary et al., 2012; Aimond et al., 2020; Ibrahim et al., 2023), analgesic, diuretic, anti-tumour, anticancer (Gurgel et al., 2009; Ding et al., 2017; Ibrahim et al., 2023), antiacetylcholinesterase (Thirgnanasampandan et al., 2008), antimicrobial (Goncalves et al., 2012; Sarkar et al., 2021, 2022; 2024; Ibrahim et al., 2023), antiangiogenic (Meade-Tollin et al., 2004; Huang et al., 2016) and phytotoxic (Rauf et al., 2012). The high antimicrobial activity of the extracts might also be contributed by the terpenoids present in the plant. Besides, the plant is also a significant constituent of the Chinese patent medicine 'FufangSanyexiangchacaiPian,' which is used to care for hepatitis B in China.

Although the extract exhibited good antibacterial potency, the study used a limited number of microorganisms. Therefore, further investigation is still warranted into the broad spectrum of microbes to explore their inhibitory action on the growth of other bacterial strains, parasites, viruses, and/or fungi. Further, more analytical research on this plant is needed to sort out novel drugs.

Conclusion

The present study's outcomes show that *Isodon ternifolius* leaf extract exhibits high antimicrobial and antioxidant properties but could not protect the neuronal cells from rotenone cytotoxicity. These vigorous antimicrobial activities established in the current study partially substantiate the traditional use of *I. ternifolius* as a disinfectant by the indigenous people of Manipur, India. Thus, the study provides evidence that *I. ternifolius* could be regarded as a potent medicinal plant for the possible source of many antimicrobial agents and natural antioxidants. However, additional studies are mandatory for the isolation and purification of the bioactive constituents present in the organic extracts of *I. ternifolius* to examine the mechanism of the observed activities. Those compounds' free radical quenching activity may further be required to study in the living system using *in-vitro* cell culture models and *in-vivo* animal models. Lastly, this is the first information on the free radical scavenging potential of *I. ternifolius*.

Acknowledgement

The authors are thankful to – (a) Prof. Lisam Shanjukumar Singh, Professor, Department of Biotechnology, Manipur University, Canchipur; (b) (Retd) Prof. Potsangbam Kumar Singh, Professor, Department of Life Sciences (Botany), Manipur University, Canchipur; (c) Prof. N Debananda Singh, Professor, Department of Biochemistry, Manipur University, Canchipur; and (d) Dr. Kh. Ranjana Devi, Professor, Department of Microbiology, RIMS. (e) Dr. Yumkham Sanatombi Devi, Guest Faculty, Department of Life Sciences (Botany), Manipur University, Canchipur.

Abbreviations

ABTS: 2, 2′-Azinobis-3-ethylbenzo-Thiozoline-6-sulphonate, $ABTS^{\cdot+}$ -2, 2′- azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) Radical Cation, $AlCl_3$: Aluminium chloride, ATCC: American Type Culture Collection, DCF-DA: 2′,7′-Dichlorofluorescein diacetate, DCF: 2′,7′-Dichlorofluorescein, DMEM: Dulbecco's Modified Eagle Medium, DMSO: Dimethyl sulfoxide, DPPH: 1, 1-Diphenyl-2-Picrylhydrazyl, EDTA: Ethylene diaminetetraacetic acid, $FeCl_3$: Ferric Chloride, FBS: Fetal Bovine Serum, GAE: Gallic Acid Equivalent, H_2O_2: Hydrogen peroxide, ME: *Isodon ternifolius* methanolic crude extract, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide, QAE: Quercetin Equivalent, RIMS: Regional Institute of Medical Sciences, ROS: Reactive Oxygen Species, RPMI: Roswell Park Memorial Institute Medium, RT: Room Temperature, TFC: Total Flavonoid Content, TPC: Total Phenolic Content.

Conflict of Interest

The authors declare no conflict of interest.

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How to cite this Article:

Aruna Chanu Hijam, Arunkumar Singh Koijam and Reena Haobam (2024). Methanol extract of *Isodon ternifolius* (D. Don) KUDO leaves has antimicrobial and antioxidant activities but no neuroprotective activity. *International Journal of Experimental Research and Review*, *39*(spl.) 200-212.

DOI: https://doi.org/10.52756/ijerr.2024.v39spl.016

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