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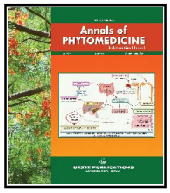

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Antioxidant and anticancer activity in leaf extracts of *Elaeocarpus serratus* (L.)

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Abstract

Research into new treatments and prevention measures is still needed since cancer is a serious worldwide health concern. In this work, we used a number of *in vitro* tests to find out the antioxidant and anticancer potential of ethyl acetate, chloroform, ethanol, and aqueous extracts from *Elaeocarpus serratus* (L).

Fresh leaves of *E. serratus* were gathered in January from Sugandhagiri in the Wayanad district of Kerala. For antioxidant activity assessment, the ethyl acetate extract exhibited remarkable IC_{50} values of 54 µg/ml and 59 µg/ml in DPPH and ABTS radical scavenging assays, respectively, surpassing the ethanol, chloroform, and aqueous extracts. In the case of anticancer activity, the ethyl acetate extract showed the highest efficacy against MCF-7 cells, as evidenced by an IC_{50} value of 196.46 µg/ml. While the standard drug, doxorubicin, exhibited superior anticancer activity with an IC_{50} value of 4.54 µg/ml, the ethyl acetate extract's performance is noteworthy and highlights its potential as an anticancer chemical resource. In conclusion, this study's findings suggest that *E. serratus* leaves have anticancer and antioxidant properties. This study lays the groundwork for more research into the bioactive substances detected in the ethyl acetate extract, which might have relevance for the creation of innovative medicines and goods high in antioxidants.

1. Introduction

In 2018, approximately 9.6 million fatalities were linked to cancer, establishing it as the 2nd leading global reason of death. Cancer is the main root cause of nearly one in six deaths on a global scale, with nations having low and moderate incomes witnessing the loss of almost 70% of their populations to this disease (Weiss *et al*., 2021). The ongoing progress in technology and a deeper comprehension of neoplastic conditions are opening avenues to mitigate the mortality rate attributed to cancer through the development of novel medications. Cancer develops across several stages, changing from a condition of precancerous lesion to one of malignant tumour state, as normal cells transform into tumour cells (Nelson *et al.,* 2020). Since the inception of ancient medicine, substances extracted from plants have been employed in the treatment of human ailments.

Over the last three decades, there has been a growing focus on natural products for their potential as innovative agents for both preventing and treating cancer. Simultaneously, mounting evidence supports the capacity of compounds derived from plants to inhibit different stages of tumorigenesis and associated inflammatory processes. This underscores the significance of these products in the realms of cancer prevention and therapy (Sujatha *et al.,* 2023).

The continued prevalence of traditional medical systems can be attributed to multiple factors. These include population growth, limited drug accessibility, the high costs of treatments, adverse effects linked to artificial drugs and the development of resistance to medications widely used for infectious diseases. As a result, there is a growing emphasis on harnessing the medicinal properties of plant materials to address a wide range of human ailments (Manasa *et al.,* 2022; Kriker *et al.,* 2021; Hemalatha *et al*., 2023). Medicinal plants serve as abundant reservoirs of bioactive compounds. The increasing interest in herbal medicine prompts researchers to explore and identify the bioactive compounds present in these plants, given their notable effectiveness in the field of medicine (Anvitha *et al.,* 2023).

The genus *Elaeocarpus*, in the family *Elaeocarpaceae*, contains tropical and continental deciduous trees and shrubs. Its 350 distinct species range from Madagascar in the southwest to New Zealand in the east.

This species can be found distributed in Southeast Asia, Southern China, Japan, Australia, Malaysia, Fiji, New Guinea and Hawaii. There are approximately thirty to forty-five species native to the Indian subcontinent, of which *E. serratus* is one of the most notable due to its medicinal and commercial importance (Baruah *et al*., 2019). The *E. serratus* fruit tree grows up to 8 to 15 meters tall with a canopy spread of 5 to 10 meters. It reaches full fruit in January after flowering from September to October. The berries are spherical or ovoid, 4 to 5 cm long, with stone seeds enclosed in a brittle shell. The ripe fruit of *E. serratus* has a unique scent and sweet and sour taste, which can make them attractive as table fruit in tropical climates (Raji *et al*., 2021). The plant is rich in various compounds, including nutrients, carotenoids polyphenolic elements (condensed tannins, flavonoids., *etc*.), and carbs. Phenolic compounds found in plant materials are recognized for their diverse biological benefits for human health (Tsui *et al*., 2021). Moreover, the leaves of *E. serratus* contain bioactive substances that support its historic usage for a variety of illnesses, including acids, ester compounds, alcohols, petroleum products, aromatic compounds, alkenes and amino acids (Geetha *et al*., 2013).

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This work aims to investigate the possible antioxidant and anticancer activities of different leaf extracts derived from *E. serratus*.

2. Materials and Methods

2.1 Sample collection

The fresh leaves of *E. serratus* were gathered in the month of January from Sugandhagiri, Wayanad district, Kerala. Their scientific authenticity was confirmed by Dr. Sreekumar V.B, Senior Scientist, Forest Botany Department, KFRI (Kerala Forest Research Institute), Peechi, Thrissur Kerala. The specimen is accessed to KFRI herbarium (KFRI Herbarium Accession Number 1835). After the plant specimens were properly cleaned by flowing freshwater to get rid of any remaining dirt, they were wrung dry in the shadow for approximately two weeks. They were then crushed into a powder and kept in a sealed bag for further research.

2.2 Extraction

Plant samples consisting of powdered leaves (100 g) are utilized in 500 ml of successive solvent extraction with increasing polarity like chloroform, ethyl acetate, ethanol and water. Initially, the powdered leaves will be defatted with petroleum ether. After that, it spends 48 h in an orbital shaker. A rotary flask evaporator will be used to concentrate the extract once the supernatant has been collected and filtered using Whatman No. 1 filter paper. After precise weighing, the dehydrated extract will be kept in vials at –20°C and utilized for the *in vitro* antioxidant and *in vitro* anticancer research.

2.3 *In vitro* antioxidant study

2.3.1 Total antioxidant capacity assay (TAC)

The phosphomolybdenum technique was used to evaluate the total antioxidant capacity of sample extracts according to a predetermined protocol. To be precise, 3 ml of reagent solution, consisting of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate, was added to 0.3 ml of the extract. Tubes containing the reaction solution were incubated for 90 min at 95°C, according to the instructions of Prieto *et al*. (1999) and Deepika *et al*. (2018).

After the solution cooled to room temperature, the absorbance was measured at 695 nm using a UV-VIS spectrophotometer in comparison to a blank. The blank utilized 0.3 ml of solvent in place of the extract. The TAC was quantified in terms of the gram equivalent of ascorbic acid. Different ascorbic acid concentrations (1000, 500, 250, 125, 62.5, and 31.25 µg/ml) were combined with the solvent to create a calibration curve.

2.3.2 $ABTS^{\bullet+}$ decolorisation assay

To create the $ABTS^{\bullet+}$ radical operating solution (Suseela *et al.,* 2010), mix 9.5 ml of ABTS (7 mM) with 245 µl of potassium persulfate (100 mM) and dilute the mixture to 10 ml with pure water. The resultant solution was diluted using potassium phosphate buffer (0.1 M, pH 7.4) after being exposed to the dark for 18 hours at room temperature unless the absorbance of 0.70 (± 0.02) at 734 nm was attained. Methanol was used to produce samples at different dilutions (µg/ml). 10 µl of the specimen and 2.99 ml containing the $ABTS^{\bullet+}$ radical solution for use were well mixed in a test tube. At 734 nm, the absorbing capacity of the resultant clear combination was measured. The proportion of antioxidant activity in the sample was determined using the formula below:

$$\% \text{ Inhibition} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where, Abs control is the absorbance of $ABTS^{\bullet+}$ radical in methanol; Abs sample is the absorbance of $ABTS^{\bullet+}$ radical solution. The sample was replaced with 10 µl methanol to create the control (Das *et al.,* 2023).

2.3.3 FRAP assay

The ferric ion-reducing antioxidant power (FRAP) reagent was created by combining 25 ml of acetate buffer (30 mM; pH 3.6), 2.5 ml of TPTZ solution (10 mM), and 2.5 ml of ferric chloride solution (20 mM). Before being applied, this mixture had been exposed for 15 min at 37°C. In this test, ascorbic acid was used as the standard. Its calibration curve was created using values of 10 µg - 50 µg in fresh water.

For the experiment, 2.85 ml of the FRAP reagent was introduced to glassware containing the standard or the specimen extract (made in ethanol) at various concentrations. The mixture was incubated for 30 min in the dark, and then the absorbance at 593 nm was measured. Rather than the plant specimen, the blank was an equivalent amount of methanol. Ascorbic acid equivalents (AAE) in micrograms per gram were used to express the results (Irshad *et al*., 2012).

2.3.4 DPPH assay

Reagent preparation: To make 0.1 mM DPPH solution, 4 mg of DPPH was dissolved in one hundred millilitre ethanol. Working procedure: Various sample extract volumes were adjusted to 40 µl with DMSO, and 2.96 ml of DPPH solution (0.1 mM) was introduced. The entire reaction mixture underwent incubation in a dark environment at room temperature for 20 min. Following the 20 min incubation, the absorbance of the mixture was measured at 517 nm using a UV-Vis Spectrophotometer. A control was established using 3 ml of DPPH (Deng *et al.,*2011).

$$\% \text{ RSA} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100$$

where, the absorbance of ethanol + DPPH radical is known as Abs control. The absorbance of the DPPH radical + extract of the sample is known as the Abs sample.

2.4 *In vitro* anticancer studies

The MCF-7 human breast cancer cell line was initially supplied by the NCCS in Pune, India. It was then grown in Dulbecco's modified Eagles medium (DMEM) obtained from Sigma Aldrich in the USA. The cell line was maintained in a 25 cm^2 culture chamber flask with DMEM enhanced with 10% FBS, L-glutamine, and sodium bicarbonate (Merck, Germany) and an antibiotic solution containing penicillin (100 u/ml), streptomycin (100 µg/ml), and amphotericin B (2.5 µg/ml). The cultivated cell lines were maintained at 37ºC in a humidified incubator with 5% CO_2 (NBS Eppendorf, Germany). To see the cells' viability up close, the MTT test method and an inverted phase-contrast microscope were employed. Trypsinizing a two-day-old consolidated overlay of cells, suspending it in a 10% growth medium, and seeding 100 µl of the cell solution (5×10^3 cells/well) into a 96-well cell culture plate. After that, the plate is incubated in a hydrated 5% CO_2 chamber at 37°C (Jayanthi *et al.,* 2022).

Using a cyclomixer, 1 mg of the material was weighed and processed in 1 millilitre of 0.1% DMSO to create the compound stock. To ensure sterility, the fluid was passed over a 0.22 µm millipore syringes filter.

After 24 h, the growth material was removed in order to serially dilute newly produced compounds in DMEM for anticancer evaluation. This was done using a two-fold dilution approach (100 µg, 50 µg, 25 µg, 12.5 µg, and 6.25 µg in 500 µl of DMEM). The matching wells were filled with three duplicates of each concentration (100 µl), and the mixture was then allowed to incubate at 37ºC in an incubator with 5% CO_2 that was hydrated. Untreated control cells were retained as well. Using an inverted phase-contrast tissue culture microscope (Olympus CKX41 with an Optika Pro5 CCD camera), direct microscopic observation was used to perform anticancer experiments (Jayanthi *et al.,* 2022).

Cytotoxicity was suspected by morphological alterations such as cell rounding or shrinkage, granulation, and vacuolization in the cytoplasm. An MTT test was also carried out. All test and cell control wells received 15 mg of MTT (Sigma, M-5655) that had been reconstituted in 3 ml PBS, filter-sterilized, and added. The supernatant was discarded after the 4 h incubation period, and MTT solubilization solution (DMSO, dimethyl sulphoxide, Sigma Aldrich, USA) was then added. The absorbance values at 540 nm wavelength were measured using a microplate reader.

Using the formula, the proportion of inhibition of growth was determined:

Percentage viability =

$$\frac{\text{Mean absorbance of samples}}{\text{Mean absorbance of control group}} \times 100$$

3. Results

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenyl-1-picrylhydrazyl) tests are commonly used techniques for determining the antioxidant activity of substances or extracts (Conforti *et al*., 2008). These tests offer important insights into the capacity of various compounds to scavenge free radicals and shield cells from oxidative damage. The DPPH assay is based on the principle of measuring the capacity of a substance (often plant extracts or antioxidants) to donate hydrogen atoms or electrons to the stable free radical DPPH. When DPPH radicals encounter an antioxidant, they become stable and change colour from purple to yellow, allowing for the quantification of the antioxidant's effectiveness. The lower the IC_{50} (half-maximal inhibitory concentration) value, the higher will be the antioxidant activity of the tested substance. Lower IC_{50} values indicate more effective scavenging of DPPH radicals.

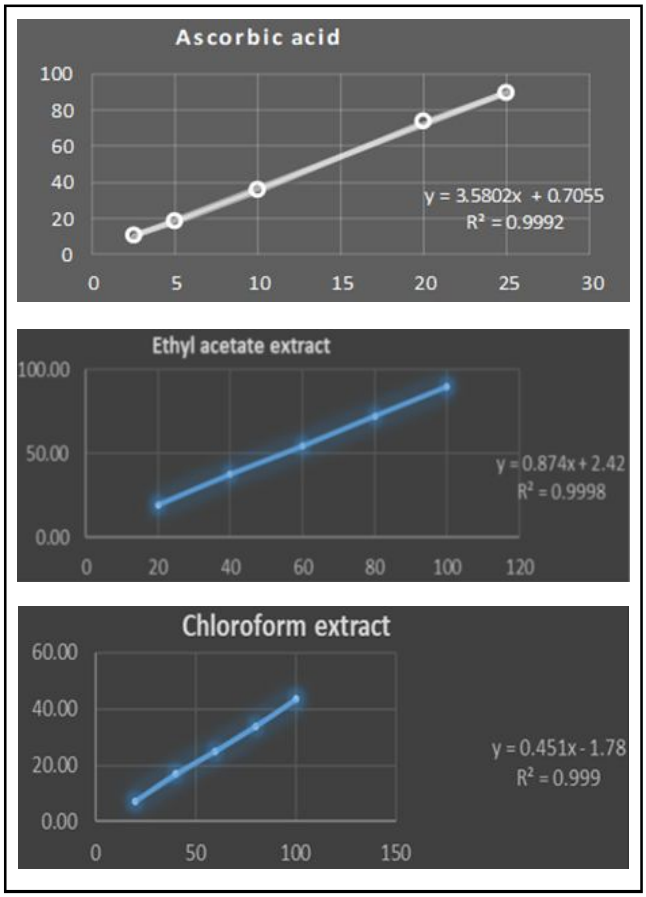
The basic principle of the ABTS Assay (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay) is the antioxidants' ability to lower the ABTS radical cation. The colour changes from green-blue to colourless as a result of this decrease, and the absorbance difference is used to measure how well the antioxidant can destroy free radicals. ABTS assay is considered to be more biologically relevant than DPPH because the ABTS radical cation is closer in structure to radicals encountered in biological systems. It can be applied to a variety of compounds and samples, including foods, beverages, and plant extracts. As with the DPPH assay, a lower IC_{50} value in the ABTS assay indicates a higher capacity to scavenge ABTS radicals and, therefore, stronger antioxidant activity.

Table 1 presents the results of the DPPH and ABTS assays for ethanol, ethyl acetate, aqueous, and chloroform extracts of *E. serratus* leaves, as well as for the standard ascorbic acid. Each extract's IC_{50} value is compared to that of ascorbic acid to determine the antioxidant activity of that extract.

Table 1: DPPH and ABTS inhibitory effects of various extracts of *E. serratus* leaves and ascorbic acid

Extracts	IC_{50} (µg/ml)	
	DPPH assay	ABTS assay
Ethyl acetate	54	59
Ethanol	68	71
Aqueous	110	119
Chloroform	115	124
Ascorbic acid	13.85	14.34

Among the several extracts tested, the ethyl acetate extract of *E. serratus* leaves exhibited the highest antioxidant activity in both the DPPH and ABTS assays. In the DPPH assay, the IC_{50} value for the ethyl acetate extract was 54 µg/ml, which was significantly lower than the IC_{50} values for the other extracts. This suggests that the ethyl acetate extract is highly effective at scavenging DPPH radicals and has strong antioxidant properties. These findings are graphically illustrated in Figure 1.



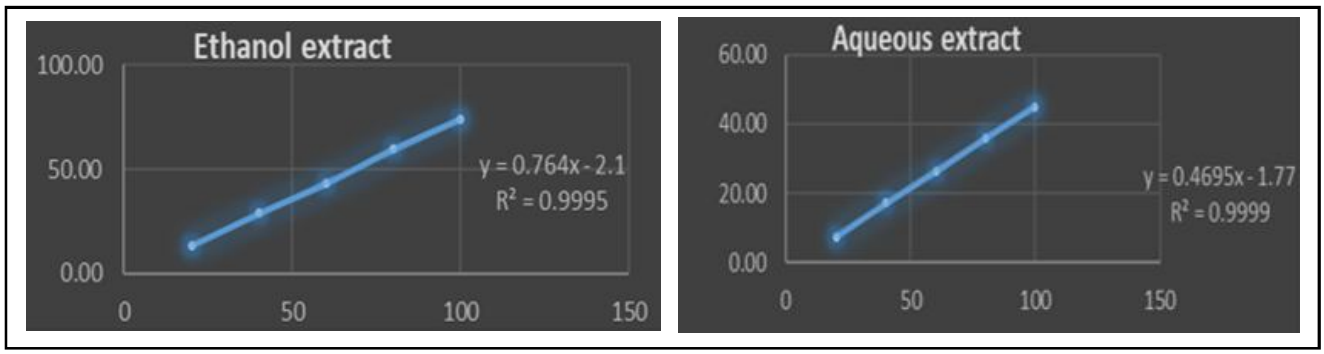


Figure 1: DPPH inhibitory assay for different extracts of *E. serratus* leaves.

Similarly, in the ABTS assay, the ethyl acetate extract also displayed the highest antioxidant activity with an IC_{50} value of 59 µg/ml. Once again, this result surpasses the IC_{50} values of the other extracts and is quite close to the IC_{50} value of ascorbic acid, indicating the remarkable antioxidant potential of the ethyl acetate extract as shown in Figure 2.

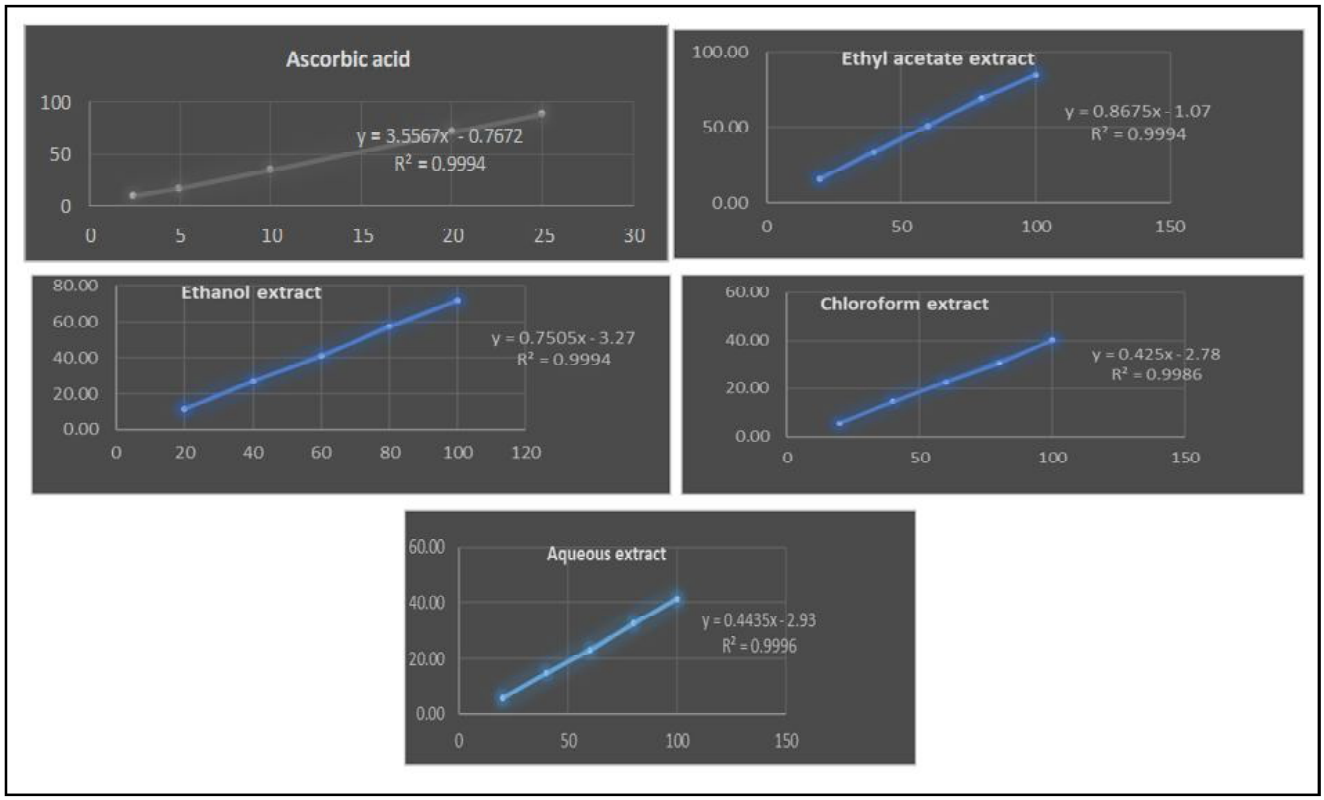


Figure 2: ABTS inhibitory assay of different extracts of *E. serratus* leaves.

The ferric reducing antioxidant power (FRAP) method is a widely used assay for evaluating the antioxidant capacity of various compounds and extracts. In this study, the presence of antioxidants in the samples resulted in the reduction of the Fe^{3+}/ferricyanide complex to the blue ferrous form. The extent of this reduction is a measure of the antioxidant capacity of the samples. The results are expressed in terms of milligrams of ascorbic acid equivalents (AAE) per mg of extract.

As seen in Figure 3, all values demonstrated a rise with increasing concentrations. Notably, the ethyl acetate extract displayed the highest reducing ability among the four extracts. This suggests that the ethyl acetate extract is particularly effective at reducing Fe^{3+} ions and, thus, has a strong antioxidant capacity.

In addition to the FRAP assay, the total antioxidant capacity (TAC) assay was employed in this study. The TAC assay is based on the reduction of phosphomolybdate ions in the presence of an antioxidant, leading to the formation of a green phosphate/MoV complex. This complex is then measured spectrophotometrically.

The TAC results, also shown in Figure 3, indicate that the ethyl acetate extract showed the highest total antioxidant activity, with an equivalent value of 376 milligrams of ascorbic acid per mg. It was followed by the ethanol extract with 290 milligrams of equivalents,

the aqueous extract with 185 milligrams of equivalents, and the chloroform extract with 168 milligrams of equivalents.

The findings underscore the exceptional antioxidant potential of the ethyl acetate extract, which consistently outperformed the other extracts in both the FRAP and TAC assays. These results emphasize the relevance of the choice of solvent in the extraction process and suggest that the ethyl acetate extract may hold promise for various applications where potent antioxidants are desired, such as in the development of natural health products.

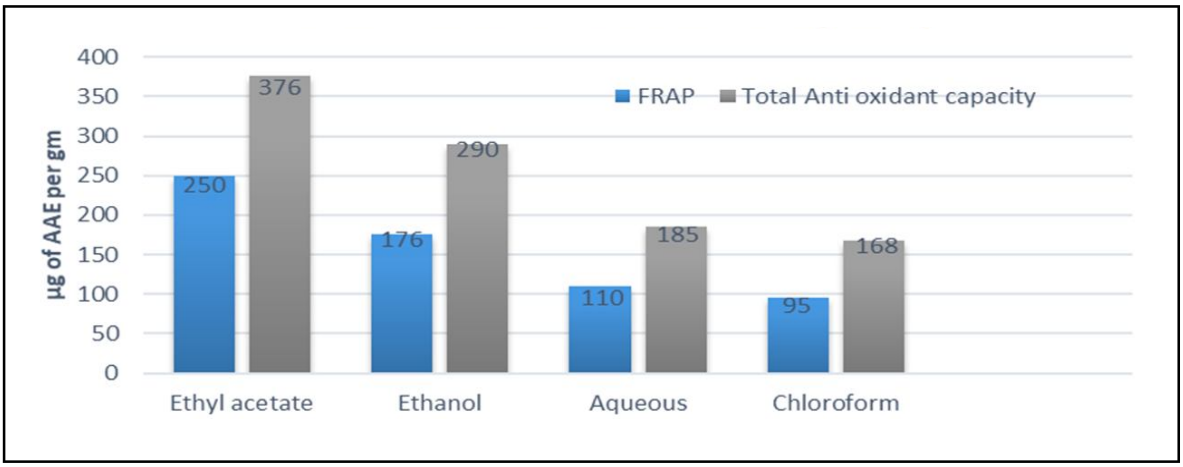


Figure 3: FRAP and total antioxidant capacity for different extracts of *E. serratus* leaves.

Table 2: IC_{50} values of several extracts of *E. serratus* leaves and doxorubicin by MTT assay method

Samples	IC_{50} (µg/ml)
Ethyl acetate	196.46
Ethanol	235.41
Chloroform	254.02
Aqueous	412.92
Doxorubicin	4.54

The anticancer properties of several extracts from *E. serratus* leaves were assessed in the MTT test and compared with the reference drug doxorubicin. The cell line MCF-7 was selected for the *in vitro* anticancer study. IC_{50} values of extracts and standard drugs are given in Table 2.

The results indicate that all the extracts from the plant exhibit anticancer activity. Figure 4 illustrates on the anticancer activity of ethyl acetate extract through the MTT assay. Figure 5 represents the anticancer impact of ethanol extract through the MTT assay. In Figure 6, the anticancer impact of chloroform extract through the MTT assay can be depicted.

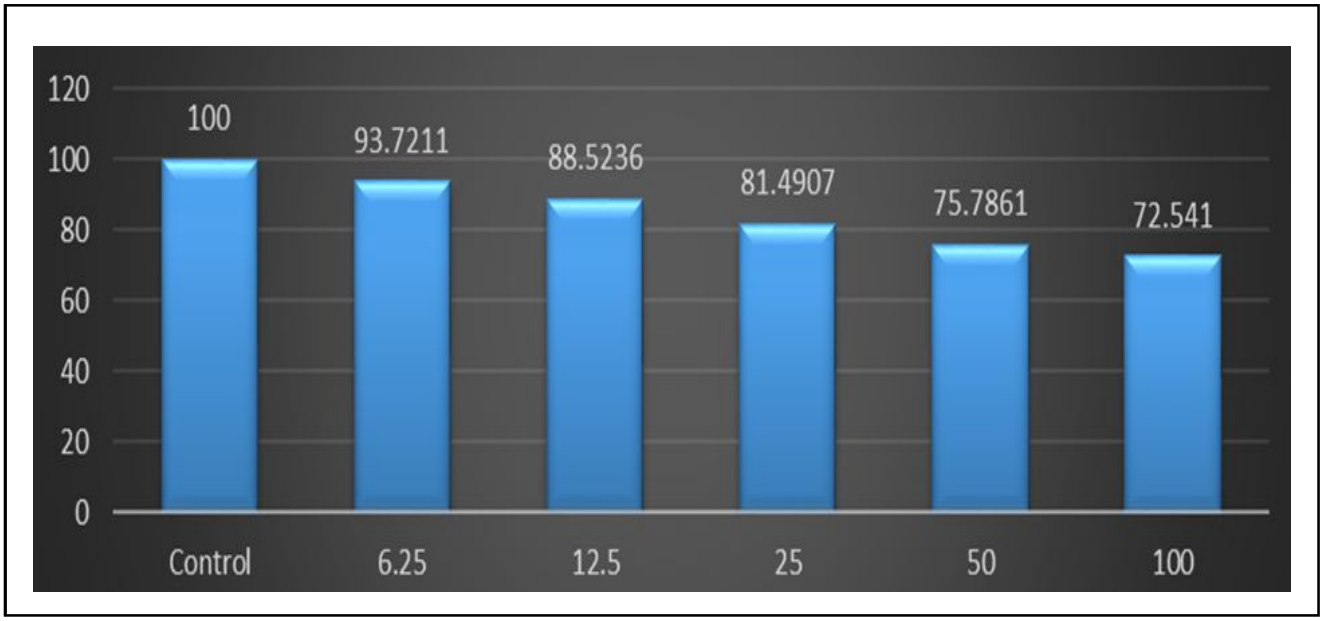


Figure 4: The graphical illustration demonstrates the anticancer effect of the ethyl acetate extract using the MTT assay. Percentage viability is plotted on the Y-axis, while different concentrations of ethyl acetate are indicated along the X-axis.

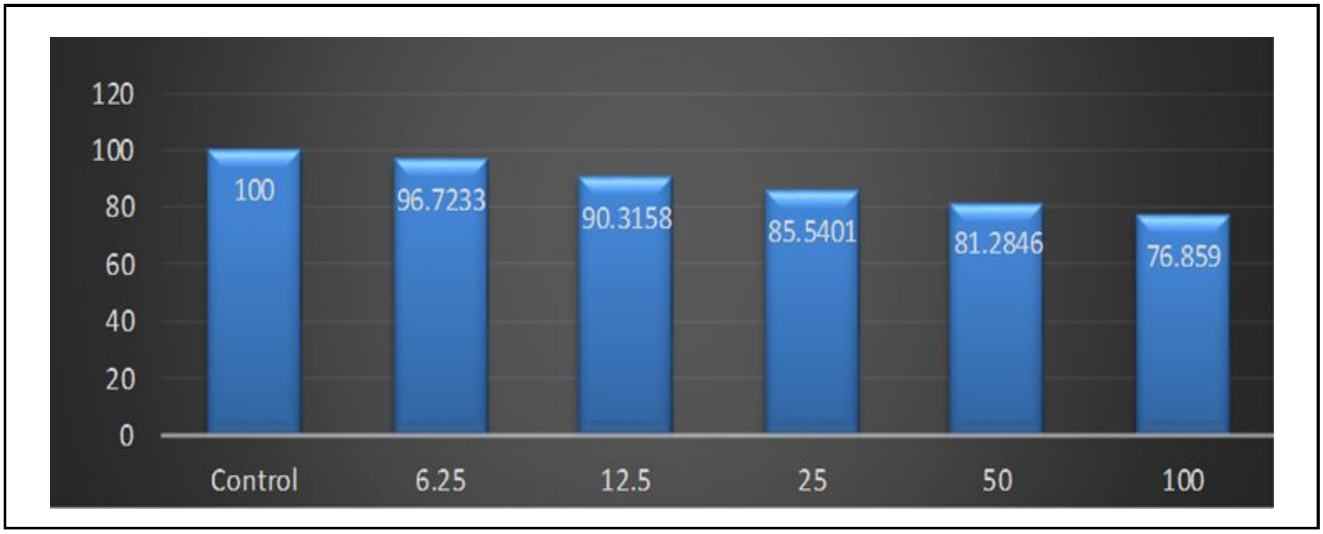


Figure 5: Graphical representation illustrating the anticancer impact of ethanol extract through the MTT assay. Percentage viability is plotted on the Y axis, and different concentrations of ethanol are shown along the X axis.

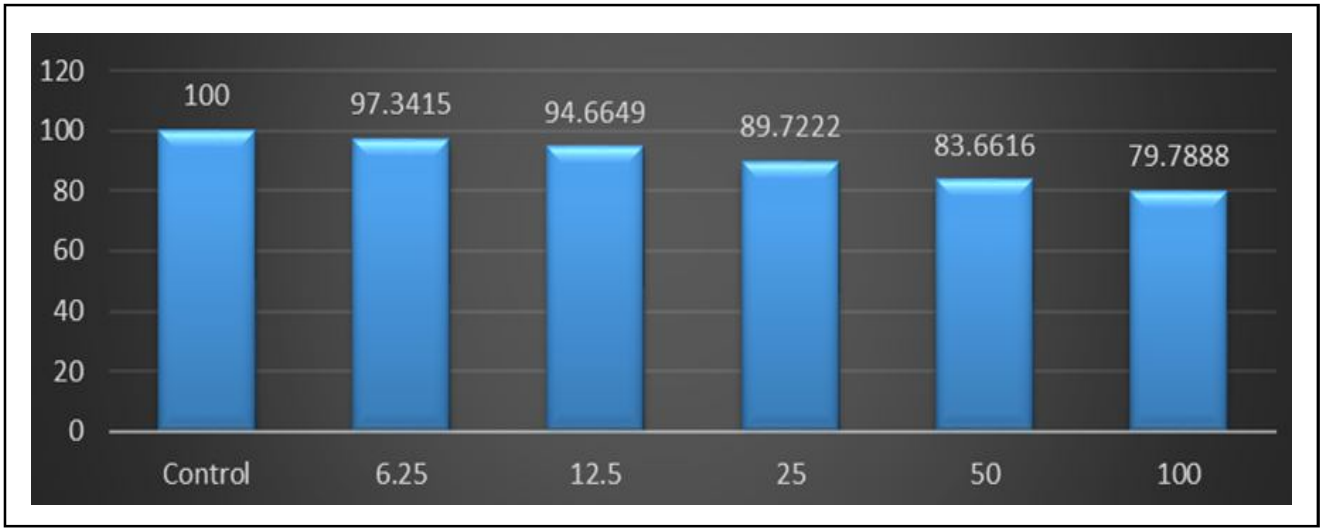


Figure 6: Graphical illustration portraying the anticancer impact of chloroform extract through the MTT assay. Percentage viability is plotted on the Y axis, while various concentrations of chloroform extract are presented along the X axis.

Among the four extracts tested, the ethyl acetate extract displayed the highest anticancer activity against MCF-7 cells, as indicated by its IC_{50} value of 196.46 µg/ml. The IC_{50} value represents the concentration at which 50% of the cells are inhibited or killed, and a lower IC_{50} value indicates stronger anti-cancer activity.

The IC_{50} values for the other extracts were as follows:

- Ethanol extract: 235.41 µg/ml
- Chloroform extract: 254.02 µg/ml
- Aqueous extract: 412.92 µg/ml

In comparison, the standard anti-cancer drug, doxorubicin, exhibited a very low IC_{50} value of 4.54 µg/ml, indicating its potent anticancer activity.

4. Discussion

Antioxidants are crucial in lowering oxidative stress, which has the potential to harm biological molecules. Damage may be caused by an imbalance between oxidants and antioxidants (Iqbal *et al*., 2015). McClements and Decker state that phenolic substances can be discovered in plants in the forms of basic phenolic, acids phenolic, cyan pigments, derivatives of cinnamic acid, flavonoids, and tannins. These compounds' structures enable them to scavenge free radicals. The oxidation rate can be effectively mitigated through the use of antioxidants, which neutralize these unstable free radicals. Nevertheless, the adverse effects and the high costs associated with antioxidant medications compel a significant portion of the population to turn to herbal remedies, known for their milder side effects. While numerous techniques are available for assessing antioxidant activity both *in vivo* and *in vitro*, only a handful of dependable methods can be employed for the analysis of plant extracts.

Furthermore, in our pursuit to assess the antioxidant potential of our plant extracts, we conducted evaluations to determine their capability to scavenge stable free radicals, specifically DPPH and ABTS cations, along with their ferric ion-reducing antioxidant power (FRAP) and total antioxidant capacity (TAC). This research aims to shed light on the benefits of plant extracts as a natural source of antioxidants and their effectiveness in combatting oxidative stress.

In the MTT assay against the MCF-7 cell line, all the tested extracts demonstrated anticancer activity. Among these extracts, the ethyl acetate extract exhibited the most potent anticancer effect, with an IC_{50} value of 196.46 µg/ml. While the standard drug, doxorubicin, exhibited superior anticancer activity with an IC_{50} value of 4.54 µg/ml, the ethyl acetate extract's performance is noteworthy and highlights its potential as an origin of anticancer substances.

These results suggest that the ethyl acetate extract from *E. serratus* leaves shows the most promising anticancer potential against MCF-7 cells among the tested extracts. While doxorubicin remains a highly effective reference drug, the findings explain the importance of natural extracts as a source of anticancer compounds and their utility in the development of new treatments or therapies for cancer. Further research is necessary to identify and define the active ingredients in the ethyl acetate extract that are responsible for this anticancer effect.

5. Conclusion

This study focused on the assessment of various extracts from *E. serratus* leaves for their antioxidant and anticancer activities. The results of antioxidant assays, including DPPH, ABTS, FRAP, and TAC, showed that the ethyl acetate extract has the highest antioxidant potential, surpassing other extracts and even the standard, ascorbic acid. This emphasizes the significance of solvent choice in extraction processes and underscores the ethyl acetate extract's potential in applications requiring strong antioxidant properties.

This research underscores the multifaceted bioactivity of *E. serratus* leaf extracts, emphasizing their promise in both antioxidant and anti-cancer applications. The findings provide a foundation for further exploration into the identification and isolation of the bioactive components within the ethyl acetate extract, which could lead to the development of novel therapeutic agents and antioxidant-rich products. Additionally, the results open doors for investigating the potential of natural sources in combating oxidative stress and cancer, offering a valuable contribution to the field of health and pharmaceutical sciences.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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