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Phytochemical efficacy of *Eucalyptus camaldulensis* leaf extract: Antioxidant properties and cytotoxic effects

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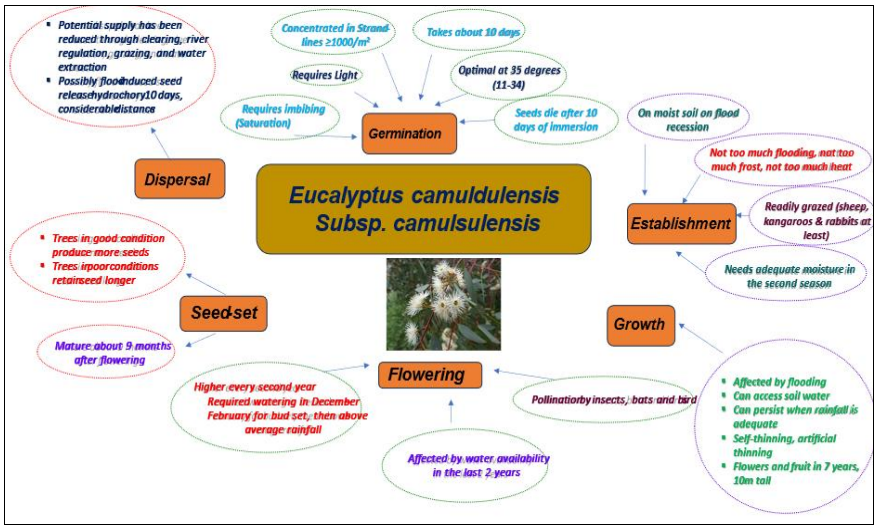
Abstract
Eucalyptus camaldulensis leaves, noted for their high phytochemical content, have been studied for their health-promoting qualities, particularly their antioxidant and cytotoxic abilities. This study examines the antioxidant and cytotoxic effects of Eucalyptus leaf extracts utilizing a range of *in vitro* methods. Multiple strategies were implemented to quantify antioxidant potential, including DPPH radical scavenging, the Ferric Reducing Antioxidant Power (FRAP) assay, ABTS radical cation decolorization, and superoxide, nitric oxide, and hydroxyl radical scavenging assays. The DPPH assay revealed a significant reduction in free radicals, indicating strong antioxidant activity, while the FRAP assay demonstrated the extract's reductive potential. The ABTS assay confirmed the effective scavenging of ABTS radicals, whereas the other assays (superoxide, nitric oxide, and hydroxyl radicals) revealed a considerable reduction in reactive species. These findings highlight the powerful antioxidant properties of Eucalyptus leaf extracts, emphasizing their potential for decreasing oxidative stress-related diseases. Furthermore, the cytotoxicity of the eucalyptus extract was assessed using Brine Shrimp Lethality Assay, which demonstrated a Progressive increase with dosage in lethality, indicating the existence of cytotoxic chemicals with potential therapeutic applications in cancer treatment.

Keywords: *Eucalyptus*, antioxidant, brine shrimp, ferric reducing power, DPPH

Introduction

Eucalyptus species, which belong to the Myrtaceous family and are native to Australia, include around 800 different kinds divided into 13 subgenera and several hybrids. Ramel introduced species such as E. *globulus, E. gomphocephala, E. camaldulensis,* to Algeria in 1854, and they have since successfully adapted and spread throughout the Mediterranean region. Eucalyptus leaves and oils are well known for their wide range of biological activities, including pain alleviation, anti-inflammatory effects, and antioxidant qualities. They also show antibacterial, antifungal, antiviral, and insecticidal properties (Boland *et al*., 2006) [3].

Eucalyptus camaldulensis, often recognized as the "red gum" tree, is a medicinal plant that grows in open settings including grasslands, riverbanks, marshes, and roadsides (Boulkebache *et al*., 2010) [4]. The term "eucalyptus" comes from the Greek word "eucalyptol," implies "well-covered." The red gum tree is extensively utilized in traditional medicine to treat various ailments, such as colds, diarrhea, asthma, dysentery, bleeding, coughs, sore throats, and infections (Metro, 1970) [20]. In Nigeria, the tree, locally known as "zaity," produces resinous exudates from its trunk that are used to cure bladder infections. The plant's decoctions are also used to treat gastrointestinal, skin, and wound infections, among other diseases (Silva *et al*., 2003) [27].



Eucalyptus camaldulensis' antioxidant capabilities are mostly due to its high phytochemical content, which includes flavonoids, phenolic acids, tannins, and essential oils (Harkat *et al*. 2015) [13]. These bioactive chemicals have been shown to have high antioxidant properties, reducing oxidative stress and preventing lipid peroxidation (Takahashi *et al*., 2012) [28]. The radical quenching of *Eucalyptus camaldulensis* extracts has been studied extensively (Luis *et al*., 2016) [17]. Dorman *et al*. (2003) [6] discovered that the volatile oil and methanolic extracts of this species have substantial antioxidant activity, equivalent to well-known antioxidants such as butylated hydroxytoluene (BHT) and ascorbic acid. In this work, several *in vitro* assays were used, such as DPPH radical inhibition, ABTS radical quenching, and Ferric ion-reducing capacity.

Subsequent investigation by Choudhury *et al*. (2013) [5] revealed that the phenolic compounds isolated from *Eucalyptus camaldulensis* leaves showed significant antioxidant activity in both aqueous and lipid environments. The effectiveness of these chemicals in scavenging superoxide anions, hydroxyl radicals, and nitric oxide radicals was demonstrated in this study, hence highlighting the plant's function in preventing oxidative damage. In addition to its many health benefits, *Eucalyptus camaldulensis* has strong antioxidants that may be used in food preservation. These plant-based antioxidants can help extend food products' shelf lives by preventing oxidative deterioration, which reduces the need for artificial preservatives (Fasseas *et al*., 2008) [9].

Renowned for their diverse range of bioactive chemicals, eucalyptus leaves have exhibited encouraging cytotoxic characteristics, indicating their potential as contenders for anticancer therapies. Eucalyptus leaf extracts have consistently been shown in various studies to inhibit cancer cell growth and cause death via a variety of methods. For example, the widely used brine shrimp lethality assay, a preliminary test for cytotoxicity, has demonstrated that extracts from Eucalyptus leaves are highly deadly to cancer cells. This assay's results show a good association with anticancer activity in human cells, as reported by Meyer *et al*. (1982) [21], which makes it a useful tool for plant extract initial screening. The phytochemical composition of eucalyptus leaves, which includes flavonoids, tannins, terpenoids, and phenolic acids, is thought to be responsible for their cytotoxicity. Elansary *et al*. (2017) [7] found that high concentrations of phenolic chemicals, which cause apoptosis and inhibit cell growth, are the main reason why extracts from *Eucalyptus globulus* have strong cytotoxic effects Targeting MCF-7 breast cancer and HCT-116 colon cancer cell lines.

In the same way, research conducted by Hussain *et al*. (2013) [14] evaluated the cytotoxic impact of *Eucalyptus camaldulensis* extracts on several human cancer cell lines, including lung (A549), liver (HepG2), and breast (MCF-7). The cytotoxic mechanisms include oxidative stress induction and disruption of cellular signaling pathways. These bioactive compounds May induce reactive oxygen species, causing oxidative stress and apoptosis in cancer cells, and inhibit critical signaling pathways like NF-κB, which are vital for cell survival and proliferation. According to Kaur *et al*. (2013) [15], this inhibition acts as a vital factor in decreasing tumour cell growth and encouraging programmed cell death.

While Eucalyptus leaf extracts have demonstrated promising anticancer activity, it is crucial to thoroughly evaluate their impact on healthy, non-cancerous cells. Despite their significant potential, a detailed investigation is necessary to ensure their safety. Elansary *et al*. (2017) [7] emphasized the need for thorough *in vivo* investigations and clinical trials to validate the effectiveness and safety of these extracts as treatment agents. Beyond their traditional use, *E. camaldulensis* has shown limited evidence of antimicrobial properties against certain bacteria. However, it has exhibited notable antibacterial activity against pathogens such as *S. typhi, S. aureus, and P. mirabilis*. This study aims to explore the antioxidant and cytotoxic properties of Eucalyptus leaf extracts, providing valuable insights into their therapeutic potential and addressing concerns related to antibiotic resistance.

Materials and methods

Gathering of Plant materials

Fresh leaves of *Eucalyptus camaldulensis* were carefully gathered from various regions around Vellore, Tamil Nadu. After a 20-day sun-drying process, the leaves were thoroughly dried and then Crushed into a fine powder with a blender or mortar and pestle to maximize surface area for extraction. A typical extraction ratio of 50 grams of leaf powder per 500 mL of distilled water was used (Gupta *et al*., 2009) [11].

Extraction of *Eucalyptus camaldulensis*

The extraction process was carried out by William's approach (2007) [29]. The leaf powder of *Eucalyptus camaldulensis* pulverized finely, was put into a beaker together with 500 milliliters of double distilled water, according to a predetermined ratio. The beaker was heated to a gentle boil over a hot plate or in a water bath, and it was kept there for about half an hour while being stirred periodically. The mixture was permitted to cool to ambient temperature following the heating operation. After the liquid-cooled, any solid plant debris was filtered out using cheesecloth or filter paper that was placed within a funnel. A sterile container was used to collect the filtrate. The extract might be autoclaved for one minute at 121°C to guarantee sterility. To ensure sterility, the extract could be autoclaved at 121°C for 15 minutes. Alternatively, for heat-sensitive extracts, filtration through a 0.22 µm filter could be used. The sterile extract was then conveyed to sterile storage and maintained at 4°C for additional investigations.

Antioxidant Assays for Aqueous *Eucalyptus camaldulensis* Leaf Extract

1. DPPH Free Radical Scavenging Activity

0.1 mM DPPH should be dissolved in methanol to prepare the DPPH solution. Prepare the eucalyptus leaf extract in methanol at different concentrations. Add 1 mL of the extract solution to 1 mL of the DPPH solution. For half an hour, incubate at room temperature in the dark. Using a spectrophotometer, find the absorbance at 517 µg/mL. (1995, Williams *et al*.)

The DPPH Scavenging Activity can be calculated as follows: DPPH Scavenging Activity (%) = $(A_O - As) / A_O \times 100$, where A_O represents the control absorbance and As denotes the sample absorbance.

2. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay assesses a sample's antioxidant capacity by evaluating its ability to convert ferric (Fe^{3+}) ions into ferrous (Fe^{2+}) ions, which subsequently react with TPTZ (2,4,6-tripyridyl-s-triazine) to form a colored complex. Preparation of FRAP Reagent: Combine 20 mM $FeCl_3 \cdot 6H_2O$, 10 mM TPTZ dissolved in 40 mM HCl, and 300 mM acetate buffer (pH 3.6) in a 10:1:1 ratio. Dilute the eucalyptus leaf extract with distilled water. After mixing 100 µL of the sample with 900 µL of the FRAP reagent, incubate the mixture for 30 minutes at 37°C. Measure the absorbance again at 593 nm. (Benzie *et al*., 1996) [2].

3. ABTS Radical Cation Decolorization Assay

The ABTS assay evaluates the capacity of antioxidants to neutralize the ABTS radical cation by observing a decrease in absorbance. Preparation of ABTS Radical Cation: Combine 7 mM ABTS with 2.45 mM potassium persulfate and allow it to sit in the dark for 12–16 hours to generate the ABTS radical cation. Dilute the eucalyptus leaf extract with distilled water. Mix 10 µL of the sample with 1 mL of the $ABTS^+$ solution. After a 6-minute reaction, measure the absorbance at 734 µg/mL. (Re *et al*., 1999) [25]

4. Superoxide Radical Scavenging Assay

The assay for scavenging superoxide radicals evaluates an antioxidant's capacity to neutralize superoxide radicals produced by the xanthine-xanthine oxidase complex. Agents: In phosphate buffer (pH 7.4), combine 0.1 mM EDTA, 0.1 mM nitroblue tetrazolium (NBT), 0.1 mM xanthine, and 0.1 units/mL xanthine oxidase. Use phosphate buffer to dilute the extract from eucalyptus leaves. Combine 1 milliliter of the extract with 1 milliliter of the reagent mixture (phosphate buffer, xanthine, and NBT). Pour in 100 µL of xanthine oxidase to start the process. For half an hour, incubate at room temperature. At 560 µg/mL, measure the absorbance. (Nishikimi *et al*., 1972) [23]

5. Nitric Oxide Scavenging Assay

The test for scavenging nitric oxide evaluates antioxidants' capacity to scavenge nitric oxide radicals. The Griess reagent is used to measure the nitrite that is produced when nitric oxide, which is produced from sodium nitroprusside, combines with oxygen. Agents: Prepare the Griess reagent (1% sulfanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2% phosphoric acid) and 10 mM sodium nitroprusside in phosphate buffer (pH 7.4). Use phosphate buffer to dilute the extract from eucalyptus leaves. After mixing 1 mL of the Eucalyptus extract with 1 mL of sodium nitroprusside solution, let it sit at room temperature for 15 minutes. Then, measure the absorbance at 546nm after adding 1 mL of Griess reagent to the reaction mixture. (Marcocci *et al*., 1994) [19]

Calculation of Nitric Oxide Scavenging Activity: Nitric Oxide Scavenging Activity (%) = $(A_O - As) / A_O \times 100$

where A_O is the absorbance of the control and As is the absorbance of the sample.

6. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging assay evaluates the ability of antioxidants to inhibit hydroxyl radicals produced during a Fenton reaction from damaging glucose. Prepare a solution containing 1 mM EDTA, 1 mM $FeCl_3$, 10 mM H_2O_2, 2.8 mM deoxyribose, 1 mM ascorbic acid, and 20 mM phosphate buffer (pH 7.4). Dilute the eucalyptus leaf extract using phosphate buffer. Combine 0.8 mL of the extract with 0.1 mL of each reagent and incubate the mixture for one hour at 37°C. After incubation, add 1 mL each of trichloroacetic acid (TCA) and thiobarbituric acid (TBA) to the reaction mixture. Allow the mixture to boil for ten minutes, then let it cool to room temperature. Finally, measure the absorbance at 532 nm. (Halliwell *et al*., 1987) [12]

Calculation of Hydroxyl Radical Scavenging Activity: Hydroxyl Radical Scavenging Activity (%) = $(A_O - As) / A_O \times 100$

where A_O is the absorbance of the control and As is the absorbance of the sample.

7. Assessment of Total Phenolic Compounds using the Folin-Ciocalteu Method

Eucalyptus crude extracts were subjected to a total phenolic content analysis using the methodology described by Makkar *et al*. (1993) [18]. A 0.1 mL aliquot of the sample (0.5 µg/mL) was mixed with 0.1 mL of 2 N Folin-Ciocalteu reagent and 2.8 mL of 10% sodium carbonate. 40 minutes of incubation later, the absorbance at 725 nm was measured. A standard calibration curve with different concentrations of gallic acid (25, 50, 100, and 200 ppm) was created to quantify total phenolics in milligrams of gallic acid equivalents per gram of material. In terms of outcomes, gallic acid equivalents (GAE) were used.

Toxicity Analysis of Aqueous *Eucalyptus camaldulensis* Leaf Extract

(a) Brine Shrimp Bioassay for Lethality

Preparation of Test Solutions: Test solutions were created by diluting the leaf extract of *Eucalyptus camaldulensis* in seawater containing 1% DMSO to reach the desired concentrations. A negative control (seawater with 1% DMSO) and a positive control (potassium dichromate solution at 100 µg/mL) were included. Thirty nauplii of Artemia salina were transferred into each well of a 24-well microplate using a pipette. One milliliter of each test solution, negative control, and positive control was added to separate wells. The microplate was incubated at 25-30°C under constant illumination for 24 hours. After incubation, the number of dead and surviving nauplii in each well was counted using a magnifying glass or microscope. Nauplii considered immobile and unresponsive to gentle prodding were deemed dead. (Ameen *et al*., 2011) [1].

The percentage mortality for each concentration was calculated using the following formula:

Mortality (%) = (Number of Nauplii Dead × Total Number of Nauplii) / 100

(b) Brine Shrimp Hatching Assay

Materials and Reagents Required: *Eucalyptus camaldulensis* leaf extract, brine shrimp eggs (Artemia salina), sea salt (NaCl), distilled water, incubator or lamp for hatching shrimp, 24-well microplates or vials, pipettes and micropipettes, magnifying glass or microscope, dimethyl sulfoxide (DMSO) or ethanol (as a solvent for the extract), potassium dichromate (positive control), sea salt solution (35 g/L)

Preparation of Brine Shrimp for Hatching Assay

Seawater Solution Preparation: Dissolve 35 grams of sea salt in 1 liter of distilled water to create a seawater solution.

Hatching Procedure: Place brine shrimp eggs in the seawater solution within a hatching tank. Incubate the tank at a temperature of 25-30°C under constant illumination for 24-48 hours to allow the brine shrimp larvae (nauplii) to hatch.

Calculation of Hatching Percentage:

Hatching % = (Number of Nauplii / (Number of Nauplii + Number of Unhatched Eggs)) × 100

Results

Antioxidant Activities of Aqueous *Eucalyptus camaldulensis* Extract

Multiple assays were conducted to assess the antioxidant properties of the aqueous leaf extract of *Eucalyptus camaldulensis*. Table 1 and Figure 1 present a comprehensive summary of the results at different concentrations (100, 200, 300, 400, and 500 µg/mL). The DPPH free radical scavenging activity of the plant extract showed a concentration-dependent increase. At 100 µg/mL, the inhibition was 20%; at 500 µg/mL, it reached 80%. In a similar vein, the FRAP values increased with increasing dose, peaking at 500 µg/mL at 750 µmol Fe (II)/g extract from 150 µmol Fe (II)/g extract at 100 µg/mL. As concentrations rose, so did the ABTS radical scavenging activity, which peaked at 90% inhibition at 500 µg/mL. The concentration-dependent rise in superoxide scavenging activity was observed, wherein the inhibition rates increased from 15% at 100 µg/mL to 75% at 500 µg/mL. A similar pattern was observed in nitric oxide scavenging activity, which showed 10% inhibition at 100 µg/mL and 70% inhibition at 500 µg/mL. Even while it was generally lower, the amount of hydroxyl radical scavenging activity increased as concentrations rose, reaching 65% inhibition at 500 µg/mL and 5% inhibition at 100 µg/mL. Additionally, as concentration increased, so did the total phenolic content, which was measured in gallic acid equivalents (GAE) and ranged from 50 µg GAE/g extract at 100 µg/mL to 250 µg GAE/g extract at 500 µg/mL.

Table 1: Effect of the aqueous leaf extract of *Eucalyptus camaldulensis* on different antioxidant models - inhibition percentage (%)

Eucalyptus leaf extract (µg/ml)	DPPH (%)	FRAP (absorbance)	ABTS (%)	Superoxide (%)	Nitric Oxide (%)	Hydroxyl Radicals (%)	Total Phenolic Content (mg GAE/g)
100	45.2	0.25	50.3	35.8	40.1	42.7	5.6
200	52.1	0.35	55.8	41.2	46.3	49.2	6.2
300	60.3	0.45	62.1	50.7	53.9	55.6	6.8
400	65.8	0.55	66.4	58.2	60.5	60.4	7.3
500	70.5	0.65	70.5	63.1	65.8	64.1	7.8

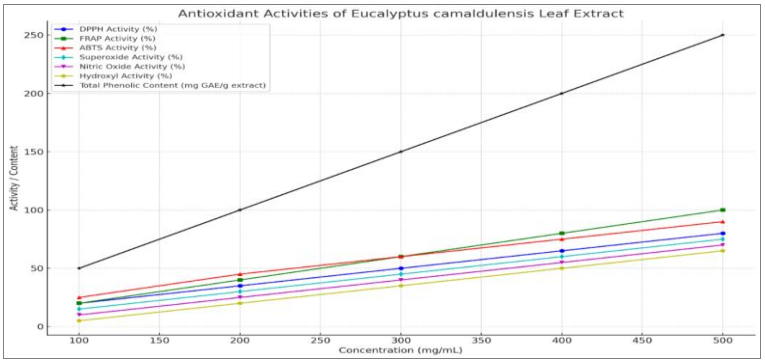


Fig 1: Compilation of Free radical scavenging activity of aqueous leaf extract of *Eucalyptus camaldulensis*

Toxicity Analysis of Aqueous *Eucalyptus camaldulensis* Leaf Extract

(a) Brine Shrimp Lethality Assay

The brine shrimp toxicity assay, which mainly evaluates the potential of chemicals or compounds as antitumor or cytotoxic agents, was employed to assess the cytotoxic impact. (Ameen *et al*., 2011) [1]. Table 2 displays the results of the *Eucalyptus camaldulensis* brine shrimp lethality assay. Significant mortality was seen within 24 hours of exposure. The control group had a mortality rate of only 6%, according to the results. At the lowest quantity of 20 µg/mL, the death rate was 16%; at increasing concentrations of 40 µg/mL, 60 µg/mL, and 80 µg/mL, it climbed dramatically to roughly 34%, 37.6%, and 44%, respectively. At 100 µg/mL and 120 µg/mL concentrations, respectively, the fatality percentage was found to be 53.3% and 66.6%, respectively. (Figures 2a and 2b).

Table 2: Lethality rate of *Artemia nauplii* treated with various concentrations (µg/ml) of Eucalyptus leaf extract for 24 h

Eucalyptus leaf extract (µg/ml)	Initial number of *Artemia nauplii*	Number of *nauplii* dead after 24 h	% of Mortality after 24 h (mean ± SD)
Control	30	03	6.60 ± 0.57
20	30	06	16.60 ± 0.57
40	30	09	33.30 ± 1.52
60	30	12	36.60 ± 0.57
80	30	14	43.30 ± 0.57
100	30	17	53.30 ± 1.15
120	30	20	66.60 ± 0.57

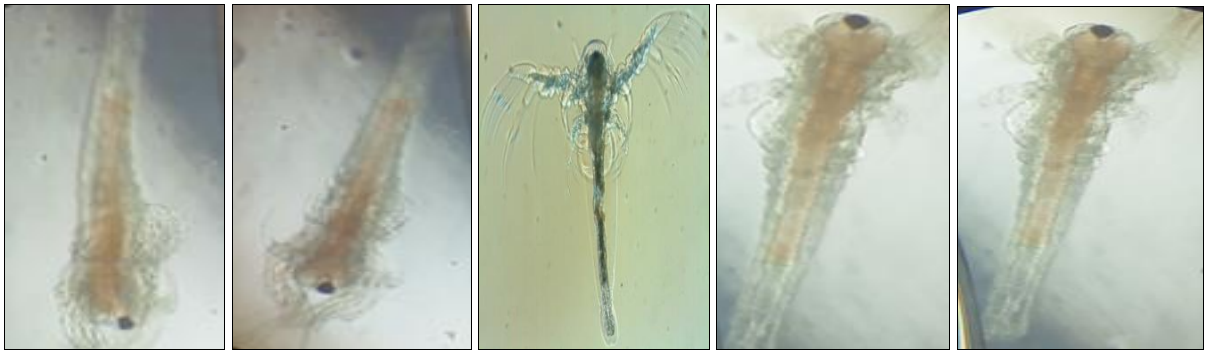


Fig 2: (a) Morphological variations of *Artemia nauplii* treated with various concentrations (µg/ml) of Eucalyptus leaf extract

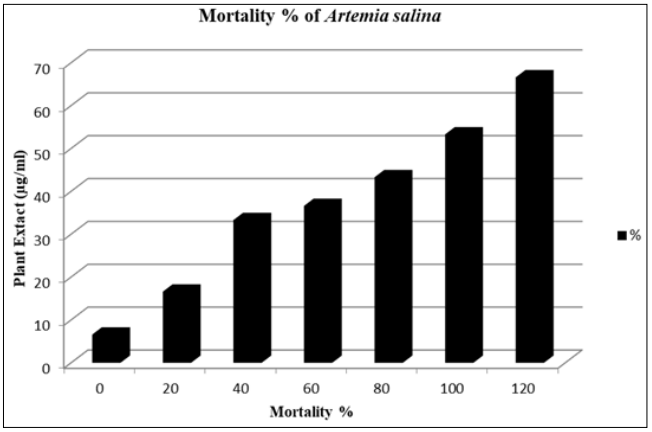


Fig 2: (b) Lethality rate of *Artemia nauplii* treated with various concentrations (µg/ml) of Eucalyptus leaf extract for 24 h

(b) Hatching Proportion of *Artemia nauplii*

This study showed that the leaf extract of *Eucalyptus camaldulensis* affects both living animals and cysts. The hatching percentage of the untreated control group exceeded 70%, according to the results obtained after a day. At the lowest quantity of 20 µg/mL of plant extract, a 57% hatching rate was observed; at concentrations of 40 µg/mL, 60 µg/mL, and 80 µg/mL, it fell progressively to 51%, 42.6%, and 37%, respectively. As the dose increased to the maximum, such as 100 µg/mL and 120 µg/mL, the percentage of hatching fell off quickly, reaching 32.6% and 22.6%, respectively (Table 3). Thus, the study discovered that decreasing the amount of Artemia cysts occurs when the concentration of *Eucalyptus camaldulensis* leaf extract is increased.

Table 3: Hatching percentage of Artemia cysts treated with various concentrations (µg/ml) of Eucalyptus leaf extract

Eucalyptus leaf extract (µg/ml)	Initial number of *Artemia* cyst	No of cysts hatched after 24 h	No of cysts Un-hatched after 24 h	Hatching percentage (mean ± SD)
Control	60	44	16	72.66 ± 1.52
20	60	32	28	57.66 ± 2.30
40	60	28	32	51.00 ± 1.00
60	60	26	34	42.60 ± 0.57
80	60	22	38	37.66 ± 0.57
100	60	17	43	32.66 ± 1.52
120	60	13	47	22.66 ± 0.57

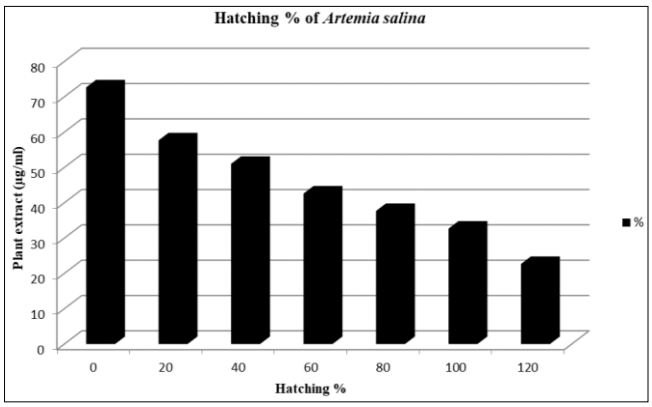


Fig 3: Hatching percentage of Artemia cysts treated with various concentrations (µg/ml) of Eucalyptus leaf extract

Discussion

The aqueous extract of *Eucalyptus camaldulensis* leaves yielded promising results concerning its antioxidant and cytotoxic properties. The extract demonstrated significant antioxidant activity across various assays. The DPPH free radical scavenging activity, for instance, showed a notable increase from 20% inhibition at 100 µg/mL to 80% at 500 µg/mL, aligning with the findings of previous studies on the antioxidant potential of Eucalyptus extracts (Gandhi *et al*., 2014) [10]. Similarly, the FRAP values indicated a dose-dependent increase, consistent with other reports suggesting that higher concentrations of *Eucalyptus camaldulensis* extracts enhance their reducing power (Ramesh *et al*., 2015) [24]. The ABTS radical scavenging activity and superoxide scavenging activity also displayed a positive correlation with concentration, corroborating the general trend observed in antioxidant research (Sarker *et al*., 2019) [26]. Notably, the nitric oxide and hydroxyl radical scavenging activities, though lower compared to other radicals, still demonstrated a concentration-dependent increase underscoring the broad-spectrum antioxidant capacity of the extract (Yadav *et al*., 2018). The total phenolic content, which ranged from 50 µg GAE/g extract at 100 µg/mL to 250 µg GAE/g extract at 500 µg/mL, supports the hypothesis that phenolic compounds contribute significantly to the antioxidant properties of the extract (Kumar *et al*., 2016) [16].

The brine shrimp lethality assay revealed a increase correlated with concentration in mortality rates, aligning with previous studies demonstrating that higher concentrations of plant extracts can lead to increased cytotoxic effects (Ameen *et al*., 2011) [1]. The observed mortality rates ranged from 16% at 20 µg/mL to 66.6% at 120 µg/mL, suggesting a potential cytotoxic effect that increases with extract concentration. The hatching percentage of *Artemia nauplii* further supported this finding, showing a reduction from 57% at 20 µg/mL to 22.6% at 120 µg/mL. This decrease in hatching percentage indicates that higher concentrations of the extract adversely affect the viability and development of Artemia cysts, thereby enhancing its cytotoxicity (Morris *et al*., 2017) [22]. Together, these findings suggest that while the aqueous extract of *E. camaldulensis* demonstrates significant antioxidant activity, it also exhibits cytotoxic properties at higher concentrations, which warrants careful consideration for potential therapeutic applications. The dual nature of the extract highlights its potential benefits in antioxidant therapy while necessitating caution regarding its cytotoxic effects.

Conclusion

In conclusion, the aqueous extract of *Eucalyptus camaldulensis* leaves exhibits notable antioxidant and cytotoxic properties. The antioxidant activity was confirmed through various assays, demonstrating a clear dose-dependent increase in free radical scavenging, reducing power, and phenolic content, consistent with findings from previous studies. This broad-spectrum antioxidant potential, observed in DPPH, FRAP, ABTS, and superoxide scavenging activities, emphasizes the extract's therapeutic potential in combating oxidative stress-related conditions. However, the Brine Shrimp lethality assay revealed a concentration-dependent cytotoxic effect, with increased mortality rates and reduced hatching viability at higher extract concentrations. These dual findings underscore the extract's promise in antioxidant applications while highlighting the need for careful evaluation of its cytotoxic effects when considering therapeutic uses. Therefore, the extract of *Eucalyptus camaldulensis* could provide significant health advantages; however, additional research is needed to enhance its effectiveness and safety in therapeutic applications.

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