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Research Article

Phytochemical profiling, biopotentiality of *Naravelia zeylanica* (L.) DC. – A potent medicinal plant from Western Ghat

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ARTICLE HISTORY

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Abstract: Plant products or their by-products have always exhibited appreciable and vast economic values, as the whole plant or part of it is beneficial for human welfare. One of the significant values is they are the treasure of medicines due to the presence of unique kinds of bioactive compounds. Hence boundless research has been underway on the plants, which leads to the innovation of medicines. To know about the presence of bioactive compounds in the selected tree species N. zeylanica was subjected to screening the preliminary phytochemicals and antioxidants in the leaf, and stem, of various solvent extracts. The results were positive for saponins, tannins, terpenoids, phenols, glycosides, steroids, flavonoids and carbohydrates. Stem methanolic extract showed the highest percentage of total phenolic content (24.919% at 50µg/mL). Leaf ethanolic extract (98.78%) showed the highest rate of free radical scavenging activity and reducing power activity was more in stem ethanolic extract with absorbance1.5683 Stem ethanolic extract showed a maximum of 47% inhibition of protein denaturation and 62% of proteinase inhibitory activity.

1. INTRODUCTION

Healing with medicinal plants is as old as mankind. Plants are a renewable resource that provides raw materials for different industrial applications significantly in pharmaceutical industries (Manisha *et al*., 2025). In the present study, *N. zeylanica* a potential medicinal plant was taken to understand its antimicrobial, anti-oxidant and cytotoxicity of leaf and stem extracts. *N. zeylanica* belongs to the family Ranunculaceae, distributed in South East Asia, throughout India. It is a climbing shrub, grows up to 8m height, young stem, leaves and buds densely villous; roots tuberous, leaflets are ovate-lanceolate, serrate or crenate, prominently nerved, flower, fragrant, in axillary and terminal panicles, sepals downy, petals linear- cravate, elongate, fruits aggregate of aches, ending in twisted feathery tails (Tamura, 1986). *Naravelia* includes many species few important species are *N. antonii, N. axillaris, N. dasyoneura, N. eichleri, N. finlaysoniana, N. laurifolia, N. lobata, N. loheri, N. pauciflora,* etc. (Ranil *et al*., 2023)

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N.zeylanica is the source of promising bioactive compounds which include carbohydrates, tannins, flavonoids, alkaloids, phytosterols, fixed oils, fats, gums, mucilages, proteins, and terpinoids (Sutharsingh *et al*., 2011). Earlier reports prove that ethanolic extract of *N. zeylanica* yielded three important benzamides i.e., 3, 4-methylenedioxybenzamide, 4-methoxybenzamide and 4-hydroxy-3-methoxybenzamide. Beriberine, an alkaloid is isolated from methanolic extract of leaves of *N. zeylanica* which has antimutagenic, anticancer, antimicrobial activity, diarrhoea, intestinal parasite infections, and ocular trachoma infections and lowers low density lipoproteins (Naika *et al*., 2007).

In Indian system of medicine Ayurveda, the plant *N. zeylanica* (Linn) has been used in the treatment of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers. From the ethno medical reviews, it has been observed that the aerial parts of *N. zeylanica* traditionally used in vitiated vata, pitta, inflammation, skin diseases (Easwaran, & Ramani, 2014). The leaves when crushed give a pungent odour which is inhaled to cure cold, all types of headaches including migraine, while root and stem paste is applied externally for psoriasis, itches and skin allergy. In Kerala, it is used as a source of drug for intestinal worms, skin disease, leprosy, and toothache (Jaroszewski, *et al*., 2005).

The traditional medicine practitioners residing in Karnataka are using the *N. zeylanica* leaf and stem juices for treating psoriasis and dermatitis. The root and stem have a strong penetrating smell and is used to relive malarial fever and headache. For treating wounds and worm infections, whole plant paste is applied externally on affected part for 2-3 days. In case of rhinitis, the stem is dried, powdered, tied in a clean cloth and the aroma from the cloth bag is gently inhaled (Umarani *et al*., 2020). In the present research work preliminary phytochemical analysis, antimicrobial, antioxidant and cytotoxicity, etc., has been carried out on root, leaf and fruits of *N. zeylanica* extracts. As nothing has been worked on antioxidant activity and *in-vitro* anti-inflammatory activities, the present study on stem and leaf of *N. zeylanica* has been undertaken (Figure 1).

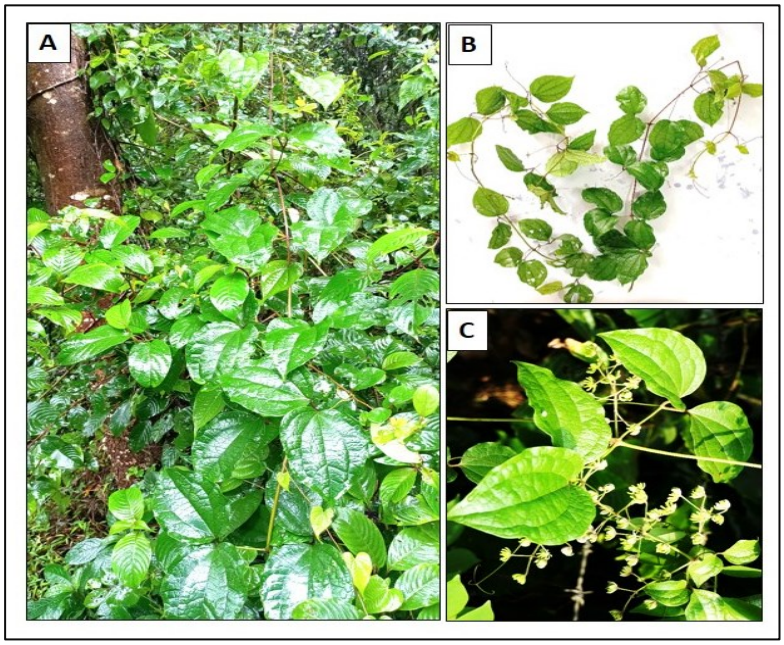

Figure 1. Morphology of *Naravelia zeylanica*; A) Habit; B) Leaves; C) Inflorescence.

2. METHOD

2.1. Collection of Plant Material and Preparation of Extracts

N. zeylanica, healthy leaf, and stem were collected from the Agumbe region of Karnataka, India. Plant was authenticated by the taxonomist and voucher number (UOMBOT24NZ06) was taken. Dried stem and leaf powder of about 50 g each were successively extracted in a Soxhlet

extractor using three different solvents such as hexane, ethyl acetate, and methanol based on their polarity index. The percentage yield was determined by using the following formula (Orban-Gyapai *et al.,* 2017). The extract was stored in an air-tight container and used for further phytochemical analysis.

$$[(W2 - W1)/W0 * 100]$$

2.2. Preliminary Phytochemical Screening

Leaf, stem, and extracts of *N. zeylanica* was subjected to preliminary phytochemical screening based on the standard procedure of Raaman (2006), Trease and Evans (2009, 1989), and Sofowora (1982, 1993).

2.3. Antimicrobial Assay

The microbes for antimicrobial activity including six bacterial (*Bacillus subtilis, Listeria monocytognes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonell a typhi*) and two fungal strains (*Candida albicans* and *Epidermophyton floccosum*) were collected from Microbial Type of Culture Collection (MTCC), Chandigarh, India.

2.3.1. Antimicrobial activity

All four solvent extracts of *N. zylanica* leaf were used to detect their antimicrobial efficacy by agar well diffusion method (CLSI, 2015). Each extract was tested at a concentration of 35 mg well^{-1} in 50 µL. Six bacterial and two fungal strains were used to check the antimicrobial effect of leaf solvent extracts with Gentamicin for bacteria and Nystatin for fungi as a positive control and incubated at 26 ± 2°C for 72 hr. The zone of inhibitions was measured after incubation, and the assay was performed in triplicates.

ELISA multi-plate reader was used to detect the minimum inhibitory concentration (MIC) of each leaf solvent extract through micro broth dilution method (LabTech 4000, Japan) (CLSI, 2017). Each extract was prepared at 35 mg mL^{-1} concentration from this stock solution, 100µL (5mg) was added to the wells and serially diluted to achieve the desired concentration (0.0024 - 5 mg mL^{-1}). Streptomycin, Nystatin, and respective solvents were used as positive and negative controls respectively. After incubation, TTC (2, 3, 5-triphenyl tetrazolium chloride) of about 10 µL well^{-1} (2 mg mL^{-1}) was added and again incubated for 30 min. The absorbance was recorded at 624 nm and no color changes were considered as a minimal concentration and the assay was performed in triplicates.

2.4. Antioxidant Activities

2.4.1. ABTS anti-oxidant assay

ABTS decolourization assay is used to assess potential *in vitro* antioxidant capacity of *N. zeylanica* leaf methanol and ethyl acetate extracts and the assay was performed as per the procedure explained by Auddy (2003). ABTS radical cations are produced by reacting ABTS and APS on incubating the mixture at room temperature in dark for 16 hours. The solution thus obtained is further diluted with PBS to give an absorbance of 1.000. Different concentrations of leaf ethyl acetate and methanol extracts (0.5, 1.0, 2.0, 2.5 µg/mL) and the reference standard Quercetin (-1.0, -0.5, 0.0, 0.5, 1.0, 1.5 µg/mL) are added to 225mL of ABTS working solution to give a final volume of 250µL, made up by adding PBS. The absorbance is recorded immediately at 734nm and the percentage of inhibition was recorded.

The percentage inhibition is calculated at different concentrations and the IC_{50} values are calculated by using Graph pad prism. The relative activity of the sample can be determined by comparing the IC_{50} value of the sample with the standard. The higher the IC_{50} value, the lower the relative activity in comparison to the standard and vice-versa.

2.4.2. DPPH radical scavenging assay

DPPH assay is used to predict antioxidant activities by mechanism in which antioxidant act to inhibit lipid oxidation, so scavenging of DPPH radical and therefore determinate free radical scavenging capacity. In the present study DPPH assay is carried out as per the method of Rajakumar *et al*. In brief, 80mL of DPPH solution; various concentrations of test samples and quantity sufficient to 240 mL with HPLC grade methanol. The different concentrations of *N. zeylanica* leaf methanol and ethyl acetate extracts (0.5, 1.0, 1.5, 2.0, 2.5 µg/mL) and reference standard Quercetin (-0.5, 0.0, 0.5, 1.0, 1.5 µg/mL) were used. The reaction mixture is mixed and incubated at 25°C for 15 minutes. The absorbance is measured at 510 nm using spectrophotometry. A control reaction is carried out without the leaf extracts. Calculating percentage growth inhibition with IC_{50} value using Graph Prism software version 5.0 by nonlinear regression analysis of percentage inhibition recorded for different concentrations of leaf solvent extracts.

$$\%\text{Inhibition} = \frac{-\text{OD of control} - \text{OD of Sample}}{\text{OD of control}} \text{X100}$$

2.4.3. Ferric reducing anti-oxidant power assay

Different concentration of leaf methanol and ethyl acetate extracts of *N. zeylanica* and 0.7mL reference standard ascorbic acid was added to 2.3 mL of working FRAP (Ferric Reducing Anti-oxidant Power) reagent to give final volume 3mL, then this mixture was kept in incubation in dark at 37°C for 30min. The absorbance was measured at 593nm was against blank having all the reagent excluding leaf solvent extracts. A stronger absorbance will indicate increased reducing power. Results were expressed in mM of ascorbic acid equivalents per gram extract.

2.5. Cytotoxicity Study Using Various Cell Lines

The cytotoxicity study is one of the biological evaluation and screening tests that use tissue cells *in vitro* to observe the cell growth, reproduction, and morphological effects of medical devices. In the present study five different cell lines such as HT-29, HEPG-2, MCF-7, Raw-264.7 were used to study the cytotoxic properties of the *N. zeylanica* leaf ethyl acetate extract. Chemicals used in the present study such as DMEM (Dulbecco's Modified Eagle Medium), FBS, Penstrep, and Trypsin were procured from Invitrogen.

2.5.1. *Sample preparation*

1mM stock of Doxorubicin used as a standard for cytotoxicity studies, further serial two-fold dilutions were prepared from 100µM to 1.56µM using plain DMEM for treatment. 32mg/mL stocks were prepared using DMSO. Serial two folds dilutions were prepared from 320µg/mL to 10µg/mL using DMEM for treatment. Final vehicle control containing plain media with 1% DMSO.

2.5.2. *Cell culture*

Different cell lines used in the present study such as HT-29, HEPG-2, NADF, MCF-7, and Raw-264.7 were obtained from ATCC. The stock cells were cultured in DMEM supplemented with 10% inactivated Fetal Bovine Serum (FBS), penicillin (100 IU/mL), streptomycin (100µg/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were dissociated using 0.05% trypsin and centrifuged at 1000 rpm for 5min. The culture media was discarded and the cell pellet was gently resuspended using 2mL DMEM. The viability of the cells was checked and a single cell suspension of 5.0 X105 cells/mL was prepared (Frei, 2011).

2.5.3. *Procedure*

100 µL of the prepared cell suspension was added to each well of the pre-labelled 96-well microtiter plate (50,000 cells/well) and incubated at 37°C with 5% CO_2. The supernatant was removed after 24hr. of incubation and the monolayer was rinsed with plain media. To each pre-designated well, 100µL of test drugs at various concentrations were added and incubated for

24hrs. After incubation, the test solutions in the wells were discarded and 100 µL of MTT reagent (5 mg/10 mL of MTT in PBS) was added to each well. The plates were incubated for 4 hr at 37°C in 5% CO_2. The supernatant was removed and 100 µL of DMSO was added and the plates were gently shaken to solubilize the formazan crystals. The absorbance was measured using a microplate reader at 590 nm (Spectramax i3X, Molecular devices). The percentage growth inhibition was calculated using the following formula;

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \text{X100}$$

IC_{50} values for cytotoxicity tests were derived from a nonlinear regression analysis (curve fit) based on a sigmoid dose-response curve (variable) and computed using Graph Pad Prism 5 (Graph pad, SanDiego, CA, USA).

2.6. Evaluation of Anti-Inflammatory Against LPS Induced TNF-α Release

Raw-264.7, a promonocytic cell line was obtained from the ATCC. Cells were cultured in DMEM supplemented with 10% inactivated FBS, Penicillin (100 IU/mL) in a humidified atmosphere of 5% CO_2 at 37°C until confluent. The cells were aspirated from the 80% confluence culture flask and centrifuged at 1500rpm for 5min. The cell pellet was then resuspended in 1mL of DMEM. 1 X 105 cells/well was seeded to each well of the 96 well microtiter plate and incubated for 24 hrs., To determine the cytokine (TNF-A) release, Raw-264.7 cells were pretreated for 2hrs after incubation with various concentrations of test compounds followed by LPS (1µg/mL) stimulation for additional 24hrs. Dexamethasone (Dex) was used as a positive control. Post incubation, the cell supernatant is aspirated from each of the wells into sterile micro centrifuge tubes and centrifuged at 1000rpm for 2-3mins to settle any cells if present. The cell supernatant is then used for evaluation of presence of TNF-A using ELISA and experiments were carried out in duplicates

2.7. Evaluation of TNF-α Release

Standards of various two fold dilution series from 450pg/mL (top standard) to 3.5pg/mL and samples of 100µL/well were added of to the designated wells in the ELISA plate. Assay diluent (1X) served as the zero standard (0pg/mL). Plate was sealed and incubated at 37°C for 2 hrs. After incubation, the plate was washed four times with wash buffer (1X) and residual buffer was blotted by firmly tapping the plate upside down on an absorbent paper. A diluted detection antibody of 100µL solution was added to each well, the plate was sealed and after 1 hr of incubation at 37°C the plate was washed 4 times with Wash Buffer (1X). Diluted Avidin-HRP of 100µL solution was added to each well; Plate was sealed and incubated at 37°C for 30 minutes followed by washing plates with Wash Buffer (1X). For this final wash, wells were soaked in Wash buffer for 30 sec. for each wash. TMB Substrate of 100µL solution was added to each well and incubated in the dark for 30 minutes at 37°C. Reaction was stopped by adding 100µL of stop solution to each well. Absorbance was recorded at 450nm immediately after adding stop solution and percentage of inhibition was calculated by using following formula;

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of sample}}{\text{OD of control}} \text{X100}$$

2.8. Statistical Analysis

Data from triplicates were analyzed for each experiment and the analysis of variance (ANOVA) was done by using IBM software SPSS Inc. 16.0 Ver. Significant effects of treatments were determined by F values ($p \leq 0.05$) after Tukey's HSD test, considering separated treatment means. Each experiment was performed in triplicates and the data is represented as mean ± SE.

3. RESULTS

3.1. Phytochemical Screening

N. zeylanica stem and leaf extracts percentage yield was determined by using various solvents based on their polarity. Leaf ethyl acetate extract showed the highest yield than stem ethyl acetate extract (11.8% and 9.4%) and the lowest percentage yield was noticed in stem hexane extract (4.6%). Overall, the yield was higher in leaf material than in stem and these significant differences may be due to the lack of chlorophyll and other associated components in the stem. Further, each extract was subjected to comparative phytochemical analysis, which revealed the presence of significant phytochemical constituents and results were represented in Table 1. Significant phytochemicals such as saponins, terpenoids, flavonoids, phenols, glycosides, tannins, steroids, and carbohydrates were found in both leaf and stem extracts of *N. zeylanica*. Notably, maximum phytochemicals tests were positive in leaf ethyl acetate extract followed by stem ethyl acetate and methanol extracts of leaf and stem. Overall, ethyl acetate extracts with the highest yield in extraction were found to possess more number of important secondary metabolites in both leaf and stem. This observation indicates that the use of ethyl acetate solvent is suitable for extraction for further studies on secondary metabolites investigation in *N. zeylanica.*

Table 1. Preliminary phytochemical evaluation of *N. zeylanica* leaf and stem extracts.

Phytochemical tests		Meth.		Eta.		Hex.	
		L	S	L	S	L	S
Saponins		+	++	+	-	-	-
Terpenoids		+	+	+	++	++	+
Flavanoids	1. Conc. HCl	+	+	-	-	-	-
	2. NaOH	-	-	++	-	-	-
	3. Magnesium reduction	-	-	-	-	-	-
Phenol	1. $FeCl_3$	-	++	++	+	-	-
Glycosides		+	+	-	-	-	-
Tannins	1. $FeCl_3$	++	-	++	+	-	-
	2. Potassium dichromate	+	++	-	-	-	-
Steroids	1. Lieberman - Burchard test	_	+	-	-	-	-
	2. H_2SO_4	_	+	+	-	-	-
Carbohydrates	1. Fehling's test	+	+	+	+	+	+
	2. Molish test	+	++	+	++	+	++
	3. Benedict's test	+	+	-	-	-	-
	4. Barfoed's test	-	-	++	-	-	-

Note: "+" - Positive with less amount, "++" - Positive with more amount, "-" - Negative, Meth. - Methanol; Eta. - Ethyl acetate; Hex. - = Hexane; 'S' - Stem; 'L' – Leaf.

3.2. Anti-Microbial Activity and MIC

N. zylanica leaf ethyl acetate extract showed significant results in preliminary phytochemical analysis compared to stem extracts and hence leaf extracts were tested for its antimicrobial properties in six bacterial and two fungal strains (Table 2). Antibacterial activity of leaf ethyl acetate extract showed significant inhibition against *P. aeruginosa* (21mm), *E. coli* (19mm), *L. monocytognes* (17), *B. subtilis* (13) and least significant results were found in *S. typhi* (11mm). MIC value ranging from 2.5 – 1.25 mg mL^{-1} and MIC value of methanol and ethyl acetate extract was same (1.25 mg mL^{-1}), hexane was (2.5 mg mL^{-1}) (Figure 2). Comparatively antifungal activity was more in leaf ethyl acetate extracts against *C. albicans* (22mm) followed by *E. floccosum* (19mm) (Figure 3). Hence, the results indicated that methanol and ethyl acetate leaf extracts were found to possess significant antibacterial activity, but antifungal activity was observed only in ethyl acetate extract.

Table 2. Antimicrobial screening of *N. zeylanica* leaf and stem various solvent extracts.

Extracts	Zone of Inhibition (mm)							
	Bacteria						Fungi	
	Gram-Positive			Gram-Negative			Dermatophyte	
	B. subtilis	*L. monocytognes*	*S. aureus*	*E. coli*	*P. aeruginosa*	*S. typhi*	*C. albicans*	*E. floccosum*
Gentamicin(10 µg)	25	26	25	22	23	24	00	00
NZM (35 mg)	00	00	00	14	15	00	00	00
NZH (35 mg)	00	00	00	00	00	00	00	00
NZE (35 mg)	13	17	11	19	21	13	22	19
Nystatin (10 µg)	--	--	--	--	--	--	18	23
	Minimum Inhibitory Concentrations (MIC)							
Gentamicin(10 µg)	0.156	0.156	0.039	0.039	0.156	0.156	--	--
NZM (35 mg)	--	--	--	--	1.25	--	--	--
NZH (35 mg)	--	--	--	--	2.5	--	--	--
NZE (35 mg)	--	--	--	--	1.25	2.5	--	--
Nystatin (10 µg)	--	--	--	--	--	--	0.156	0.156

Note: NZM: *N. zeylanica* methanol extract; NZH: *N. zylanica* hexane extract; NZE: *N. zeylanica* ethyl acetate extract.

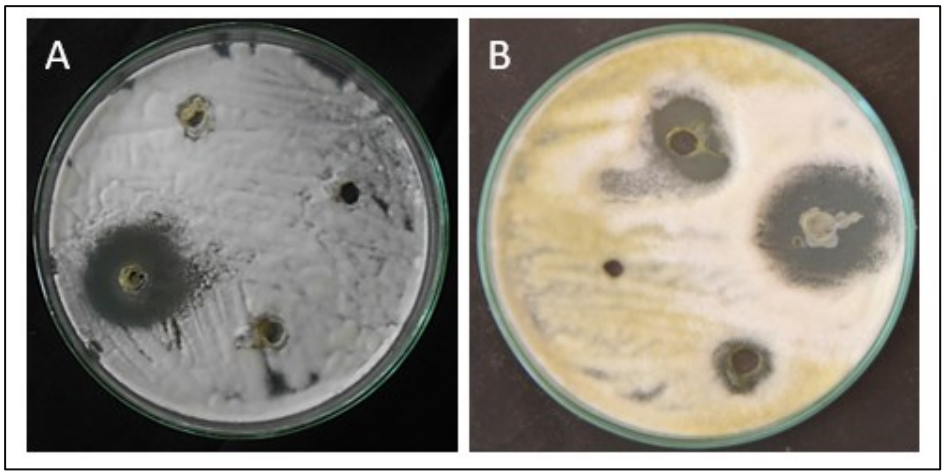

Figure 2. Antifungal screening of *N. zeylanica* leaf extracts: A) *E. floccosum*; B) *P. aeruginosa.*

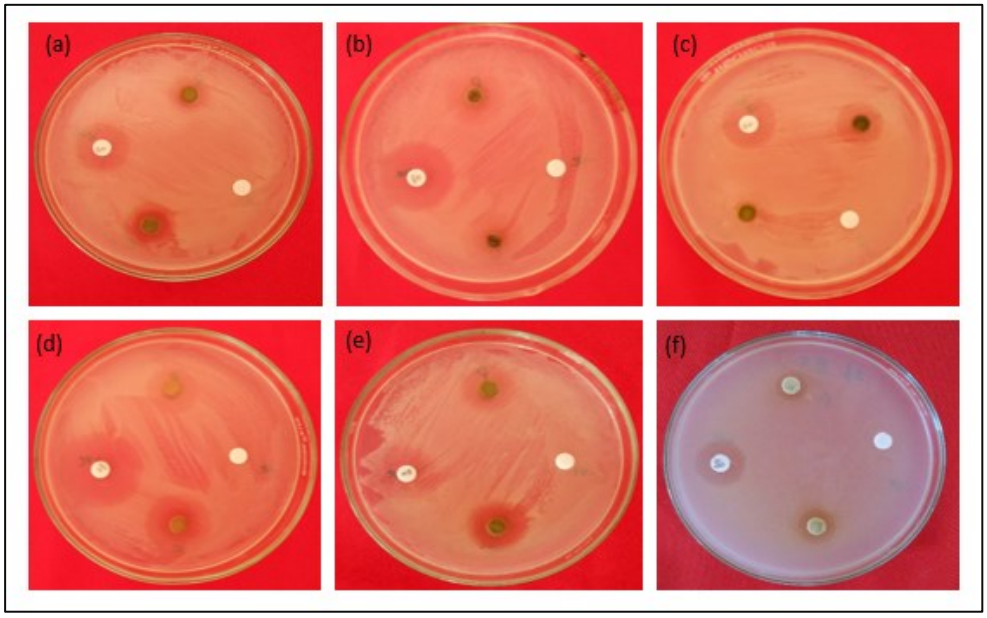

Figure 3. Antibacterial screening of *N. zeylanica* leaf extracts.a) *B. subtilis,* b) *L. monocytognes,* c) *S. aureus,* d) *E. coli*, e) *P. aeruginosa* and f) *S. typhi.*

3.3. Anti-Oxidant Activity of *N. zeylanica* Leaf Extracts

3.3.1. ABTS anti-oxidant assay

Leaf ethyl acetate and methanol extracts of *N. zeylanica* were tested for antioxidant properties by ABTS assay. The relative activity of the sample can be determined by comparing the IC_{50} value of the sample with standard Quercetin (Supp. Tables 1 and 2). The results showed an activity of 98.78% in ethyl acetate extract (Figure 4A) and 71.26% in methanol extract (Figure 4B) at 50 µg/mL concentration. Both ethyl acetate and methanol leaf extracts showed activity having an IC_{50} value of 24.2µg/mLand 58.69µg/mL, respectively. Standard Quercetin has showed 81.66% of inhibition with IC_{50} value of 1.366 µg/mL (Figure 5).

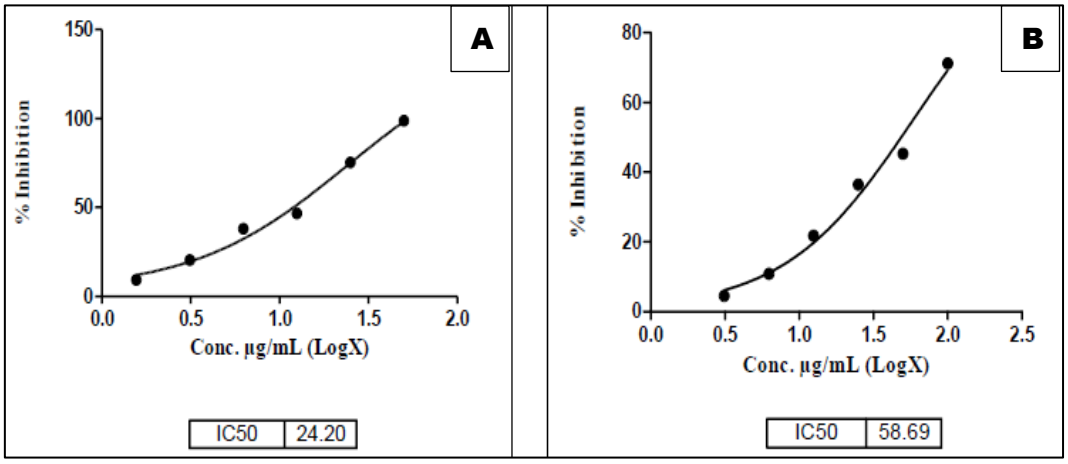

Figure 4. ABTS radical scavenging activity of *N. zeylanica* leaf extracts; A) Ethyl acetate extract; B) Methanol extract.

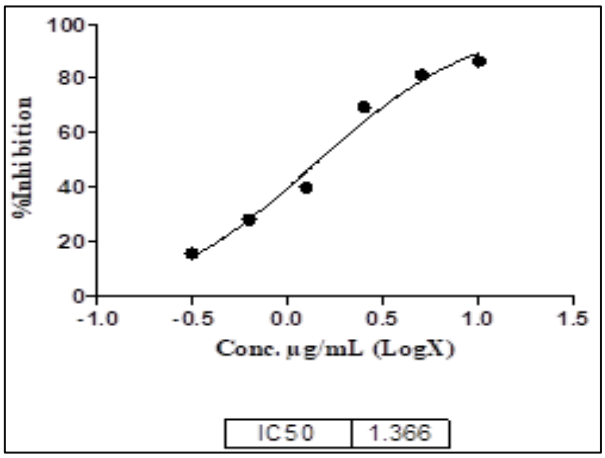

Figure 5. ABTS radical scavenging activity of standard Quercetin.

3.3.2. *DPPH radical scavenging assay*

The DPPH (2, 2-diphenyl-1-picrylhydrazyl radical) radical scavenging activity of *N. zylanica* leaf methanol and ethyl acetate extracts revealed significant results of inhibition ($p \leq 0.05$) in a dose-dependent manner (Supp. Table 3 & 4). The decrease in absorbance indicated an increase in free radical scavenging activity concerning the color changes from deep purple to pale yellow color. The leaf ethyl acetate and methanol extracts exhibited the highest percentage of radical scavenging activity of 69.55% and 48.52% at maximum concentration of 100µg $mL^{-1}$with an IC_{50} of 70.92 µg $mL^{-1}$and 147 µg mL^{-1} respectively (Figures 6 A and B). The standard Quercetin showed 89.17% (IC_{50}: 1.519 µg mL^{-1}) (Figure 7). Hence, the results of this assay indicate that, the plant possesses significant antioxidant properties.

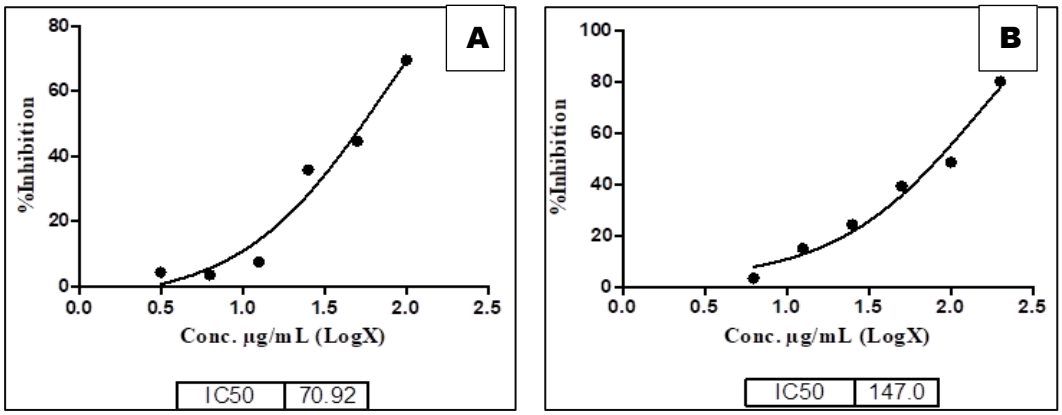

Figure 6. DPPH radical scavenging activity of *N. zeylanica* leaf extracts. **A**) Ethyl acetate extract; **B**) Methanol extract.

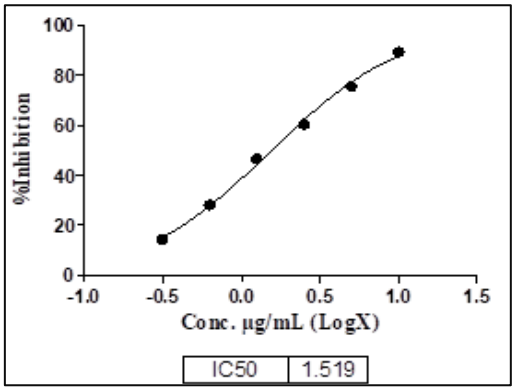

Figure 7. DPPH radical scavenging activity of standard Quercetin.

3.3.3. *FRAP assay*

N. zeylanica leaf methanol and ethyl acetate extracts and standard Ascorbic acid showed a reduction of Fe^{3+} to Fe^{2+} (Supp. Table 5 & 6). Results at higher concentrations showed FRAP value of 98.84 mM EAA/g in leaf methanol and 200.75Mm EAA/ g in leaf ethyl acetate extracts (Figure 8). FRAP assay was used to calculate the FRAP value of samples using a linear regression of the standard curve and standard ascorbic acid exhibited 1.441% at 20µM concentration (Figure 9).

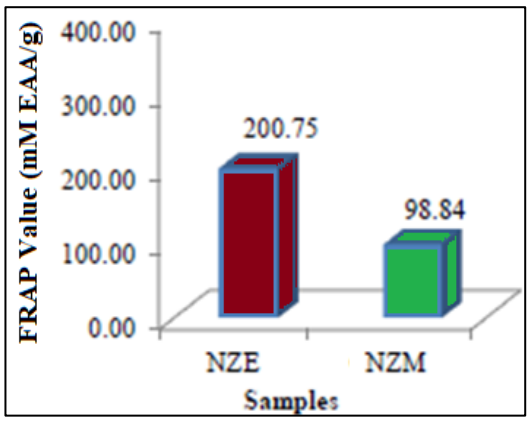

Figure 8. FRAP assay of methanol and ethyl acetate assay of *N. zeylanica* leaf.

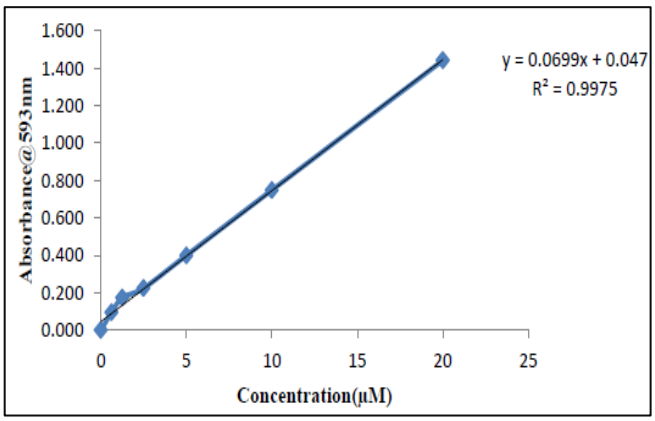

Figure 9. FRAP assay of standard ascorbic acid.

3.4. Cytotoxicity Study Using HT-29 and HEPG-2 Cell Line

The cytotoxicity study results suggest that leaf ethyl acetate extract of *N. zylanica* has not shown marked cytotoxicity in HT-29 cells. Reference standard Doxorubicin have shown the IC_{50} value of 25.43 µM against HT-29 cells with a maximum inhibition up to 89.28 % at the highest

concentration tested at 100µM (Figure 10). Similarly both the extracts do not show the cytotoxicity in NADF AND HEPG-2 cell lines, but standard Doxorubicin shows the IC_{50} value of 21.73 µM against HEPG-2 cells with a maximum inhibition up to 86.85% at the highest concentration (100µM) (Figure 11).

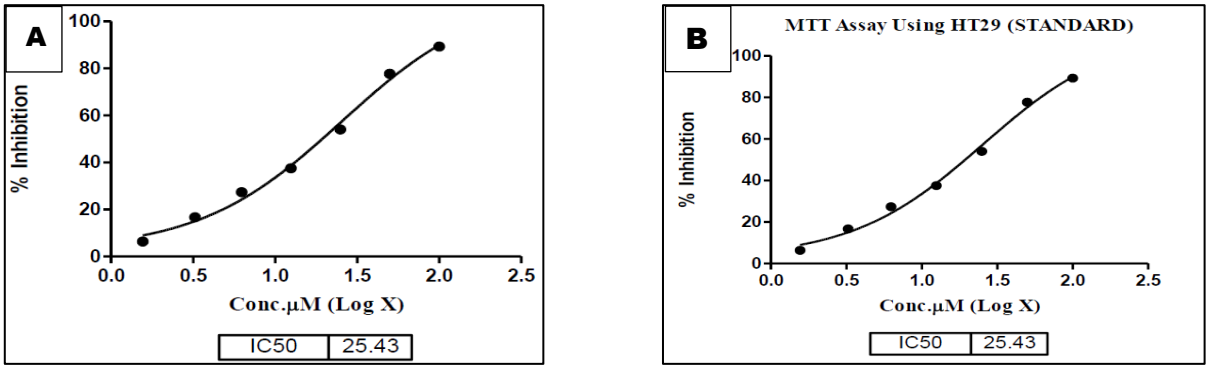

Figure 10. Cytotoxicity study using HT-29 cell line; **A**) *N. zeylanica* leaf ethyl acetate extract; **B**) Standard.

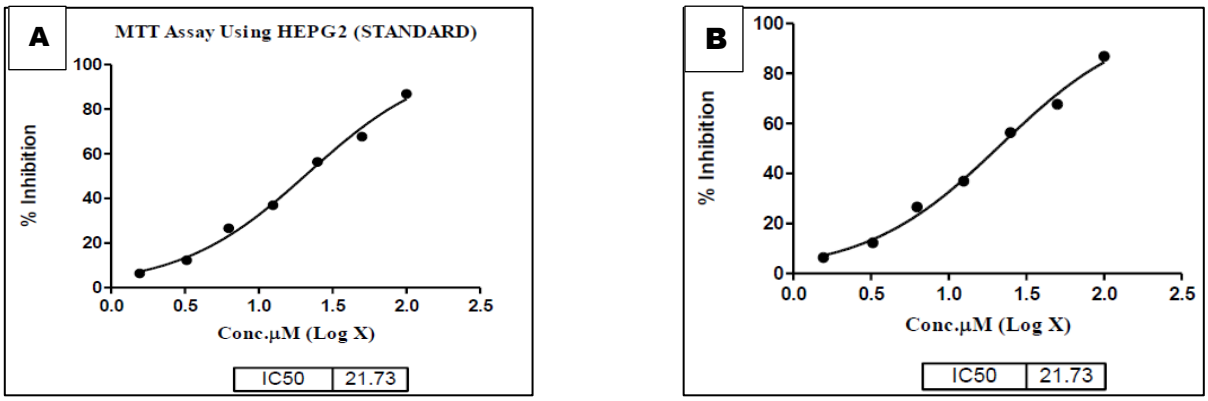

Figure 11. Cytotoxicity study using HEPG-2 cell line; **A**) *N. zeylanica* leaf ethyl acetate extract; **B**) Standard.

3.5. Cytotoxicity Study Using MCF-7 and RAW 264.7 Cell Line

N. zeylanica leaf methanol extracts showed significant results in Raw -264.7 cells and MCF-7 cells of 73.50% and 67.13% cytotoxicity with IC_{50} 239.8 µg/mL (Figure 12 A) and 205.9µg/mL at 320µM concentration (Figure 13 A) respectively. Reference standard Doxorubicin was shown the IC_{50} value of 21.73 µg/mL (Figure 12 B) against Raw-264.7 cells with a maximum inhibition up to 90.28 % and IC_{50} value of 20.29 µg/mL (Figure 13 B) against MCF-7 cells with inhibition of 86.85% at 100µM concentration (Supp. Table 10 & 11).

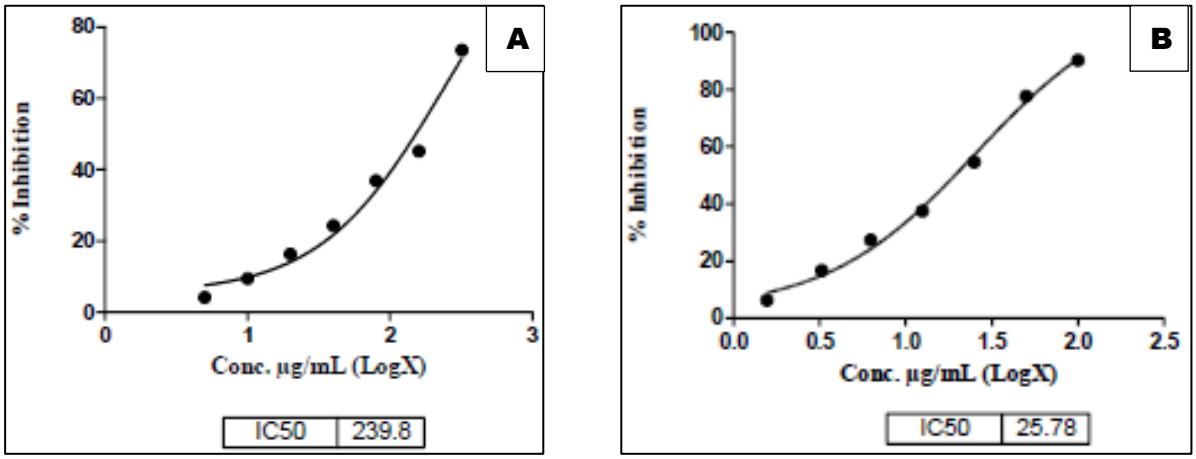

Figure 12. Cytotoxicity of test compounds in Raw -264.7 cell line; **A**) *N. zeylanica* leaf ethyl acetate extract; **B**) Standard.

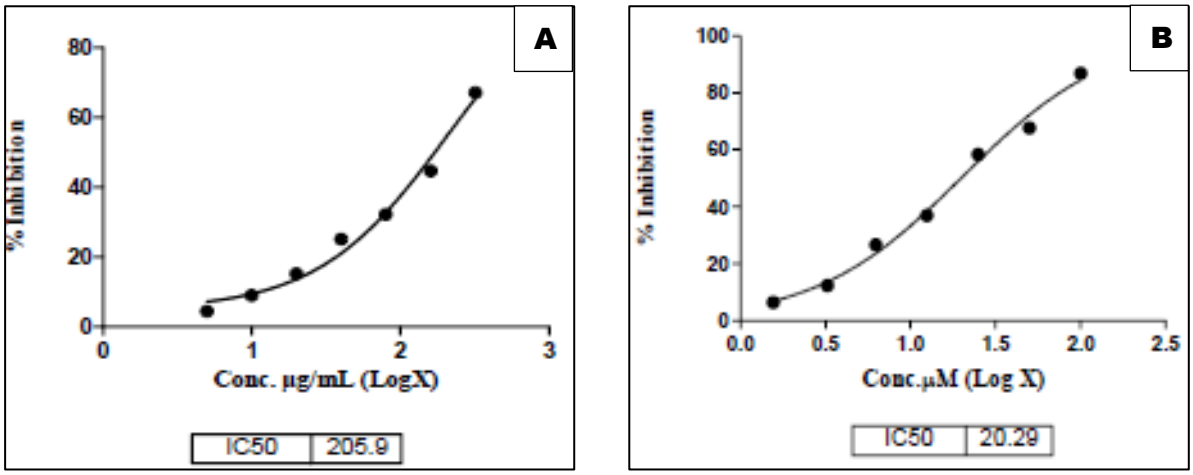

Figure 13. Cytotoxicity of test compounds in MCF -7 cell line; **A**) *N. zeylanica* leaf ethyl acetate extract; **B**) Standard.

3.6. Evaluation of Anti-Inflammatory Effects Against LPS Induced TNF-α Release

Raw 264.7 cells were treated with various concentrations of test compounds to evaluate the inhibitory effects on LPS induced TNF-α release. TNF- α release within cells was induced by treating the cells with LPS at 1µg/mL concentration for 24 hrs with and without various concentrations of test compounds. The results suggest that the sample GGM has shown inhibition of LPS induced TNF- α release of more than 50% up to 52.13%, at the highest concentrations tested at 200 µg/mL. Samples NZM has shown inhibition of TNF-A release less than 50% at the highest concentration treated i.e., at the highest concentrations tested at 200 µg/mL. Positive control Dexamethasone at 2µg/mL has shown up to 81% inhibition. Obtained data is represented in the Figures 14 and 15.

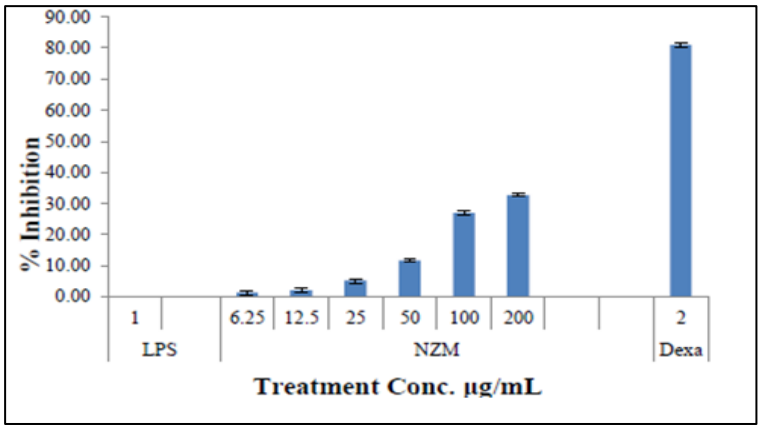

Figure 14. Inhibition of LPS induced TNF-α in Raw 264.7 cells treated with test compounds.

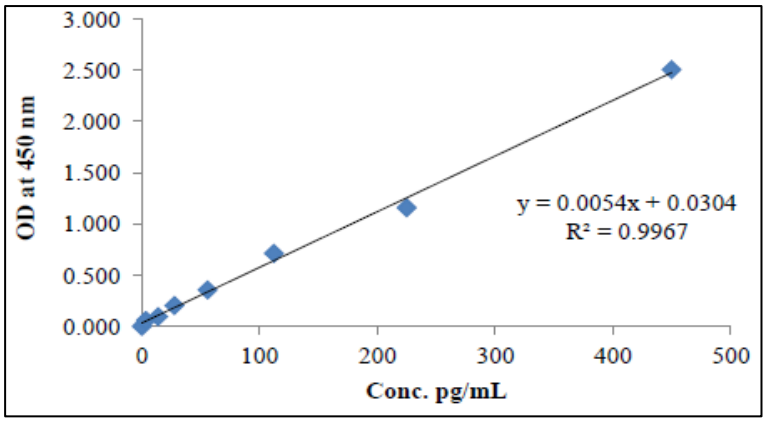

Figure 15. TNF-α Standard plot.

4. DISCUSSION

Many pharmaceutical industries in India are engaged in the production of skin ointments from this plant. Roots are crushed and inhaled to cure headache; fresh stem is chewed to releave toothache. Young leaf paste is applied for skin diseases, ulcers and headaches. Crushed roots are inhaled against cold and fever. Crushed roots are boiled in water and the vapours are inhaled to cure cold and fever. To treat rheumatism leaves are boiled in water and used to bathe (Mohammed *et al*., 2023; Mohammed *et al*., 2024; Sevindik *et al*., 2023).

Preliminary phytochemical analysis done for the different solvent extracts of stem and leaf parts of *N. zeylanica* showed more phytoconstituents like saponins, terpenoids, flavonoids, phenol, glycosides, tannins, steroids and carbohydrates in ethanolic and methanolic extracts compared to other solvent extracts. Terpenoids and carbohydrates were present in all the extracts while proteins, alkaloids, anthroquinone, phlobotanins, fixed oils and fats were absent in all the extracts. Thomas *et al*., (2013) reported the presence of terpenoids, cardioglycosides, flavanoids, phenols, saponions, tannins, and carbohydrates in *D. chloroxylon* in acetone and methanol extracts. Saponins, anthraquinones, terpenoids, tannins, and polyphenols were present in the roots, twigs and leaves of *D. lycioides*. Flavonoids and polyphenols and tannins were seen in roots, leaves and twigs but tannins were absent in the roots (Nyambe. 2014). In human being and animals, cholesterol-lowering activity can be done by saponins and they also possess the property of anti-cancerous, anti-microbial, anti-inflammatory (Aziz *et al*., 2019). Similarly tannins also possess antimicrobial property, phenols and flavonoids play an important role in defense mechanisms. Rashed (2013) reported more radical scavenging activity of methanolic (89.6%) and ethanolic (70.9%) extracts of *D. ebenum* while aqueous (47.8%) extract showed less activity. The total phenolic content of *D. melanoxylon* also showed high activity in methanolic (1.78 ± 0.06) and ethanolic (1.36 ± 0.08) extract compared to other solvent extracts viz., acetone (1.16 ± 0.04) and aqueous (1.10 ± 0.10) extracts (Jaiswal *et al*., 2012).

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli which is frequently linked with pain and involves many biological occurrences, such as an increase in vascular permeability, an increase in protein denaturation, and membrane alteration (Ferrero-Millani, *et al.,* 2007). The results obtained from our studies on stem ethanolic extract have shown a potential anti-inflammatory activity. This indicates that *Naravelea zylanica* is more useful in studies of inflammation and various physiological studies aging, and diseases such as cancer, neurological disorder etc.

The results of anti-inflammatory activity were compared with the control, which showed significant protection and hemolysis. The extract exhibited membrane stabilization by inhibiting hypotonicity-induced lysis of the erythrocyte membrane and the results were in concordence with the report of Chou (1997). The results of heat-induced haemolysis exhibited 55% inhibition at 3mg/mL concentration. A similar study conducted by Reshma *et al*., (2014) in plant extracts of *Aegle marmelos* and *Ocimum sanctum* was effective in inhibiting heat-induced haemolysis at 95.64µg/mL and 42.17µg/mL, respectively. The proteinase inhibitory action of stem ethanolic extract showed significant action. It was found to be maximum at 15mg/mL concentration (62%), and the minimum at 5mg/mL concentration with 27% inhibition.

5. CONCLUSION

The current study reveals the presence of promising phytochemicals in all solvent extracts of the stem and leaf of *Naravelea zylanica*. Ethyl acetate and methanolic extracts of leaf and stem exhibited more antioxidant properties indicating the presence of active biochemical compounds. Furthermore, the anti-oxidant activity of the leaf ethyl acetate extract showed moderate activity. Therefore, we believe that the research work presented here paves the way for initiating the production of high-valued biomolecules using biotechnological approaches.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Neha Rani: Collection of resources and investigation. **Ramesh Kolathuru Puttamadaiah:** Experimental set up, analysis and writing. **Nuthan Bettadapura Rameshgowda**: Review and editing. **Sampath Kumar Kigga Kaadappa**: Plant identification, drafting and finalization. **Satish Sreedharmurthy:** Supervision, validation and approved for submission.

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