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IN-VITRO ANTIOXIDANT AND CYTOTOXIC ACTIVITIES OF SYZYGIUM ZEYLANICUM (LINN) DC

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Abstract

The present study was aimed to evaluate the antioxidant and cytotoxic activities of methanolic extracts of the leaves, root, and fruit of *Syzygium zeylanicum*. Antioxidant activity was assessed using DPPH, ABTS, superoxide radical scavenging, hydroxyl radical scavenging assays and lipid peroxidation inhibition assay. The in vitro cytotoxic activity was evaluated against the Dalton's Lymphoma Ascites (DLA) cell line. All extracts exhibited significant antioxidant activity, with the root extract showing the highest free radical scavenging potential in most assays. Cytotoxic studies revealed notable activity of the root extract against DLA cells, while leaf and fruit extracts showed lower cytotoxic effects. These findings suggest that *Syzygium zeylanicum* possesses strong antioxidant activity and a moderate cytotoxic potential.

Keywords**:** Epidemiologica, Atioxidant , Healthcare, Metabolism, Atherosclerosis

Introduction

Medicinal plants have been an important source of therapeutic agents since ancient times and continue to play a vital role in primary healthcare systems worldwide. According to the World Health Organization, about 80% of the global population relies mainly on traditional medicine and plant-based preparations, for their primary healthcare needs (Farnsworth *et al*., 1985). Epidemiological studies have shown that increased consumption of plants and plant products reduces the risk of chronic diseases such as cardiovascular disorders, cancer, diabetes, and Alzheimer's disease.

Reactive oxygen species (ROS), such as superoxide radicals, hydroxyl radicals, hydrogen peroxide etc are generated as byproducts of normal aerobic metabolism. These can damage cellular lipids, proteins, and DNA, initiating a cascade of events that lead to various pathological conditions (Chessman and Slater, 1993). Oxidative stress has been strongly implicated in the development of atherosclerosis, carcinogenesis, neuro-degenerative disorders and aging (Beck, 2000; Frei, 1994). Antioxidants play a crucial role in protecting biological systems by neutralizing free radicals and preventing oxidative damage. While endogenous antioxidant enzymes, dietary antioxidants, particularly those derived from plant sources are essential for maintaining redox balance and preventing chronic diseases.

Syzygium zeylanicum (L.) DC., belonging to the family Myrtaceae, is traditionally used in folklore medicine for its stimulant, antirheumatic, and vermifugal properties (Pullaiah, 2006). Previous studies have reported the presence of polyphenols and macrocyclic ellagitannins in its leaves. The present study aims to evaluate the antioxidant potential and cytotoxic effects of *Syzygium zeylanicum* extracts on murine tumour cell lines.

Materials and methods

Collection and Extraction of plant materials

Syzygium zeylanicum was collected from the Amala Nagar, Thrissur, Kerala, India. The plants were collected, washed with dechlorinated water, the leaves, fruits and root were separated and dried. The powdered plant parts were extracted with methanol. The filtrate was evaporated until solvents were completely evaporated to get the solidified crude extracts.

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The crude extracts thus obtained was stored in clean vials and maintained at 4°C in a refrigerator.

In-vitro Antioxidant activity of *Syzygium zeylanicum* extract

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of the extracts was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, with modifications to the method described by Coruh *et al*. (2007). A 0.05 mg/ml solution of DPPH in methanol was prepared, and extract solutions of varying concentrations were dissolved in methanol. A 0.1 ml portion of each extract solution was added to 1.4 ml of the DPPH solution. The absorbance at 517 nm was measured after 5 minutes of incubation at room temperature. The percentage of DPPH radical scavenging was calculated using the formula:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

ABTS Radical Scavenging Activity

The assay was performed by evaluating the interaction of the extract with a stable free radical derived from 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS). The production of the ABTS radical cation was carried out as described by Long and Halliwell (2001), with some modifications. Briefly, a stock solution of ABTS (7 mM) was prepared in water. To this solution, ammonium persulfate (2.45 mM final concentration) was added, allowing for the partial oxidation of ABTS to generate the ABTS radical. The ABTS radical solution was then diluted to achieve an absorbance of 0.75 at 734 nm in phosphate-buffered saline (PBS, pH 7.4). For the assay, 10µl of extract at different concentrations was added to 1 ml of the ABTS radical solution. The absorbance was measured spectrophotometrically after 6 minutes of incubation, using PBS as a reference. The percentage of inhibition was calculated using the formula:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

Superoxide Radical Scavenging Activity

The reaction mixture contained 3 mg of KCN dissolved in EDTA (6 µM), riboflavin (2 µM), NBT (50 µM), various concentrations (10 – 1000 µg/ml) of the extract, and phosphate buffer, with a final volume of 3 ml. The tubes were exposed to uniform illumination with an incandescent lamp for 15 minutes. Absorbance was measured at 530 nm before and after illumination (McCord and Fridovich, 1969). The percentage of superoxide radical inhibition was calculated using the formula:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

Hydroxyl Radical Scavenging Activity

Hydroxyl radical scavenging activity of the extract was measured by studying the competition between deoxyribose and test compounds for the hydroxyl radicals generated from Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Fenton reaction). The hydroxyl radicals attack deoxyribose, which eventually results in the formation of thiobarbituric acid reacting substances (Elizabeth and Rao, 1990). The reaction mixture contained deoxyribose (2.8mM), ferric chloride (0.1mM) EDTA (0.1mM), H_2O_2 (1mM), ascorbate (0.1mM), KH_2PO_4-KOH (20mM, pH 7.4) and various concentrations of the sample in a volume of 1ml was incubated for 1hr at 37^0C. Deoxyribose degradation was measured as thiobarbituric acid reactive substrate by the method of Ohkawa *et al* (1979). The inhibition produced by different concentration was calculated compared to control. Percent inhibition of hydroxyl radical was calculated using the equation:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

Lipid Peroxidation Assay

Lipid peroxidation levels were measured following the method of Ohkawa *et al*. (1979). Extract (10–1000 µg/ml) was incubated with 0.1 ml of 25% rat liver homogenate containing 30 mM KCl, Tris-HCl buffer (0.04 M, pH 7.0), ascorbic acid (0.06 mM), and ferrous ion (0.16 mM) in a total volume of 0.5 ml for 1 hour. After incubation, 0.4 ml of the reaction mixture

was treated with 0.2 ml of SDS (8.1%), 1.5 ml of TBA (0.8%), and 1.5 ml of acetic acid (20%, pH 3.5), and then heated in a boiling water bath at 100°C for 1 hour. The mixture was cooled, followed by the addition of 5 ml of a pyridine-butanol mixture (15:1), mixed thoroughly, and centrifuged at 3000 rpm for 10 minutes. The absorbance of the clear supernatant was measured at 532 nm against the pyridine-butanol mixture as a blank. The percentage inhibition of lipid peroxidation was calculated as:

Percentage of inhibition = (Control OD – Treated OD/ Control OD) x100

In vitro Cytotoxicity of *Syzygium zeylanicum* Methanol extracts

Maintanance of Cell Lines

The DLA cells were maintained in the intraperitoneal cavity of mice. At first $1x10^6$ (100µl) cells were injected into the intraperitoneal cavity of mice. After days the cells were aspirated using a 1ml syringe and Phosphate Buffered Saline (PBS). The cells were washed in PBS and the number of cells was counted using a haemocytometer and makes up as 100µl PBS containing $1x10^6$ cells. Then the cells were injected into the intraperitoneal cavity of other mice and continued in every 15 days intervals.

In vitro Cytotoxicity Assay Procedure

The cytotoxicity was determined by Trypan blue exclusion method (Babu *et al*, 1995). The exclusion assay is used to determine the number of viable cells present in a cell suspension. It is based on the principle that live cells possess intact cell membranes that exclude the dye and the dead cells do not. In the assay presented here, under light microscope a viable cell will have a clear cytoplasm whereas a nonviable cell will have a blue cytoplasm. For the assay DLA cells aspirated from mice intraperitoneal cavity and were counted to a density of 1×10^6 in 0.1 ml phosphate buffer saline (PBS, pH 7.4). To 0.8 ml of PBS add 0.1 ml of cell suspension containing 1×10^6 cells and different concentration of extract (0.01µg – 1mg/ml). These were incubated for 3 hours at 37°C. After incubation, 0.1 ml trypan blue dye was added and incubated for 2 min at room temperature. Apply a drop of tryphan blue cell mixture to a hemocytometer and count the stained (non-viable) and unstained (viable) cells separately under a microscopic field.

Statistical Analysis

The *in vitro* experiments were done in triplicates and the values were expressed in Mean ± SD.

Results and Discussion

The present study evaluates the antioxidant and cytotoxic activities of methanolic extract of *S. zeylanicum* leaves, root and fruits. The antioxidant activities are studied by performing DPPH radical scavenging, ABTS radical scavenging superoxide radical scavenging, hydroxyl radical scavenging assays and lipid peroxidation inhibition assay. For evaluating in vitro cytotoxicity tryphan blue exclusion method in DLA cells were done. The results of antioxidant and cytotoxicity were expressed in their IC_{50} values.

Antioxidant Activity of *Syzygium zeylanicum* extract

The antioxidant capability *S. zeylanicum* extract was studies using DPPH, ABTS, superoxide, hydroxyl radical scavenging assays, and lipid peroxidation inhibition. The results were expressed in IC_{50} values, representing the concentration required to neutralize 50% of the free radicals in the reaction mixture. The extracts showed varying levels of antioxidant activity for different assays.

In the DPPH radical scavenging assay, the IC_{50} values of extracts to scavenge the DPPH radical was found to be 13 µg/ml for root extract, 19.5 µg/ml for leaf extract and 17 µg/ml for fruit extract. From the results all the extracts showed high DPPH radical scavenging activity (Table 1, 2 & 3). Similarly, all the extracts showed different degrees of ABTS radical scavenging activities as per their IC_{50} values (Table 1, 2 & 3). The root extract showed an IC_{50} value of 14.5 µg/ml followed by fruit extract with 17.5 µg/ml and the leaf extract with 18.5 µg/ml. The ability of the extract to scavenge superoxide radicals was found to be 130 µg/ml for fruit extract, followed by root extract with IC_{50} value 140 µg/ml and leaf

extract showed the least activity with 190.5 µg/ml (Table 1, 2 & 3). The extracts were found to be more effective in scavenging hydroxyl radical. The root extract showed more activity with an IC_{50} value of 190 µg/ml followed by leaf extract with of 225 µg/ml. The fruit extract showed the least activity with an IC50 value of 245 µg/ml (Table 1, 2 & 3). The lipid peroxidation inhibition assay showed great activity in reducing lipid peroxidation. The root extract showed an IC_{50} of 185 µg/ml, the leaf extract showed 210 µg/ml and the fruit extract showed IC_{50} value of 190 µg/ml (Table 1, 2 & 3).

Antioxidants are substances that prevent or delay oxidative damage caused by free radicals in biological systems. The results of antioxidant activity demonstrated the strong free radical scavenging ability of *S. zeylanicum* extracts. The extracts effectively neutralized DPPH radicals by electron or hydrogen donation (Naik *et al*., 2003) and scavenged ABTS and superoxide radicals that are known to damage cellular macromolecules (Pardini, 1995; Beckmann, 1990). The extracts also inhibited lipid peroxidation in a dose-dependent manner, indicating their role as chain-breaking antioxidants. Since oxidative stress is associated with aging, cancer, and cardiovascular diseases, plant-based antioxidants play an important protective role (Yamaguchi *et al*., 1998). However, endogenous antioxidant systems are insufficient to completely prevent oxidative damage, highlighting the importance of dietary antioxidants (Henmani and Parihar, 1998).

In vitro cytotoxic effects of *Syzygium zeylanicum* Extracts

The leaves fruit and root extracts were tested in vitro for their toxic effect on mouse cancer cell line DLA. IC_{50} value is the amount of extract needed to cause death of 50% of DLA cells. Assays were done in triplicate and the average was taken The results show that the extracts exhibited varying effect on these cell lines. Against DLA cells the leaves and fruit extract showed least activity. They showed only 15% and 10% cell death respectively up to 200 µg/ml extract treatment. While the root extract showed more activity with an IC_{50} value of 84µg/ml. The in vitro cytotoxicity assay used is the preliminary test to check the ability of the extract in causing death or inhibiting the growth of cancer cells. Qualitative analysis of the extracts of *S. zeylanicum* revealed the presence of various chemical constituents such as terpenes, polyphenols, flavonoids, steroids, and glycosides. Many of these compounds are known for their antioxidant and cytotoxic activities. Therefore, the observed antioxidant and cytotoxic effects of the extracts may be attributed to the presence of these bioactive compounds.

Table 1. Antioxidant activities of *S. zeylanicum* leaf extract

Antioxidant Assay	*IC_{50} Value (µg/ml)
DPPH radical scavenging activity	19.5 ± 3.04
ABTS radical scavenging activity	18.5 ± 2.29
Superoxide radical scavenging activity	190.5 ± 5.56
Hydroxyl radical scavenging activity	225 ± 7.21
Lipid peroxidation inhibition activity	210 ± 7.00

*IC_{50} Value is the amount of extract needed to inhibit or scavenge 50% of free radicals produced in the reaction mixture.

Table 2. Antioxidant activities of *S. zeylanicum* root extract

Antioxidant Assay	*IC_{50} Value (µg/ml)
DPPH radical scavenging activity	13 ± 4.58
ABTS radical scavenging activity	14.5 ± 3.60
Superoxide radical scavenging activity	140.5 ± 5.07
Hydroxyl radical scavenging activity	190 ± 6.55
Lipid peroxidation inhibition activity	185 ± 6.00

*IC_{50} Value is the amount of extract needed to inhibit or scavenge 50% of free radicals produced in the reaction mixture.

Table 3. Antioxidant activities of *S. zeylanicum* fruit extract

Antioxidant Assay	*IC_{50} Value (µg/ml)
DPPH radical scavenging activity	17 ± 2.64
ABTS radical scavenging activity	17.5 ± 5.89
Superoxide radical scavenging activity	130 ± 5.00
Hydroxyl radical scavenging activity	245 ± 7.93
Lipid peroxidation inhibition activity	190 ± 6.76

*IC_{50} Value is the amount of extract needed to inhibit or scavenge 50% of free radicals produced in the reaction mixture.

Table 4. *In vitro* cytotoxic effect of *S. zeylanicum* extracts

Extracts	*IC_{50} Value (µg/ml)
Leaf	No activity up to 200 µg/ml
Root	84 ± 6.24
Fruit	No activity up to 200 µg/ml

* IC50 Value is the amount of extract needed to cause death of 50% of DLA cells. Assays were done in triplicate and the average was taken.

Conclusion

The present study demonstrates that methanolic extracts of *S. zeylanicum* leaves, roots, and fruits exhibit significant antioxidant activity against various free radicals. Among the extracts, the root extract showed the highest antioxidant and cytotoxic activity. The results suggest that *S. zeylanicum* could be a potential source of natural antioxidant and anticancer compounds, and need further detailed studies.

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