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Evaluation of Antioxidant Activity and Phytochemical Screening of Diverse Polar Extracts from Eucalyptus citriodora

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ABSTRACT

Parkinson's disease is a neurological ailment that progresses over time and affects millions of people worldwide. It is characterized by a complex combination of motor and non-motor symptoms. The study focused on bioactive components derived from *Eucalyptus citriodora* leaf extracts through qualitative and quantitative examination of antioxidant scavenging activity, along with in vitro cytotoxicity assessment and antimicrobial activity. The findings of the study revealed the IC_{50} values for the plant extract in various antioxidant assays like Nitric oxide scavenging and ferric reducing power showed an IC_{50} of 85.87µg/ml, Hydrogen peroxide scavenging assay exhibited an IC_{50} of 206 µg/ml. Additionally, the Phosphomolybdenum assay indicated a plant extract content of 21.45µg/ml. To assess the relative amounts of different components, TLC chromatography was conducted, and the *Eucalyptus* extract was compared with quercetin, revealing a retention factor (Rf) of 2.33.

The cytotoxic assay to determine the percentage of cell viability in isolated liver cells also exhibited maximum flavonoid compared to alkaloids compound. The IC_{50} value was identified as 147ug/ml. According to the findings, *Eucalyptus citriodora* has a high potential for antioxidants, which are molecules that are thought to be physiologically active.

Keywords: *Cytotoxicity, Antimicrobial, Hydrogen peroxide scavenging, Neurodegenerative disorder, Parkinson's disease*

1. INTRODUCTION

Parkinson's disease is a frequently encountered neurodegenerative condition linked to aging, distinguished by the gradual and enduring deterioration of motor functions (1). Globally, approximately 500,000 Americans are identified as PD diagnosed. India is anticipated to have the highest absolute number of Parkinson's disease patients worldwide, given the prevalence rates ranging from 15 to 43 per 100,000 in the population. Notably, around 40-45% of these individuals encounter the onset of motor symptoms between the ages of 49 (2). Parkinson's disease poses a significant global economic burden due to its widespread prevalence and the associated costs of healthcare and productivity losses. Characteristic motor symptoms, including slowness, balance issues, tremors, freezing, and rigidity, define Parkinson's disease (3). Furthermore, typical non-motor symptoms include melancholy, anxiety, exhaustion, constipation, sleep behaviour disturbance, rapid eye movement, and cognitive impairment (4). Nowadays, levodopa (L-DOPA) stands as the most effective treatment for the initial motor symptoms of PD, although it does not serve as a cure for the condition (5). At physiological assets the pH, dopamine primarily exists in its ionized (protonated) form and encounters challenges in traversing the blood-brain barrier (BBB) (6). Previous studies have indicated that the ability of phytochemical extracts to penetrate the BBB is contingent on several factors, including the presence of specific compounds within the extract. Few plant-derived compounds are investigated for their potential to effectively penetrate the BBB and exert their characteristics when evaluating their ability to cross the blood-brain barrier (7, 8). *E. citriodora* is extensively cultivated globally and holds a prominent presence in the international pharmacopoeia (9). *Eucalyptus citriodora* belongs to the *Myrtaceae* family, native to Africa, India, and Tasmania. It is lemon scented and recognised as a good potent source of antioxidant, anti-inflammatory, and antibacterial

properties (10). *Eucalyptus citriodora* contains essential oil, which is traditionally used for treating wounds and various ailments (11). *Eucalyptus* essential oils serve as a compelling natural source of antioxidants, offering a viable alternative to synthetic antioxidants (12). Various experimental evidence indicates involvement of oxygen free radical species (ROS) leads to Parkinson's disease (13). Free radicals are characterized by an unpaired number of electrons in atoms or groups of atoms and can be generated through the interaction of oxygen with certain molecules (14). These highly reactive radicals can start a chain reaction once they arise as free radicals. Because these radicals interact with essential biological components like DNA or the cell membrane, they represent a serious risk to the health of the cells (15). Oxidative stress in biological systems occurs when there is an imbalance in the production of oxygen free radicals (ROS) and the inherent capacity to effectively neutralize or detoxify these transient species, as highlighted by Gabriel Pazzino. Reactive oxygen species (ROS), comprising molecules such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals, naturally arise as by-products during cellular processes like metabolism. The body has defense mechanisms, including antioxidants, to regulate and counteract the effects of ROS. Antioxidant assay is a quick and reliable method; when it reacts with an antioxidant, the color changes from purple to yellow, indicating the scavenging of free radicals (16). *Eucalyptus citriodora* plants may possess an array of phytochemicals, such as phenolic and flavonoid compounds, recognized for their beneficial impact on antioxidant activity. Apart from the nitric oxide (NO), *Eucalyptus citriodora* serves as an indirect signalling molecule, engaging pathways that encompass the activation of enzymes such as nitric oxide synthase (NOS) and NADPH oxidases (17). Overexpression of these enzymes can lead to oxidative stress, and their association with conditions like Parkinson's disease and other illnesses has been explored. Apart from Nitric oxide, Hydrogen peroxide (H_2O_2) can also be generated within the peroxisomes (18). The catalase present in peroxisomes converts H_2O_2 into water, preventing its accumulation. However, if peroxisomes are compromised, experiencing damage or a reduction in enzyme activity, hydrogen peroxide is released into the cytosol. In the context of cells, particularly when reduced metals like (Fe^{2+}) H_2O_2 fenton reaction are present, a typically dangerous kind of ROS (19). Antioxidants extracted from plants play a crucial role in preventing cellular damage, thereby hindering the shared pathway associated with conditions like aging and various diseases. Various phyto extracts, oils, and formulations have demonstrated their significance as essential antioxidant agents, serving as valuable complements to the body's innate defense mechanism to prevent cellular damage, according to a study by (20). The total radical scavenging assay, measuring oxygen radical absorbance capacity (ORAC), was employed in the radical monocations of Phosphomolybdenum were generated by dissolving in water(7mM) and incorporating potassium persulfate, allowing it to stand in the dark at room temperature overnight. Before the assay, the radical solution was diluted with ethanol to achieve an absorbance at 734nm, as described by (21). The antioxidant properties were assessed through in vitro evaluations, including tests for free radical scavenging activity, Ferric reducing antioxidant power assay, and hydrogen peroxide, Phosphomolybdenum assay. In the current study, antioxidant assay was utilized to analyse the chemical composition of *Eucalyptus* essential oil. *Eucalyptus* essential oils also exhibit preservative qualities, extending the shelf life of food products and serving as a flavoring agent (22). Numerous studies highlight its effective antimicrobial properties, which are capable of inhibiting the growth of a diverse array of microorganisms (23). The essential oil is predominantly composed of both oxygenated and un oxygenated monoterpenes along with oxygenated sesquiterpenes, which sets the foundation for exploring its antimicrobial activity.

The study focuses on employing TLC to analyze the plant extract, aiming to identify and characterize the various compounds present. The presence of various bands in the TLC analysis suggests the complex nature of the *Eucalyptus citriodora* plant extract. The Rf values obtained for each band can be correlated with known standards. Column chromatography is a separation technique that is commonly used for isolating and purifying compounds from complex mixtures, such as those found in plant extracts (24). *Eucalyptus citriodora*, or lemon eucalyptus, contains various compounds, including citronellal, which is used in the fragrance industry (25). When there is an excessive amount of free radical formation, Fenton's reaction occurs, causing oxidative stress in the liver cells; this is why isolated goat liver cells were subcultures. The combination of hydrogen peroxide and iron(II) chloride, a compound containing ferrous ions (Fe^{2+}), results in the production of hydroxyl radicals(OH^-). Due to their exceptionally reactive nature, hydroxyl radicals have the potential to cause damage to DNA, proteins, and lipids within cells. This process can contribute to oxidative stress, leading to a simplified fenton reaction. Fe2+ react can indeed induce oxidative stress in biological systems, which includes isolated liver cells. Reactive oxygen species (ROS) are produced by Fenton–like reactions in this process. Because of their extreme reactivity, hydroxyl radicals may harm DNA, lipids, and Proteins within the cells. To cause oxidative stress in liver cells that have been isolated, scientists might include $Fecl_2$ into the growth medium. Controlling the dosage and exposure duration is of cells (26). Experiments to confirm oxidative stress induction frequently include appropriate controls and antioxidant testing. Ethical criteria for the use of experimental models, including isolated cells, can be followed in the study, and the length of exposure should be carefully evaluated.

2. MATERIAL AND METHODS

2.1 Materials

Fresh leaves of *Eucalyptus citriodora* were collected and air dried for a week. These leaves were collected from Kattigenahalli Lake. The plant species was authenticated and identified with accession number UASB 5652, from the University of Agricultural Sciences, Bengaluru.

2.2 Qualitative analysis of phytochemicals

The process described involves the extraction of oil from fresh plant material using a Soxhlet apparatus setup. The fresh plant material is cut into small pieces and places in a round bottomed flask with distilled water (27). The mixture is boiled in a cyclic leaf are collected and ground into fine powder, which is then filtered using a sieve filter. Water is removed with a rotary evaporator, and the Soxhlet apparatus setup Clevenger equipment is used to eliminate water vapor and prevent overpressure (Table 1).

The method for preliminary phytochemical screening followed the protocol outlined by Kokate in (28) and Harborne (29).

2.2.1 Mayer's test (Determination of Alkaloids)

A 2ml sample of the extract was combined with an equal volume of Mayer's reagent, a solution containing potassium iodide. The presence of alkaloids was identified through the observation of a precipitate, which could manifest as white, yellow, or cream-colored.

2.2.2 Libermann Burchard test (Steroid test)

Upon mixing 0.5 ml of the extract with 1 ml of acetic anhydride and 1 ml of strong sulphuric acid, the development of a violet to green precipitate was observed, indicating the potential presence of steroids,

2.2.3 Salkowski test (terpenoid test)

A mixture was prepared by combining 2 ml of chloroform, 1 ml of the extract, and adding a few drops of sulphuric acid. The emergence of a reddish –brown ring signified the presence of Terpenoids.

2.2.4 Alkaline reagent assay for flavonoids

The combination of 1 ml of the extract with a few drops of strong HCl and diluted ammonium solution revealed the presence of flavonoids, as evidenced by the development of a yellow precipitate.

2.2.5 Saponins test (Froth test)

Upon combining 1 ml of extract with 5 ml of distilled water and vigorously shaking the mixture, the formation of forth became evident, suggesting the presence of Saponins.

2.2.6 Lead acetate test, or the phenol test

The detection of phenols is signalled by the appearance of a precipitate when 1 ml of lead acetate solution is introduced to one ml of the extract.

2.2.7 Ferric chloride test, or tannin test

1 ml of the extract is mixed with an equal of ferric chloride solution, the indication of tannins becomes apparent as the color shifts to hues of blue, black or brownish green.

2.2.8 Keller killani test

Keller- Killani test for cardiac glycosides starts by adding distilled water to a milliliters of extract and allowing it to evaporate completely. The resulting dry sample is then combined with 2 ml of glacial acetic acid containing a small amount of ferric chloride solution. Along the inner walls of the tube, 1 ml of concentrated sulphuric acid is gently introduced. The appearance of a brown ring accompanied by a blue overlay signifies the presence of cardiac glycosides.

2.2.9 Amino acid testing (Ninhydrin test)

To test for the presence of amino acids, add 3-4 drops of Ninhydrin solution to a 1 ml sample and immerse it in a water bath for 10 minutes. The manifestation of a purple or blue color signifies the presence of amino acids.

2.2.10 Protein assay (Biuret test)

Combine 1ml extract with two drops of 1 % copper sulphate solution and 1 ml of 40 % sodium hydroxide solution. The emergence of a violet color indicates the presence of proteins.

2.2.11 Bradford test for carbohydrates

Mix 2 ml of the extract with 1 ml of Bradford's reagent, then heat the mixture in a water bath for a few minutes. The formation of a reddish- brown precipitate signifies the detection of carbohydrates.

2.2.12 Fehling's test: Sugar reduction method

In the sugar reduction method known as Fehling's test, equal amounts of Fehling solutions. A and B were introduced to a 1 ml extract. The observation of a brick-red precipitate indicates the existence of reducing sugars.

2.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The ferric reducing antioxidant power experiment, as described by Yildrim, Mavi, and Kara, entails blending samples with concentrations spanning 10 to 70 mg/ml with a phosphate buffer (0.1M, pH 6.5) and 1.25ml of potassium ferricyanide (2%weight/vol). Following this solution, incubation occurred at 50°C for 30 minutes. Subsequently, 1.25ml of tricholoroacetic acid(10%w/v) was introduced, followed by centrifugation at 1.25 ml of tricholoroacetic acid (10%w/v) was introduced, followed by centrifugation at 4000g for 10 minutes. After centrifugation, the supernatant was extracted, and the pellet was discarded. The resulting solution was combined with 1.25 ml of trichloroacetic acid (10%w/v) and left undisturbed for minutes. Absorbance was then measured at 700nm concerning the standard of ferric chloride and distilled water. An increase in OD (Optical density) in the reactant mixture indicated greater reduction activity. L-DOPA served as the positive control in this assay (30).

2.5 Hydrogen peroxide scavenging activity

Hydrogen peroxide (H_2O_2) scavenging activity was assessed using the method described by Ruch, Cheng, and Klaunig (1989). Various amounts of the oil (10-50mg/ml) and its components (10-70µg/µl) were added to a solution containing 0.6-40mmol/L of H_2O_2 in phosphate buffer (PO_4^{3-}) at pH 7.2. After 10 minutes of incubation, the absorbance at 230nm was measured using a sample in a buffer devoid of H_2O_2 included in the blank. L-DOPA served as the positive standard in this study (31).

2.6 Phosphomolybdenum Assay

The total antioxidant capacity was determined by reducing Mo (VI) to Mo (V) complex in an acidic pH environment. In this method, 0.3 ml of the extract was dissolved in methanol and mixed with 3 ml of a reagent solution comprising 0.6mol/L sulfuric acid, 28mmol/L sodium phosphate, and 4 mmol/L ammonium molybdate. The combinations were incubated at 70ºC for 90 minutes and then cooled to room temperature. The absorbance was then measured at 695nm. The overall antioxidant activity was measured in terms of equivalents of L-DOPA, ascorbic acid, and quercetin (32).

2.7 Thin Layer Chromatography

The TLC method is employed for the separation of non-volatile mixtures. The technique entails applying a thin layer of an absorbent substance onto a glass, plastic, or aluminum foil sheet. To facilitate separation, solvents are prepared using different ratios of ethyl acetate and hexane. Specifically, one solvent is prepared with 4:6 ratio ethyl acetate to hexane, another with 4:6 ratios, and a third with a ratio of 2:8. This approach allows for diverse elution conditions, contributing to the method's versatility in resolving complex mixtures. This technique is valuable for monitoring the progress of reactions, identifying compounds in a mixture, and assessing substance purity. A small quantity of the plant extract is applied to the sheet, and then the sheet is immersed in the solvent to separate its components (33, 34).

2.8 Cell cytotoxicity screening

To determine cytotoxicity, a modified micro culture MTT technique was used. Initially, liver cells were disassociated using a solution of 0.1% trypsin, 0.01% EDTA, and 0.025% glucose in PBS. After verifying cell viability and centrifugation, cells were planted at a density of 3×10^4 cells per well in a microplate reader plate and incubated for 24 hours at 37ºC with 5% CO2. After initial incubation, 100µl of different chemical concentrations were applied to the microliter plates. The plates were incubated at 37ºC for 24 hours with 5.0 % CO2. After incubation, remove the test solutions and add 100µl of MTT solution (5mg/10ml in PBS) to each well. The plates were then incubated for another four hours at 37°C in a 5% CO2 environment. After incubation, aspirate the supernatant and add 100µl of DMSO. The plates were gently shaken to dissolve the generated formazan, and the absorbance was measured with a microplate reader at a wavelength of 590nm, as described by Sanjay Patel in 2009. The percentage of cell viability was determined using the proper formula (35).

% Inhibition activity= 1-T/C×100

T= Absorbance of the test sample

C Absorbance of Control sample

2.9 Counting cell viability- Trypan blue staining

To assess cell viability using the Trypan blue exclusion technique, first make a cell suspension in a predetermined volume, typically 1 ml. Mix 10 µL of this solution with an equal volume of Trypan blue using a pipette to ensure proper mixing. Transfer the resulting mixture to a haemocytometer capable of distinguishing between alive and dead liver cells. Begin counting within 5 minutes of staining to minimize prolonged exposure and potential dye absorption by the cells. Apply a small quantity of the cell suspension and trypan blue stain to the haemocytometer and cover with a slip. Capillary action will draw the cell suspension behind the coverslip unless there is an air bubble. When placing the coverslip on the grid, take care not to overfill the wells and to maintain it stable. Position the haemocytometer onto the stage of an inverted microscope and fine-tune the focus and magnification settings until a solitary counting square occupies the field. Employ the prescribed formula, as outlined by Shen et al, to calculate both the number of cells per millimetre and the overall cell count (36).

Determine the percentage viability using the formula: % viability = (live cell count) × 100

3. RESULTS

3.1 Phytochemical screening

The methanolic extract obtained from *Eucalyptus citriodora* leaves underwent a thorough phytochemical screening, revealing the qualitative presence of various compounds. The analysis demonstrated the occurrence of Saponins, Quinone, carbohydrates, tannin, phenol, fat, and flavonoids, with the notable absence of proteins (Table 1).

Components	Methanolic	Aqueous	Ethyl acetate
Alkaloids	-	-	+
Carbohydrates	+	-	+
Cardiac glycosides	+	+	-
Flavonoids	+	+	+
Phenols	-	+	+
Phlobatanins	+	-	-
Amino acids and Proteins	-	+	-
Saponins	+	-	+
Tannins	+	-	+
Sterols	+	+	+
Terpenoids	+	-	+
Quinones	-	+	+
Oxalates	-	+	+

Table 1: Phytochemical Screening of *Eucalyptus citriodora*

(+) denotes presence of phytochemical compound (-) denotes absence of phytochemical compound

3.3 Ferric reducing antioxidant power assay

The antioxidant activity measured by the FRAP method was observed in all extracts. In general, the values of antioxidant activity of extracts of *Eucalyptus citriodora* were higher concerning dried samples. The highest values were 88.4± 0.10 and 120±0.23µM in methanolic extracts of *Eucalyptus citriodora*. It was observed that the antioxidant activity measured by FRAP was positively correlated to the concentration of the polyphenols. Only the methanolic extracts of dry samples were not positively correlated to the concentration of the polyphenols. The values obtained were the relatively low antioxidant activity of *Eucalyptus citriodora* with 132.20µM de $FeSO_4$/g (fig 1).

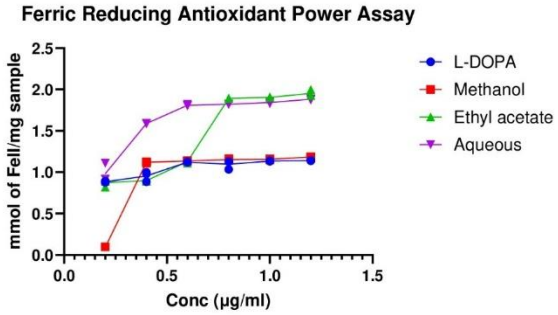

Fig 1. Ferric reducing antioxidant power for different concentration IC_{50} *Eucalyptus citriodora*

3.5 Hydrogen peroxide radical scavenging assay

The ability of the extracts to scavenge hydrogen peroxide was determined according to the method. A hydrogen peroxide (H_2O_2) scavenging assay performed using the methanolic extract was found to be 85.87 µg/ml. The results showed that methanolic extract possessed significantly higher hydrogen radical scavenging activity as compared with the L-DOPA drug (fig 2).

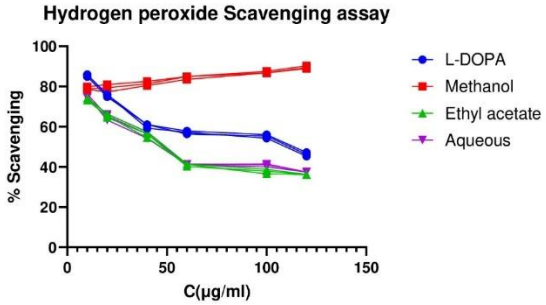

Fig 2. Hydrogen peroxide scavenging assay for different concentration IC_{50} *Eucalyptus citriodora*

3.6 Phosphomolybdenum scavenging assay

Phosphomolybdenum scavenging activity extract was measured using the reduction of Mo (VI) in Mo (V), leading to the formation of a complex Mo (V) of green phosphate. All determinations were performed in triplicates. The IC_{50} value of the methanolic extract was found to be 10.70µg/ml. The Phosphomolybdenum scavenging activity was considerably significantly greater than antioxidant activity as compared with the L-DOPA drug (fig 3).

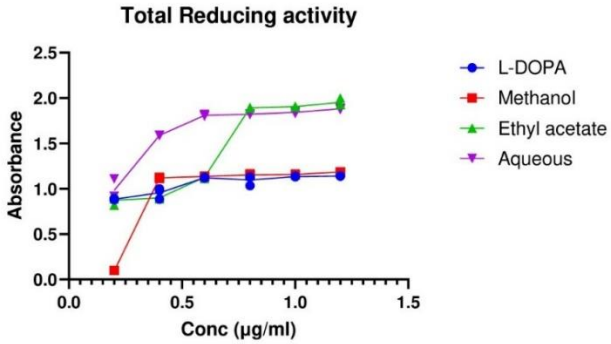

Fig 3. Total reducing activity Phosphomolybdenum assay for different concentration IC_{50} *Eucalyptus citriodora*

3.7 TLC analysis

TLC was conducted using a solvent of 40% ethyl acetate and 60% hexane. The resulting thin-layer chromatogram revealed an Rf value of 2.33, indicating the relative mobility of the compounds under the specified conditions. To improve the separation of *Eucalyptol*, a modified TLC solvent comprising 20% ethyl acetate and 80% hexane was employed. Despite this adjustment, the Rf value in the solvent system remained 5, suggesting that the incomplete separation of *Eucalyptol* have impacted the results (fig. 4).

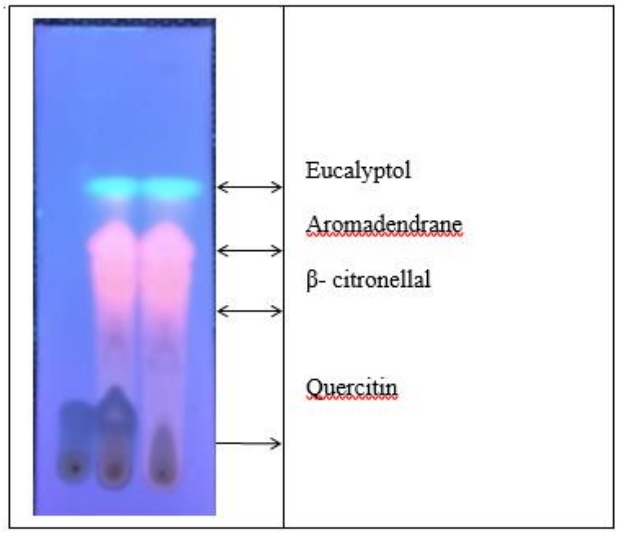

Fig 4. TLC profiles of the methanol leaf extract of *Eucalyptus citriodora* and reference standard Quercetin.

3.4 Cytotoxicity assay

Eucalyptus citriodora samples were distributed into four treatment groups: a control group (untreated) and three groups treated with different concentrations of *Eucalyptus citriodora* plant extract (5,10, 50 and 100 µg/ml). Additionally, a separate group was exposed to a constant concentration of 100 µg/ml of *Eucalyptus citriodora* for a 48-hour duration. To determine cell viability, the MTT assay was performed. The cells were seeded at a density of 0.315×10^{6}cells/ml in a cell culture medium and incubated for 4 hours at 37°C. After incubation, the reaction was stopped by adding 20 µl of dimethyl sulphoxide (DMSO) to each well for 15 minutes. The absorbance was then measured at 490 nm using a 96-well microplate reader. This experimental design allowed us to assess the effect of different doses of *Eucalyptus citriodora* plant extract on cell viability over a set period. The MTT test quantified cellular activity and responsiveness to the plant extract treatments. The cell cytotoxicity assay showed the viability of cell count as 85%, which was analysed by cell viability percentage (fig 5).

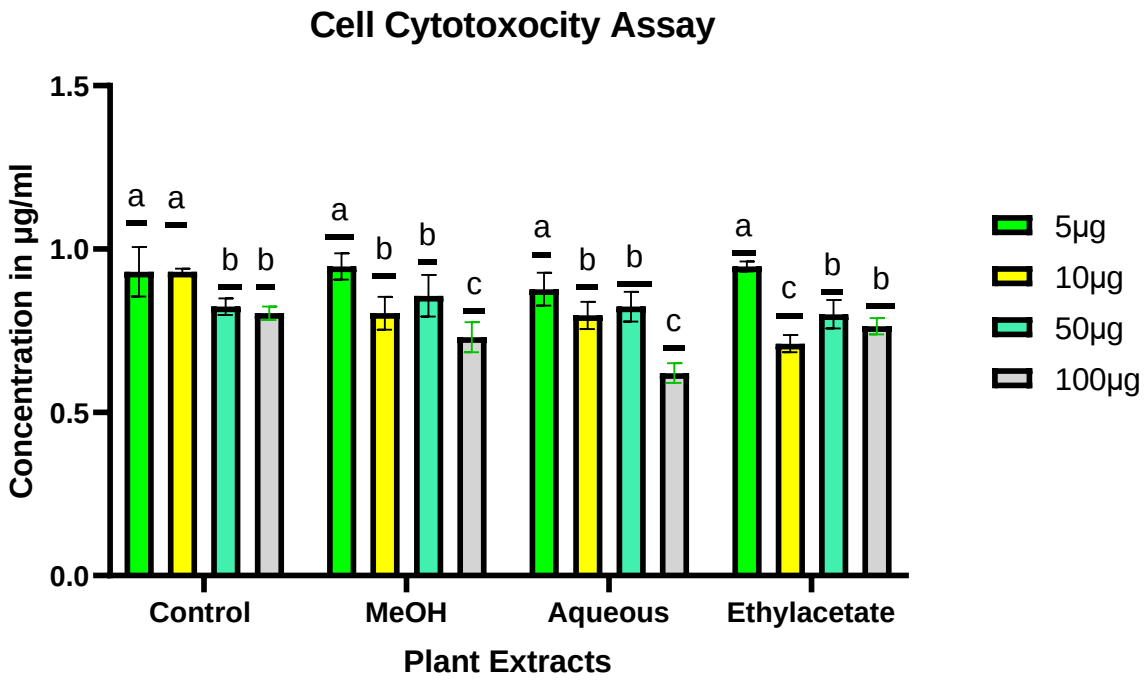

Fig 5. Comparison with control cells (viability) cells were compared among all groups using Tukey Kramer post hoc test to compare treated cells with control. Comparison of all groups using two-way ANOVA with multiple comparisons (Tukey Kramer post hoc) to compare with control group, p<0.001 value between different solvents.

4. DISCUSSION

Parkinson's disease, being the major neurological ailment affecting the motor neurons, has affected the population worldwide, leading to social and economic burdens. Our studies emphasize screening the phytochemical compounds of *Eucalyptus citriodora* acting as potential neuroprotection. The conventional methods of screening of these phytochemicals were carried out. A study of Babayi et al. ((37) found similar constituents in the methanolic extracts, except for quinone, flavonoids, tannins, and phenols when compared to their investigation of the methanolic extract. In their comparison with the ethyl acetate extract. Furthermore, comparing the *Eucalyptus* extract to the aqueous extract revealed the presence of carbohydrates, cardiac glycosides, flavonoids, phlobatanins, Saponins, Quinone's, and oxalates. When *Eucalyptus citriodora* extract was compared to the conventional medication L-DOPA, the methanolic extract showed greater activity. The total phenolic activity of *Eucalyptus citriodora* was analysed. When evaluating the activity, the methanolic extract outperformed the L-DOPA medication. In comparison to the total flavonoid content of *Eucalyptus citriodora*, the methanolic extract showed enhanced activity. The IC_{50} value for hydrogen peroxide scavenging was 52.78 µg/ml in the methanolic extract. The evaluation of the total reducing agents within the plant extract was found to be 88.28% in the methanolic extract. For nitric oxide scavenging assay and Phosphomolybdenum assay, statistics underscore the distinct antioxidant capacities between the essential oil and the broader plant extract, emphasizing the importance of considering the specific components and formulations when assessing antioxidant properties. After TLC, further optimization of the *Eucalyptol* may have impacted the results. Further optimization of the chromatographic conditions may be necessary for achieving better resolution and separation of *Eucalyptol* in future analyses. MTT assay was performed to know the cell viability of the compounds reacting to produce formazon compound. The cell viability of 3×10^4 cells was seeded to analyse the plant secondary metabolites of the *Eucalyptus citriodora*. The trypan blue exclusion method was used to analyse the number of viable cells in the suspension, and the Viability of the cells was analysed as 85% by analysing the percentage of cell viability. The significant findings showed the potential impact of the bioactive compounds derived from *Eucalyptus citriodora, which were* found to be effective in the neuroprotection of the brain. Similar observation were carried out by many researchers on active constituents from medicinal plants and their products (38-40).

5. CONCLUSION

Eucalyptus citriodora is a plant that has considerable medicinal significance and enjoys global use owing to its efficacy against bacteria and fungi. Extracts derived from the dried leaves of the Eucalyptus plant, obtained through a Soxhlet apparatus, demonstrated noteworthy antimicrobial and antioxidant properties. Two different extracts, methanolic and ethyl acetate, were obtained and submitted to qualitative and quantitative phytochemical analysis. The methanolic extract included cardiac glycosides, flavonoids, phlobatanins, saponins, quinones, and oxalates. The methanolic extract retained a higher concentration of phytochemicals from *Eucalyptus* compared to the ethyl acetate extract. This underscores the potential of *Eucalyptus* as a viable source of bioactive compounds, particularly in its methanolic form, showcasing significant phenolic content, flavonoids, and methanol, which was notable for antioxidant activity. Neurotransmitters play a crucial role in the communication between neurons. Modulating their activity can have implications for cognitive function and protection against neurodegenerative conditions. Eucalyptol has vasodilatory effects, meaning it can widen blood vessels. This could potentially boost blood circulation, leading to improved delivery of oxygen and nutrients to the brain. Adequate blood supply is critical for the overall health and neural tissue. Cytotoxicity of *Eucalyptus* extract using MTT assay provides valuable insights into the potential impact on cell viability. The experimental data revealed a concentration-dependent response, indicating that varying concentrations of the *Eucalyptus* extract influenced the cellular health of the tested cells. The observed response provides a basis for further exploration of the extract's impact on cellular mechanisms and warrants consideration in the context of potential therapeutic applications or precautions. However, it is essential to acknowledge the need for additional studies to elucidate the specific cellular pathways affected by the *Eucalyptus* extract and to establish a comprehensive understanding of its cytotoxic potential. Further investigations, including molecular and mechanistic studies, are recommended to unravel the underlying cytotoxic potential. Further research, including molecular and mechanistic studies, is needed to understand the underlying processes and examine the extract's safety profile.

These findings contribute to the growing body of knowledge regarding the biological effects of *Eucalyptus* extract and underscore the importance of cautionary considerations when utilizing it in various applications, particularly those involving direct contact with living cells. For the Nitric oxide scavenging assay, these statistical underscores the distinct antioxidant capacities between the essential oil and the broader plant extract, emphasizing the importance of considering the specific components and formulations when assessing antioxidant properties.

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CONSENT FOR PUBLICATION

Not applicable.

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