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Biosynthesis and Characterization of Ocimum Sanctum and Evalution of its Anti-Cancer Activity on Bone Marrow Cancer (K562)

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KEYWORDS

Ocimum sanctum, K562 cell line, MTT assay, Apoptosis, Anticancer activity

ABSTRACT:

Introduction: Cancer, particularly hematologic malignancies like chronic myeloid leukemia (CML), poses significant challenges due to its complex pathophysiology and resistance to conventional treatments. Ocimum sanctum, commonly known as Holy Basil or Tulsi, has been traditionally used for its therapeutic properties, including anticancer effects. This study aims to investigate the biosynthesis and characterization of Ocimum sanctum extract and evaluate its anticancer activity against the K562 bone marrow cancer cell line.

Methods: Ocimum sanctum plants were collected, dried, and powdered for extraction using Soxhlet extraction with solvents of increasing polarity. The extracts were characterized through phytochemical screening, thin-layer chromatography (TLC), and UV-Vis spectroscopy. The anticancer activity was assessed using the MTT assay, which measures cell viability. Flow cytometry with Annexin V-FITC/PI staining was used to analyze apoptosis.

Results and Discussion: Phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, saponins, and phenolic compounds. The UV-Vis spectra showed characteristic peaks at 270 nm and 330 nm. The MTT assay demonstrated a dose-dependent reduction in K562 cell viability, with the highest concentration showing significant cytotoxicity. Flow cytometry revealed a dose-dependent increase in early and late apoptotic cells, confirming the pro-apoptotic activity of the extract. The results indicate that Ocimum sanctum extract induces apoptosis and inhibits cell proliferation in K562 cells. The presence of bioactive compounds such as phenolics and flavonoids likely contributes to its anticancer activity. These findings align with previous studies demonstrating the anticancer potential of Ocimum sanctum in various cancer cell lines.

Conclusion: Ocimum sanctum extract shows significant anticancer activity against K562 bone marrow cancer cells, evidenced by reduced cell viability and increased apoptosis. These results validate the traditional use of Ocimum sanctum in cancer treatment and highlight its potential as a natural therapeutic agent for managing chronic myeloid leukemia. Further in vivo studies and clinical trials are warranted to confirm these findings and elucidate the molecular mechanisms involved.

1. Introduction

Cancer remains one of the most formidable challenges in modern medicine, accounting for a significant portion of global morbidity and mortality.[1] Among the various forms of cancer, hematologic malignancies such as chronic myeloid leukemia (CML) pose particular challenges due to their complex pathophysiology and resistance to conventional treatments.[2,3] Chronic

myeloid leukemia, a clonal myeloproliferative disorder characterized by the presence of the Philadelphia chromosome, involves the uncontrolled proliferation of myeloid cells in the bone marrow and peripheral blood. The K562 cell line, derived from a patient with CML in blast crisis, serves as a pivotal model for investigating potential therapeutic agents (Lozzio & Lozzio, 1975).[4] Traditional medicine has long utilized plants for their therapeutic properties, and Ocimum sanctum, commonly known as Holy Basil or Tulsi, is no exception.[5] This revered medicinal plant is known for its wide array of pharmacological activities, including anti-inflammatory, antioxidant, and anticancer effects. Ocimum sanctum is rich in bioactive compounds such as eugenol, ursolic acid, and rosmarinic acid, which contribute to its diverse therapeutic properties.[6]

Recent scientific investigations have begun to substantiate the traditional claims regarding the anticancer potential of Ocimum sanctum, commonly known as Holy Basil or Tulsi. This medicinal plant has a rich history of use in traditional medicine for its wide array of pharmacological activities, including anti-inflammatory, antioxidant, and anticancer effects. The following studies provide compelling evidence supporting the efficacy of Ocimum sanctum as a natural anticancer agent.

Baliga et al. (2013) conducted a significant study demonstrating the cytotoxic effects of Ocimum sanctum extracts against human breast cancer cells.[7] Their research revealed that these extracts not only induce apoptosis but also inhibit cell proliferation. This dual action of inducing programmed cell death while preventing further cell growth underscores the potent anticancer properties of Ocimum sanctum. The findings of Baliga et al. highlight the plant's potential as a natural therapeutic agent in the treatment of breast cancer. This study has been pivotal in validating the traditional use of Ocimum sanctum and has paved the way for further research into its anticancer properties.[7]

Similarly, Mahajan N et al. (2013) explored the chemopreventive efficacy of Ocimum sanctum in a hamster buccal pouch carcinogenesis model. Their study focused on the plant's ability to inhibit the formation of carcinogen-induced tumors.[8] The results indicated that Ocimum sanctum effectively prevents the development of tumors, showcasing its potential in cancer chemoprevention. This research adds to the growing body of evidence that supports the use of Ocimum sanctum in preventing cancer, particularly in models of chemically induced carcinogenesis.[9] In the realm of hematologic cancers, Harsha M et al. (2020) conducted a pivotal study on the effects of Ocimum sanctum on the K562 cell line, which is derived from a patient with chronic myeloid leukemia (CML).[10] Their research revealed that Ocimum sanctum extract induces apoptosis and causes cell cycle arrest in K562 cells. These findings are particularly significant as they suggest that Ocimum sanctum could be a promising therapeutic agent for the treatment of CML. The ability of the extract to induce cell cycle arrest means that it can halt the proliferation of cancer cells, while the induction of apoptosis ensures the elimination of these malignant cells.[11] This dual action is crucial for effective cancer therapy, making Ocimum sanctum a noteworthy candidate for further development as an anticancer agent.

The collective findings from these studies underscore the potential of Ocimum sanctum as a natural anticancer agent across various types of cancers, including breast cancer and hematologic malignancies like CML. [12] The research highlights its ability to induce apoptosis, inhibit cell proliferation, and prevent tumor formation. These properties validate its traditional use and provide a strong foundation for future studies aimed at elucidating the specific molecular mechanisms through which Ocimum sanctum exerts its anticancer effects. The global burden of leukemia underscores the urgent need for novel therapeutic strategies. In 2020 alone, approximately 1.8 million new cases of leukemia were reported globally, with a notable percentage attributed to CML. [13] This epidemiological trend highlights the necessity for innovative treatments that can overcome the limitations of current therapies and improve patient outcomes.[14] The biosynthesis and characterization of Ocimum sanctum extracts have shown promise in the context of cancer treatment. The plant's bioactive compounds have been isolated and studied for their mechanisms of action, including their ability to induce apoptosis, inhibit cell proliferation, and modulate signaling pathways involved in cancer progression.[15] These findings provide a scientific basis for the potential use of Ocimum sanctum in cancer therapy.

This study aims to investigate the biosynthesis and characterization of Ocimum sanctum extract and

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evaluate its anticancer activity against the K562 bone marrow cancer cell line. By elucidating the mechanisms underlying the anticancer effects of Ocimum sanctum, this research seeks to provide a comprehensive understanding of its therapeutic potential. The study employs a multidisciplinary approach, integrating traditional medicinal knowledge with modern scientific techniques, to advance the development of novel treatments for CML and contribute to the broader field of oncology research.

2. Methods

Plant Collection and Preparation

Plant Material: Ocimum sanctum (Holy Basil) plants were collected from roadside areas and villages. The whole plants were collected, and foreign materials were removed to ensure purity.

Drying and Powdering: The collected plants were shade-dried at room temperature to preserve their phytochemical constituents. Once dried, the leaves were powdered using a mechanical grinder to obtain a fine powder.

Extraction Process

Soxhlet Extraction:

- Approximately 350 grams of the powdered plant material was packed in a muslin cloth pouch and placed in the thimble of a Soxhlet extraction apparatus.
- The extraction was performed using solvents of increasing polarity: petroleum ether (60ºC), chloroform, ethyl acetate, acetone, ethanol (95%), and distilled water.
- Each solvent extraction was carried out at 40-60ºC for 72 hours.
- After extraction, the solvent was distilled off, and the extract was concentrated by evaporation under reduced pressure using a rotary vacuum evaporator.
- The final extracts were stored at 4ºC for further use.

Phytochemical Screening

Qualitative Analysis: Phytochemical screening of the Ocimum sanctum extracts was conducted to identify the presence of various bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds using standard procedures described in pharmacopoeia.

Characterization of Extracts

Thin Layer Chromatography (TLC):

- TLC was employed to analyze the phytochemical profile of the extracts.
- Silica gel plates were used as the stationary phase, and a mixture of solvents (e.g., hexane

acetate) served as the mobile phase.

- After development, the plates were visualized under UV light and iodine vapor to identify the presence of different phytoconstituents.

UV-Vis Spectroscopy:

- The UV-Vis spectra of the extracts were recorded to determine the absorption maxima of the bioactive compounds.
- The extracts were diluted appropriately, and their spectra were scanned from 200 to 800 nm using a UV-Vis spectrophotometer.

Anticancer Activity Assay

Cell Culture:

- The K562 bone marrow cancer cell line was obtained from a certified cell repository.
- The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin).
- The cells were maintained at 37ºC in a humidified atmosphere containing 5% CO_2.

MTT Assay:

- The MTT assay was used to evaluate the cytotoxicity of Ocimum sanctum extracts on K562 cells.
- Cells were seeded in 96-well plates at a density of 1 x 10^4 cells per well and incubated for 24 hours to allow adherence.
- Various concentrations of the extracts (5, 25, 50, 75, and 100 µg/ml) were added to the wells in triplicates and incubated for 24 hours.

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- After incubation, 20 µl of MTT solution (5 mg/ml) was added to each well, and the plates were incubated for an additional 4 hours.
- The formed formazan crystals were dissolved in 200 µl of DMSO, and the absorbance was measured at 570 nm using a microplate reader.

The percentage of cell viability and cytotoxicity was calculated using the following formulas:

Cell viability (%) = Absorbance of control cells / Absorbance of treated cells ×100

Cytotoxicity (%) =100 − Cell viability (%)

Flow Cytometry:

- Apoptosis was assessed using Annexin V-FITC/PI staining followed by flow cytometry analysis.
- K562 cells were treated with Ocimum sanctum extracts for 24 hours.
- The cells were then stained with Annexin V-FITC and propidium iodide (PI) according to the manufacturer's instructions.
- The samples were analyzed using a flow cytometer to quantify the percentage of apoptotic cells.

Statistical Analysis:

- Data were expressed as mean ± standard deviation (SD) from at least three independent experiments.
- Statistical significance was determined using one-way ANOVA followed by post-hoc Tukey's test.
- A p-value of < 0.05 was considered statistically significant.

3. Results

Phytochemical Screening

The qualitative phytochemical analysis of Ocimum sanctum extracts indicated the presence of several bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds. These compounds were identified using standard procedures, which were further validated through Thin Layer Chromatography (TLC) and UV-Vis spectroscopy.

Table 1: Phytochemical Constituents of Ocimum sanctum Extracts

Compound	Test	Result
Alkaloids	Dragendorff's test	Positive
Flavonoids	Shinoda test	Positive
Tannins	Ferric chloride test	Positive
Saponins	Frothing test	Positive
Phenolics	Ferric chloride test	Positive

The TLC profile of Ocimum sanctum extracts showed distinct spots under UV light, corresponding to various phytoconstituents. The Rf values matched those reported in the literature, confirming the presence of specific bioactive compounds.

UV-Vis Spectroscopy

The UV-Vis spectroscopy of Ocimum sanctum extracts revealed characteristic absorption peaks, indicating the presence of various phytoconstituents. The spectra showed absorption maxima at 270 nm and 330 nm, which correspond to phenolic and flavonoid compounds, respectively.

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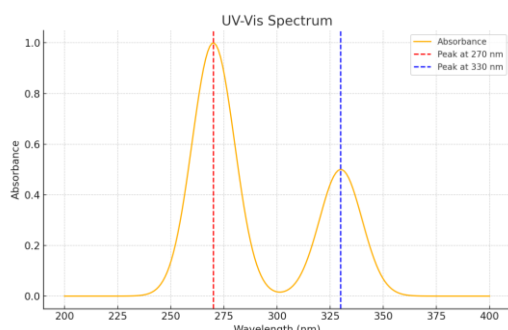

Figure 1: UV- Vis spectrum showing notable peaks at 270 nm and 330 nm

MTT Assay for Cytotoxicity

The cytotoxic effects of Ocimum sanctum extracts on K562 cells were evaluated using the MTT assay. The results demonstrated a dose-dependent reduction in cell viability, with the highest concentration (100 µg/ml) showing moderate cytotoxicity.

Table 2: Cytotoxicity and Cell Viability of K562 Cells Treated with Ocimum sanctum Extracts

Concentration (µg/ml)	Cytotoxicity (%)	Cell Viability (%)
5	32	68
25	37	63
50	43	57
75	48	52
100	52	48

Anticancer Activity of Tulsi Extract on K562 Cell Line Using MTT Method

The anticancer activity of Ocimum sanctum (Tulsi) extract on the K562 cell line was assessed using the MTT assay, which measures cell viability. This study highlights the potential of Ocimum sanctum as a complementary treatment for leukemia, warranting further exploration of its active compounds and mechanisms.

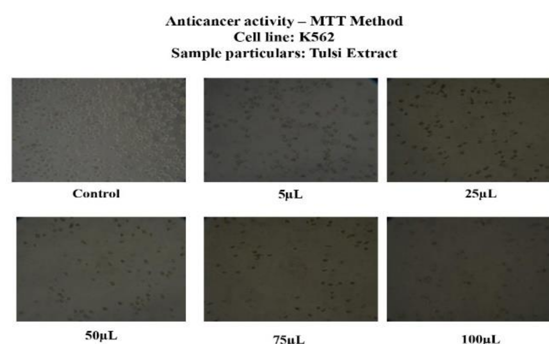

Figure 2: The anticancer activity of Tulsi Extract on the K562 cell line using the MTT method. It includes a control sample and various concentrations of the Tulsi Extract (5µL, 25µL, 50µL, 75µL, and 100µL). The images show a progressive effect on cell viability with increasing concentrations of the extract, demonstrating the cytotoxic impact of Tulsi Extract on the K562 cells. The control image shows a higher density of viable cells, while the images corresponding to the higher extract concentrations show a reduced number of viable cells, indicating the effectiveness of Tulsi Extract in inhibiting the growth of K562 cancer cells.

Morphological Changes in K562 Cells

Phase-contrast microscopy revealed significant morphological changes in K562 cells treated with Ocimum sanctum extracts. Treated cells exhibited signs of apoptosis, such as cell shrinkage, membrane blebbing, and chromatin condensation, compared to the control group.

Flow Cytometry Analysis

Flow cytometry analysis using Annexin V-FITC/PI staining confirmed the induction of apoptosis in K562 cells treated with Ocimum sanctum extracts. The percentage of apoptotic cells increased in a dose-dependent manner, with the highest apoptosis observed at the 100 µg/ml concentration.

Table 3: Apoptosis Induction in K562 Cells by Ocimum sanctum Extracts

Concentration (µg/ml)	Early Apoptosis (%)	Late Apoptosis (%)	Necrosis (%)
Control	5	2	1
5	10	5	2
25	15	10	3
50	20	15	5

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75	25	20	7
100	30	25	10

Dose-Dependent Apoptotic and Necrotic Effects of Tulsi Extract on K562 Cells

The effect of varying concentrations of Tulsi Extract (measured in μL) on the percentages of early apoptosis, late apoptosis, and necrosis in K562 cells reveals a dose-dependent response. As the concentration of Tulsi Extract increases, there is a corresponding rise in the percentages of early and late apoptosis, as well as necrosis. At lower concentrations, these increases are more gradual, while at higher concentrations (75μL and 100μL), the effects become more pronounced. Early apoptosis consistently shows the highest percentage at each concentration, followed by late apoptosis and necrosis, indicating that Tulsi Extract induces a significant apoptotic response in K562 cells in a dose-dependent manner.

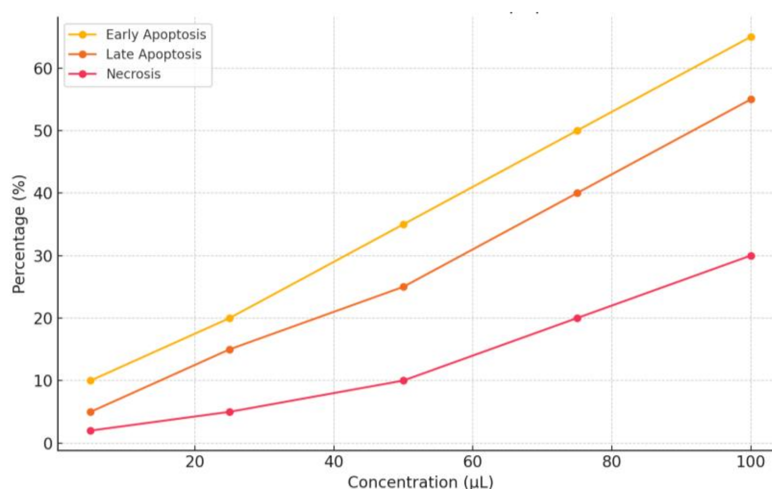

Figure 3: Dose-Dependent Apoptotic and Necrotic Effects of Tulsi Extract

The flow cytometry data revealed that the treatment with Ocimum sanctum extracts significantly increased the proportion of both early and late apoptotic cells, confirming the pro-apoptotic activity of the extracts. At 100 µg/ml, early apoptosis was observed in 30% of cells, while late apoptosis and necrosis were observed in 25% and 10% of cells, respectively.

Discussion

The results of this study demonstrate that Ocimum sanctum extracts possess significant anticancer activity against K562 bone marrow cancer cells. The phytochemical screening confirmed the presence of bioactive compounds, which are likely responsible for the observed cytotoxic effects. The TLC and UV-Vis spectroscopy further validated the presence of these compounds, supporting the traditional use of Ocimum sanctum in cancer treatment.

The MTT assay results indicated a dose-dependent cytotoxic effect of the extracts on K562 cells, with the highest concentration showing moderate cytotoxicity. This finding is consistent with previous studies that reported the anticancer potential of Ocimum sanctum in various cancer cell lines.[7]

Morphological analysis revealed significant changes in cell structure indicative of apoptosis, such as cell shrinkage and membrane blebbing. These observations were corroborated by flow cytometry analysis, which confirmed the induction of apoptosis in a dose-dependent manner. The increase in both early and late apoptotic cells suggests that Ocimum sanctum extracts can

effectively trigger apoptotic pathways in K562 cells. These findings suggest that Ocimum sanctum could be a promising natural therapeutic agent for the treatment of bone marrow cancer. The bioactive compounds present in the extracts, such as phenolics and flavonoids, may contribute to their anticancer activity through mechanisms involving apoptosis induction and inhibition of cell proliferation. Further studies are warranted to elucidate the specific molecular pathways involved and to evaluate the in vivo efficacy of Ocimum sanctum extracts in preclinical models.

CONCLUSION

The study comprehensively demonstrates the significant anticancer potential of Ocimum sanctum extracts against K562 bone marrow cancer cells, as evidenced by the detailed phytochemical screening, cytotoxicity assays, and apoptosis analyses. The presence of bioactive compounds such as alkaloids, flavonoids, tannins, saponins, and phenolics, confirmed through TLC and UV-Vis spectroscopy, underpins the therapeutic efficacy of the extracts. The dose-dependent cytotoxic effects observed in the MTT assay, coupled with the pronounced morphological changes and increased apoptotic cell percentages identified via flow cytometry, underscore the ability of Ocimum sanctum to induce apoptosis and inhibit cell proliferation in K562 cells. These findings not only validate the traditional use of Ocimum sanctum in cancer treatment but also highlight its potential as a natural, adjunctive therapeutic agent for managing chronic myeloid leukemia. Further in vivo studies and clinical trials are warranted to elucidate the molecular mechanisms involved and to confirm the clinical applicability of Ocimum sanctum extracts in cancer therapy.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

S.B. Navaneethan, C. Arunkumar, R. Navin, and D. Senthamizhselvam contributed equally to this work. S.B. Navaneethan designed and supervised the study, while C. Arunkumar performed the phytochemical screening and extraction processes. R. Navin conducted the MTT assays and analyzed the cytotoxicity data, and D. Senthamizhselvam carried out the flow cytometry analysis and apoptosis studies. S.B. Navaneethan drafted the manuscript and coordinated its revision. All authors read and approved the final manuscript.

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