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IN-VITRO STUDY OF ETHANOLIC EXTRACT OF *OCIMUM SANCTUM* LEAVES FOR ANTI-CANCER, ANTI-DIABETIC, AND ANTI-INFLAMMATORY ACTIVITY

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Abstract:
Ocimum sanctum, commonly known as Tulsi, is an important medicinal plant extensively used in traditional systems of medicine because of its diverse pharmacological properties. The present study was carried out to evaluate the in-vitro anticancer, anti-diabetic, and anti-inflammatory activities of the ethanolic extract of Ocimum sanctum leaves. The leaves were collected, shade dried, powdered, and extracted using ethanol through the solvent extraction method. The prepared extract was subjected to various in-vitro biological assays for the evaluation of its therapeutic potential.
The anticancer activity of the extract was assessed using suitable cell line studies, where it exhibited significant cytotoxic activity against cancer cells in a dose-dependent manner. Anti-diabetic activity was evaluated by α-amylase and α-glucosidase inhibition assays, which showed effective inhibition of carbohydrate metabolizing enzymes, indicating its potential in blood glucose regulation. The anti-inflammatory activity was studied by protein denaturation and membrane stabilization methods, and the extract demonstrated considerable inhibition of inflammatory responses.
The findings of the study indicate that the ethanolic extract of Ocimum sanctum leaves possesses significant pharmacological activities, which may be attributed to the presence of phytoconstituents such as flavonoids, tannins, phenolic compounds, and alkaloids. Thus, Ocimum sanctum can be considered a promising natural source for the development of herbal medicines. Further in-vivo and clinical investigations are necessary to establish its therapeutic safety and efficacy.
Keywords: *Ocimum sanctum, Tulsi, Ethanolic extract, Anticancer activity, Anti-diabetic activity, Anti-inflammatory activity, In-vitro study, Phytochemicals, Herbal medicine.*

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INTRODUCTION:

Ocimum sanctum, commonly known as Tulsi or Holy Basil, is one of the most sacred and medicinally important plants in India. It belongs to the family Lamiaceae and has been widely used in traditional systems of medicine such as Ayurveda, Siddha, and Unani for centuries. Tulsi is often referred to as the "Queen of Herbs" because of its remarkable medicinal, spiritual, and therapeutic properties. The plant is cultivated throughout India and in many tropical and subtropical regions of the world. Different parts of the plant, including leaves, seeds, roots, and stems, are used for the treatment of various diseases and disorders. Among these parts, the leaves are considered highly beneficial due to the presence of numerous biologically active phytoconstituents.

According to the World Health Organization (WHO), a large proportion of the world population depends on herbal medicines for primary healthcare needs. Herbal medicines are considered safer, economical, and easily available compared to synthetic drugs. In recent years, interest in plant-based medicines has increased significantly because synthetic drugs used for chronic diseases often produce adverse side effects, toxicity, and drug resistance. Therefore, scientific evaluation of medicinal plants has become essential to discover new therapeutic agents with improved safety and efficacy.

Ocimum sanctum contains a wide variety of phytochemical constituents such as flavonoids, alkaloids, glycosides, tannins, phenolic compounds, terpenoids, saponins, steroids, and essential oils. Major active compounds present in Tulsi leaves include eugenol, ursolic acid, rosmarinic acid, linalool, carvacrol, apigenin, and methyl chavicol. These bioactive constituents are responsible for various pharmacological activities such as antioxidant, antimicrobial, anti-inflammatory, anti-diabetic, anticancer, hepatoprotective, cardioprotective, immunomodulatory, and adaptogenic effects.

MATERIALS AND METHODS:

Collection and Authentication of Plant Material

Fresh leaves of *Ocimum sanctum* were collected from a local herbal garden and washed thoroughly with distilled water to remove dust and impurities. The leaves were shade dried at room temperature for 7–10 days and then powdered using a mechanical grinder. The powdered material was stored in an airtight container for further use. The plant material was authenticated by a qualified botanist or pharmacognosist.

Preparation of Ethanolic Extract

About 100 g of dried leaf powder of *Ocimum sanctum* was extracted using ethanol by the Soxhlet extraction method. The powdered leaves were packed in a thimble and extracted with 95% ethanol for 6–8 hours until complete extraction was achieved. The obtained extract was filtered using Whatman filter paper and concentrated using a rotary evaporator under reduced pressure. The concentrated extract was dried and stored in a refrigerator at 4°C for further experimental analysis.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the ethanolic extract was carried out to identify the presence of various phytoconstituents such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, steroids, and phenolic compounds using standard qualitative methods.

In-Vitro Biological Assays

Anticancer Assay (MTT Assay): The anticancer activity was evaluated against selected cancer cell lines (such as MCF-7 or HeLa). Cells were maintained in DMEM supplemented with 10% FBS and incubated at 37°C with 5% CO&sub2;. Cells were seeded in 96-well plates, incubated for 24 hours, and then treated with extract concentrations of 25, 50, 100, 200, and 400 µg/mL. After another 24 hours, MTT reagent (5 mg/mL) was added. The purple-colored formazan crystals were dissolved in DMSO, and absorbance was recorded at 570 nm.

Anti-Diabetic Assays: For the α-Amylase inhibition assay, starch iodine method was utilized. Plant extract was mixed with α-amylase enzyme solution, incubated, and then 1% starch solution was added. The reaction was terminated with iodine reagent, and absorbance was measured at 620 nm. The α-Glucosidase assay utilized p-nitrophenyl-α-D-glucopyranoside as a substrate, and the reaction was terminated with sodium carbonate, measuring absorbance at 405 nm. Acarbose served as the standard reference drug.

Anti-Inflammatory Assays: Protein denaturation was carried out using bovine serum albumin (BSA). The assay mixture was incubated at 37°C and then heated to 70°C, measuring absorbance at 660 nm with Diclofenac sodium as a standard. Human red blood cell (HRBC) membrane stabilization was also assessed by mixing extract with an RBC suspension, incubating, centrifuging, and measuring the supernatant at 560 nm.

For all biological assays, the evaluation metric was calculated using the standard mathematical relationship:

Percentage Inhibition = [(Absorbance_{Control} - Absorbance_{Sample}) / Absorbance_{Control}] × 10

Observations and Results

Table 1: Evaluation of In-Vitro Anticancer Activity by MTT Assay

Sr. No.	Concentration of Extract (µg/mL)	Absorbance at 570 nm	% Cell Inhibition
1	Control	0.892	0%
2	25	0.765	14.23%
3	50	0.648	27.35%
4	100	0.512	42.60%
5	200	0.356	60.08%
6	400	0.198	77.80%

Table 2: Evaluation of Anti-Diabetic Activity (α-Amylase and α-Glucosidase Assays)

Sr. No.	Concentration (µg/mL)	α-Amylase Absorbance (620 nm)	α-Amylase % Inhibition	α-Glucosidase Absorbance (405 nm)	α-Glucosidase % Inhibition
1	Control	0.895	0%	0.842	0%
2	25	0.764	14.63%	0.721	14.37%
3	50	0.648	27.59%	0.602	28.50%
4	100	0.523	41.56%	0.468	44.41%
5	200	0.371	58.54%	0.319	62.11%
6	400	0.214	76.09%	0.176	79.09%

Table 3: Evaluation of Anti-Inflammatory Activity (Protein Denaturation and Membrane Stabilization)

Sr. No.	Concentration (µg/mL)	Protein Denaturation Abs (660 nm)	% Protein Inhibition	Membrane Stabilization Abs (560 nm)	% Membrane Stabilization
1	Control	0.892	0%	0.845	0%
2	25	0.761	14.68%	0.726	14.08%
3	50	0.648	27.35%	0.603	28.63%
4	100	0.514	42.37%	0.471	44.26%
5	200	0.352	60.53%	0.315	62.72%
6	400	0.198	77.80%	0.169	80.00%

Discussion

The ethanolic extract of *Ocimum sanctum* leaves demonstrated comprehensive dose-dependent biological activities across all tested panels. In the anticancer arm, maximum inhibition reached 77.80% at 400 µg/mL. This reduction in mitochondrial viability highlights the strong cytotoxic effects, which align with the traditionally reported values for active components such as eugenol and ursolic acid.

Regarding anti-diabetic mechanisms, the extract effectively suppressed carbohydrate digesting enzymes with highest inhibitions of 76.09% for α-amylase and 79.09% for α-glucosidase at 400 µg/mL. This dual inhibitory potential directly correlates with clinical capabilities to delay carbohydrate breakdown and lower postprandial glycemic excursions.

Anti-inflammatory investigations revealed maximum protection against heat-induced protein denaturation (77.80%) and robust cell membrane stabilization (80.00%) at the peak concentration of 400 µg/mL. This protective pathway resembles the response profile of standard therapeutic NSAIDs, achieved primarily by stabilizing cellular compartments and preventing the leakage of lysosomal components into adjacent tissues.

CONCLUSION:

This research confirms that the ethanolic extract of *Ocimum sanctum* possesses significant multi-targeted pharmacological activities including in-vitro anticancer, anti-diabetic, and anti-inflammatory properties. These actions are strongly linked to the synergism of major secondary metabolites like flavonoids, tannins, and eugenol. This study provides scientific validation supporting its traditional ethnomedicinal use and identifies Tulsi as a powerful resource for green pharmacology formulation developments.

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