Original article

A comprehensive investigation on alleviating oxidative stress and inflammation in hyperglycaemic conditions through *in vitro* experiments and computational analysis

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ABSTRACT

Protein glycation, hyper-inflammatory reactions, and oxidative stress play a crucial role in the pathophysiology of numerous diseases. The current work evaluated the protective ability of ethyl alcohol extract of leaves from holy basil (*Ocimum sanctum* Linn) against inflammation, oxidative stress, glycation and advanced glycation endproducts formation. Various *in vitro* assays assessed prementioned properties of holy basil. In addition, molecular docking was conducted. The highest hydrogen peroxide reduction activity (72.7 %) and maximum percentage of DPPH scavenging (71.3 %) depicted its vigorous antioxidant abilities. Furthermore, it showed the most excellent protection against proteinase activity (67.247 %), prevention of denaturation of egg albumin (65.29 %), and BSA (bovine serum albumin) (68.87 %) with 600 μg/ml. Percent aggregation index (57.528 %), browning intensity (56.61 %), and amyloid structure (57.0 %) were all reduced significantly using 600 μg/ml of extract. Additionally, the antimicrobial potential was also confirmed. According to a molecular docking study, active leaf extract ingredients were found to bind with superoxide dismutase, catalase, and carbonic anhydrase. As a conclusion, *O. sanctum* has a variety of health-promoting properties that may reduce the severity of many diseases in diabetic patients. However, in order to ascertain the mechanisms of action of the components of its leaves in disease prevention, more thorough research based on pharmacological aspects is needed.

1. Introduction

Numerous diseases have been reported to be linked with oxidative stress and reactive radicals, including ROS (reactive oxygen species) (Rahmani et al., 2022). Oxidative stress is indicated by accumulating many reactive oxygen species (ROS), such as superoxide ions (Anwar et al., 2020a). Besides, ROS have been reported to act as mediators and regulators of vascular function in cell signaling. As signaling molecules, ROS control the development, contraction, and relaxation of vascular smooth muscle cells. The endothelium regulates vascular tone, inflammatory response, vascular growth, platelet aggregation and coagulation, and inflammation (Chen et al., 2018).

An imbalance between ROS and antioxidants can be brought on by some pathophysiological conditions, and this imbalance causes endothelial dysfunction and a number of cardiovascular disease disorders (CVD). The direct oxidizing effects of ROS on macromolecules like proteins, lipids, and DNA are reported to be implicated in cell damage, necrosis, and apoptosis (Moris et al., 2017).

ROS' formation by mitochondria is further associated with several cardiovascular issues in diabetic patients. Numerous cardiac conditions, including cardiac hypertrophy, heart failure, metabolic syndrome, myocardial fibrosis, diabetes, and myocardial infarction, have already been linked to an increase in ROS generation (Dubois-Deruy et al., 2020). An increase in ROS production causes cellular components to sustain structural damage, which in turn causes inflammation, cancer, and other diseases. Because mitochondrial metabolism increases the production of reactive oxygen species (ROS) during oncogene transformation and subsequent cancer progression, it plays a significant role in the induction of carcinogenesis (Ivanova and Yaneva, 2020).

Prolonged inflammation, frequently called chronic inflammation, is represented by the concurrent damage and repair of tissue resulting from the inflammatory process. In addition, it causes a progressive

change in the kind of cells available around the inflammation's location. Pain is often associated with inflammation, characterized by various processes, including increased vascular permeability, protein denaturation, and altered membranes (Gunathilake et al., 2018). Rheumatoid arthritis linked to diabetes has several characteristics, such as a reduced defense against oxidants, neutrophil invasion, increased ROS, a strong response of the body's defenses against trauma, infection, surgery, acute inflammation, ischemia event reperfusion, or cancer, and coronary artery disease with narrowed arteries (Qi et al., 2017).

Chronic hyperglycemia is caused by decreased insulin secretion in the body and impaired responsiveness to insulin in people with diabetes mellitus. One of the most frequent side effects of type 2 diabetes (T2DM) is atherosclerosis (AS). Compared to patients without diabetes, those with diabetes had larger necrotic cores in their coronary arteries as well as increased inflammation involving T-lymphocytes and macrophages. T2DM patients with AS have been found to have elevated serum levels of advanced glycation end products (AGEs), which may be related to the emergence of vascular problems. Glycation of proteins involved in lipid metabolism can disrupt lipid function, cause a disturbance in lipid metabolism, and ultimately result in vascular problems in diabetes (Li et al., 2020a,b). Glycation greatly affects serum albumin. The levels of glycated albumin are two or three times higher in diabetic patients, which can cause irreparable damage like retinopathy, nephropathy, neuropathy, and coronary artery disease. Therefore, the development of these diabetic problems is linked to glycation and AGEs. In addition, a large number of therapeutic medications are distributed systemically, in part due to albumin. Thus, glycation of albumin can affect this process (Guerin-Dubourg et al., 2012).

Chronic inflammation might produce advanced glycation end products (AGEs) (Anwar et al., 2021a; Byun et al., 2017). Increased AGEs generation is more common in diseases like diabetes mellitus (DM), which are linked to hyperglycemia (Anwar et al., 2021a; Nowotny et al., 2015). AGEs may have noteworthy implications Xu et al. (2023), such as cardiovascular morbidities among individuals with diabetes mellitus, owing to their capability to connect intracellular and extracellular matrix proteins, alter tissue framework, functioning, and mechanical characteristics, and contribute to cellular processes by binding to cell surface receptors referred to as receptors for AGEs (RAGE) (Taguchi & Fukami, 2023).

Chronic inflammation is believed to be causally associated with the creation and buildup of AGEs. In addition, AGEs were reported for their implications for CVD. Higher AGEs levels are implicated in a higher risk of vascular diseases in diabetics and non-diabetics (Byun et al., 2017).

Additionally, inflammation is associated with the majority of diseases related to age, and inflammation is a major risk factor for morbidity and mortality in the older people. To reduce the chronic inflammatory process in the human body, which includes dermatitis, colitis, arthritis, neurological diseases, and cancer, it is always important to develop new anti-inflammatory medications (Ferrucci and Fabbri, 2018). The current standard of care for inflammatory conditions such as RA (rheumatoid arthritis) in people with diabetes includes the use of biologics, NSAIDs, DMARDs, steroids, and painkillers. Since the majority of these methods have drawbacks, adverse effects, or both, tailored therapies that target RA's pathways are far more appropriate. Small molecule inhibitors are currently the subject of extensive research for a wide range of diseases and are thought to be one of the most effective forms of targeted therapy (Almarfadi et al., 2022).

Many herbal remedies are believed to have demonstrated beneficial qualities in managing and preventing various ailments (Anwar et al., 2020b; Rahmani et al., 2023). The prototype for contemporary synthetic compounds is found in natural sources. To prevent diseases and alter immune function, a great deal of study has been done on immunomodulators that come from natural sources (Najmi et al., 2022). Significant antioxidant and other actions are exhibited by a number of naturally occurring compounds that have been found from medicinal plants. Thus, the use of medicinal plants has become crucial to meeting both immediate and long-term health needs (Rashid et al., 2016). Plant-based pharmaceutical approaches provide a safe, efficient, and economical substitute for traditional methods involving animal cell cultures or microbial fermentation in the drug-development process. Consequently, pharmaceuticals made from plant-based natural chemicals can provide patients with more rapid and easier access to treatment (Chaachouay and Zidane, 2024).

Current research aims to offer insightful information into the oxidative stress-reducing capacity, AGEs formation-reducing activity, and antimicrobial and inflammation suppression actions found in *O. sanctum*. The *in silico* study is focused on exploring the docking interaction of naturally occurring components in *O. sanctum*, viz., phenolic molecule, phytosterol, terpenoid, fatty alcohol, and polyphenolic acids with superoxide dismutase, catalase, and carbon anhydrase.

2. Material and methods

2.1. Procedure for extraction

The leaves of *O. sanctum* were harvested from G. B. Pantnagar University, Uttrakhand, India, in March. A master gardener, who kept the records of different plants, their families, genera, and species with photos in a record register, guided us to the plant's location. The plant was further identified by a doctoral student on the basis of the height and shape of the plant as well as the form, size, and shape of the leaves. It was later compared with the herbarium of the Department of pharmacy, Mohan Institute of Nursing and Paramedical Sciences, Bareilly, India. A previously described approach (Anwar et al., 2021b) was used to extract 70 g of powdered leaves employing 500 ml of ethanol as a solvent. The extract was then used for further *in vitro* evaluation studies. Similar phytochemical analyses of *O. sanctum* were carried out by previous researchers with slight modifications on extracts prepared in different solvents (Chaudhary et al., 2020; Panchal and Parvez, 2019).

2.2. 2.2. Phytochemical screening

According to previously published procedures, phytochemical tests were done to identify various chemical components like flavonoids, carbohydrates, saponins, tannins, glycosides, and alkaloids (Rahmani et al., 2022).

2.3. Total phenol content

In many tubes, Folin-Ciocalteu's reagent (2.5 ml; 10 %) and 500 μl of crude ethanol extract of *O. Sanctum* leaves (1 g/l) were added. After adding sodium carbonate (2 ml; 7.5 %), the resultant was placed in the absence of light for half an hour (Anwar et al., 2020a). The measurement of absorbance was done at 760 nm. To compute the entire phenolic quantity in milligrams of gallic acid equivalents (GAE), a typical calibration curve using different amounts of gallic acid (50–250 μg/ml) was established. Every test was run three times.

Total phenolic content = $(Y \times V)/W$

where W represents the weight of the plant extract (g), V represents the total volume of the extract (ml), and Y denotes the amount of gallic acid (mg/ml).

2.4. Total flavonoid content

AlCl$_3$ solution (0.5 ml; 2 %) was mixed with extract (50 μg/ml; 0.5 ml). After a 10-minute incubation period at room temperature (in the dark) and vigorous shaking, the mixture was submitted to analysis of absorbance at 420 nm. Quercetin (concentration range: 20–250 μg/ml) was utilized as the reference compound for quantitative analysis. The

findings were presented as the quercetin equivalent (Anwar et al., 2020a).

Total flavonoid content = Y × V/W

where V is the volume of plant extract (ml), W shows the weight of the ethanol solution of leaves (g), and Y denotes the amount of quercetin (mg/ml).

2.5. Hydrogen peroxide-reducing(H_2O_2) activity

The H_2O_2 solution was made in phosphate buffer (pH 7.4), and 40 mM H_2O_2 (1 ml) solution was combined with 0.1 ml of extract concentrations (600, 500, 400, 300, 250, 200, 150, 100, 75, 50 μg/ml) or ascorbic acid (100 and 200 μg/ml) in order to assess the extracts' ability to scavenge hydrogen peroxide (H_2O_2). After 10 min, H_2O_2's absorbance at 230 nm was measured in comparison to a blank solution that contained phosphate buffer but no H_2O_2· H_2O_2 reducing capacity of the extract and the reference material, ascorbic acid, was measured (Ruch et al., 1989; Anwar et al., 2020a) using following equation.

Reducing capacity (%) = [(C–S)/C] × 100

S is the sample's absorbance (extract with H_2O_2 solution). C denotes the absorbance of the H_2O_2 solution alone or under control.

2.6. DPPH assay

Based on the previously published articles regarding DPPH radical (1,1-diphenyl-2-picryl-hydrazyl) reducing potential, the extract's anti-oxidant capability and the reference were examined (Almatroodi et al., 2020; Anwar et al., 2021b). Briefly, 1 ml of freshly made 0.3 mM DPPH solution and 1 ml of *O. sanctum* extract at various concentrations (50–600 μg/ml) were added in methanol and then left in the dark for half an hour. The measurements were done at 517 nm against a blank. Ascorbic acid was used as a reference.

Percent DPPH scavenging potential = [(C–S)/C] × 100.

S is the sample's absorbance (a DPPH solution containing extract), and C is the control's absorbance (DPPH in methanol).

2.7. Denaturation of bovine serum albumin by heat: Inhibition assay

About 0.2 ml of hen's egg albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4), and 2 ml of extract in various concentrations were mixed to prepare 5 ml of reaction mixture. Double-distilled water in a comparable volume served as the control. After roughly 15 min of incubation at 37 °C in an incubator, the mixture was heated to 70 °C for five minutes. Following cooling, a pure blank was used to test their absorbance at 660 nm. For the purpose of determining absorbance, diclofenac sodium, a standard medication, was utilized (Anwar et al., 2021b). This is the calculation used to get the percentage inhibition of protein denaturation:

Inhibition Percentage = [(C–S)/C] × 100.

The control has an absorbance of C. However, the test solution containing extract or diclofenac has an absorbance of S.

2.8. Heat-induced albumin denaturation inhibition

With a small modification, the procedure outlined by Anwar et al. (2021b) and Sakat et al. (2010) was used to investigate the potential of the given extract to suppress albumin denaturation. Extract was synthesized at ten different concentrations (600–50 mg/mL) using Tris buffer (0.05 M, pH 6.6). A 1 % (w/v) aqueous solution of bovine albumin fraction V (Sigma-Aldrich, Saint-Louis, MI, USA) was combined with 500 μl of each sample. After twenty minutes of incubation at 37 °C, the

reaction mixture was heated for five minutes at 70 °C. The mixture's A600 was ascertained after it cooled to room temperature. The positive control was diclofenac (ibuprofen). Three duplicates of each experiment were set up.

Heat-induced BSA denaturation inhibition (%) = [(C–S)/C] x 100.

The control solution contained neither extract nor ibuprofen and had an absorbance of C; the test solution containing ibuprofen or extract had an absorbance of S.

2.9. Evaluation for proteinase action inhibition

Extract (50–600 μg/ml) was added to a reaction mixture (2.0 ml), with Tris-HCl buffer (1.0 ml, 25 mM; pH 7.4) and 250 μl of trypsin. Incubation of the mixture lasted five minutes at 37 °C (Anwar et al., 2020a; Sakat et al., 2010). Further, casein (1.0 ml, 0.8 % w/v) was incorporated into each vial. For a further twenty minutes, the mixture was incubated. Finally, 2.0 ml of perchloric acid (70 % v/v) were included in each vial. The supernatant after centrifugation was analyzed at 280 nm for its absorbance against buffer. The results were compared with diclofenac sodium. The investigation was carried out three times.

Inhibition of proteinase action (%) = [(C–S)/C] × 100

When an experimental solution contains either extract or diclofenac, its absorbance is S, while the absorbance of a control solution, which is a control solution without either component, is C.

2.10. Screening for the antiglycation property of O. sanctum extract

2.10.1. Preparation of glycated BSA
BSA was glycated using previous reports (Brownlee et al., 1968; Anwar et al., 2020a). For this purpose, BSA (10 mg/ml) and glucose (0.500 M) were placed together in phosphate buffer, pH 7.4, in sterilized tubes with numerous amounts of prepared extract (50–600 μg/ml). The tubes were kept in the dark for a fortnight at room temperature. The molar extinction coefficient method was used to investigate the BSA content. The storage of all vials was allowed at −20 °C unless a needful examination was feasible. Three duplicates of every trial were conducted.

2.10.2. Investigation of browning intensity
The glycation of various samples contributes to the browning (Anwar et al., 2020a). A spectrophotometer recorded each sample's optical density at 420 nm (Anwar et al., 2020a; Kumar & Ali, 2019; Brownlee et al., 1986). All of the tests were run three times. The relative percentage of browning intensity was marked using the formulae provided below (Anwar et al., 2020a).

Percentage relative browning intensity = [(C–S)/C] × 100.

S is the absorbance of glycated BSA kept in *O. sanctum*. In contrast, C is the sign of the absorbance of glycated BSA without extract.

2.10.3. Protein aggregation index
Numerous detrimental situations, such as the deterioration of therapeutically essential proteins and neurological diseases, have been connected to protein aggregation. Glycation is the process that produces Schiff bases by attaching reducing sugars to the arginine and lysine residues. Significant changes in native protein structure and function, including the formation of covalent cross-links with neighboring proteins, have been associated with glycation, and this can be evaluated using a spectrophotometer (Rahmani et al., 2022). The absorbance of different glycated samples with or without extract was assessed at 280 and 340 nm.

Aggregation index (%) = [I_{340} / (I_{280} − I_{340})] × 100

I_{340} = Absorbance of numerous preparations at 340 nm, and I_{280} = Absorbance or optical density of various samples at 280 nm.

2.10.4. Inhibition of fibrilization

Congo red binds to proteins that resemble amyloid via interactions between two positively charged amino acid residues from two different protein molecules and two negatively charged sulfonic acid groups of Congo red, which are correctly oriented due to the peptide backbone's beta-pleated sheet conformation (Klunk et al., 1999). This assay was performed for the investigation of the formation of amyloid fibrils in glycated BSA (Kumar & Ali, 2019). Congo red was prepared according to our previous publication (Anwar et al., 2020a). Individual absorbance measurements were taken for glycated-BSA with *O. sanctum* extract (50–600 μg/ml), glycated protein kept alone, native protein, and the Congo red background. As a summary, Congo red (0.100 ml; 0.100 mM) and 0.500 ml of AGEs-BSA/native BSA (0.100 mM) were simultaneously incubated for 10 min at room temperature. The wavelength for the spectrophotometric study was 530 nm.

Amyloid inhibition (%) = [(Ic–Is)/Ic] × 100

Ic stands for BSA and glucose system's absorbance, whereas Is stands for absorbance of glycated BSA having extract.

2.11. Biophysical studies to investigate AGEs formation inhibiting properties of the extract

500 mM glucose and 0.2 mg/ml of BSA were kept in sodium phosphate buffer (20 mM, pH 7.4) for fortnights at room temperature in a shaking water bath with extract (50–600 μg/ml) or without extract. In the control group, BSA was incubated with phosphate buffer (20 mM, pH 7.4). Bacterial contamination was avoided by adding three mM of sodium azide. Each sample was dialyzed in the same buffer with the same pH used in the experiment but a different molarity (0.1 M) after the completion of incubation.

The absorbance study, AGEs specific fluorescence, and thioflavin T (ThT) fluorescence study were used for biophysical studies according to procedures described in our previous article (Anwar et al., 2014).

2.12. Antimicrobial activity

The extract's antimicrobial properties were investigated. *Candida albicans*, two gram-positive cocci strains (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213), and three gram-negative bacilli strains (*Klebsiella pneumonia* ATCC 700603, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) were used in this study. The microbiology lab at Aligarh Muslim University in India provided the microbial strains.

2.12.1. Dilutions and inoculum preparations

Fresh, pure strains of bacteria and fungi were employed to establish an inoculum with Muller-Hinton broth. Furthermore, the McFarland standard 0.5 was utilized for all bacterial and fungal samples. Two different extract strengths (25 and 50 mg/ml) were obtained by periodically diluting the *O. sanctum* extract stock solution in sterile, distilled water.

3. Procedure for performing the well diffusion test

The antibacterial efficacy against the chosen pathogens was investigated using an agar well diffusion experiment (Anwar et al., 2021b). The Muller-Hinton agar was infused with bacteria. Two wells were created in the agar plate using the blue micropipette tips on the back. Molten MHA agar was included to seal the bottom of the wells after ten minutes of waiting for the melted agar to solidify. Fill two separate wells with different volumes of extract after that. Erythromycin 15,

vancomycin 30, imipenem 30, amikacin 30, and fluconazole 10 were the positive controls. Following incubation, the zone of inhibition surrounding the wells was examined, and the diameter of the zone was established in millimeters using a ruler (mm).

3.1. Statistical analysis

Each experiment was run three times, and the results were reported using the standard error of ± . ANOVA was implemented to perform the statistical analysis. For a given experiment, a probability of $p < 0.05$ was deemed statistically significant.

3.2. Docking investigations

3.2.1. Receptors

To forecast potential interactions between the ingredients and metabolites of *O. sanctum* extract, the cellular enzymes evolved for antioxidant activities (superoxide dismutase and catalase) and anti-inflammatory activity (carbon anhydrase) were chosen for this investigation. Three-dimensional structures of enzymes are available on the Protein Databank homepage (https://www.rcsb.org/, accessed on January 15, 2024) and downloaded in PDB format. The resolution, source organisms, coupled ligand accessibility for active site reference, and active site residues were considered while choosing the protein structures.

3.2.2. The ligands

The active ingredients of tulsi, or *O. sanctum* Linn, are found to include phenolic molecules (Eugenol), phytosterol (Beta-Sitosterol), terpenoid (Ursolic acid), fatty alcohol (1-Triacontanol), and polyphenolic acids (Salvianolic acids, Rosmarinic acid) (Baliga et al., 2013; Beltrán-Noboa et al., 2022; Prakash and Gupta, 2005). The NCBI PubChem compounds database (https://pubchem.ncbi.nlm.nih.gov/) provided the structures of all ligands in 3D-structure-data file (SDF) format. The data was retrieved on January 15, 2024. All files were changed into PDB format using the Open Babel program (https://openbabel.org/wiki/Main_Page, accessed on January 15, 2024).

3.2.3. Docking

This technique is used for assessing ligand binding to a receptor's active region. To determine how the substrate and enzyme interacted, molecular docking was used (Trott and Olson, 2010). The proteins and ligands are prepared and then run for interaction through AutoDock Vina v.1.2.0 (https://vina.scripps.edu/, accessed on January 15, 2024), and visualization of interactions is done by BIOVIA Discovery (https://discover.3ds.com/discovery-studio-visualizer-download/, accessed on January 15, 2024). The workspace was cleared of any water molecules. Table No. 1 shows the SBDSite_Sphere XYZ coordinates and radius for each ligand.

Each protein receptor received polar hydrogens before the docking experiment, and the energy was reduced using Kollman's partial atomic charges. The AutoDock PDBQT file format, which contains hydrogens in all polar residues, was used to record the processed receptor structure. With the aid of the program MGL Tools Version 1.5.7 (https://ccsb.scripps.edu/mgltools/downloads/, accessed on January 15, 2024; Morris et al., 2009), the receptors and ligands were supplemented with hydrogen atoms. The limit on the number of runs for the procedure was fixed at ten.

The posture with the highest affinity was used to determine the ligands' docking conformation, which is the most negative Gibbs' free energy of binding/ΔG. The best dockings were considered for additional analysis focused on the free energy of binding (shown as negative numbers).

4. Results

Diabetes mellitus is an epidemic metabolic disease with a high rate of mortality and morbidity among people suffering from it. Diabetes and its complications are still largely untreated, despite significant studies into the molecular causes of this disease and the development of novel drugs. Glycation of proteins within the body is linked to a number of late-onset diabetes problems (Anwar et al., 2020a). Therapeutic compounds, particularly those that combat diabetes, have long been abundant in the natural flora. The protein-glycation cascade is known to involve oxidative processes. Plant-derived antioxidants have a variety of biological effects and have fewer negative effects than many synthetics. These antioxidant properties might be due to their potential to scavenge free radicals, donate hydrogen atoms or electrons and/or chelate metal cations, absorb and neutralize free radicals, quench singlet and triplet oxygen, or break down peroxides. As a result, natural antioxidants may be able to reduce the incidence and mortality rates of a number of diseases linked with oxidative stress that affect humans (Siddiqui et al., 2016).

This manuscript evaluated the antioxidant, anti-inflammatory, antiglycating, and AGEs formation inhibiting activities of the ethanol leaf extract of *O. sanctum*. In addition, we confirmed the antimicrobial potential of the extract. The purpose of this study was to ascertain whether this crude extract could be able to protect against inflammation, glycation, and AGEs formation. Our molecular docking study validated the molecular mechanisms of selected phytoconstituents of *O. sanctum* against oxidative stress and inflammation, as well as its ability to protect enzymes and other proteins from conformational changes, as well as to connect various potential molecules of it to their unique chemical properties. This further confirms our hypothesis that *O. sanctum* extract might be capable of protecting these molecules from denaturation caused by glycation as well as defending against oxidative stress-mediated various health ailments. As per the best of our knowledge, all of these topics have not been covered together in a single previous study till now. Further, the most recent studies were reviewed, and they added new realms to the existing knowledge in a new and innovative way. All of those are compiled in the present study, making it noble and significant compared to previous researches for future investigations and drug development.

This study focuses on the antioxidant, protein denaturation inhibition, anti-glycation, and anti-AGEs formation characteristics of *O. sanctum*, which is considered a scared plant in India (see Fig. 1).

There are several studies that have identified the presence of numerous compounds present in *O. sanctum* extract that might be responsible for its pharmacological activities. A Few of previous reports indicated the presence of a mixture of limatrol, carvacrol, methylchavicol, eugenol, and caryophylline in the leaf essential oil of *O. sanctum*. Besides, the presence of ursolic acid and n-triacontanol was also confirmed in its leaves. However, the seed oil of *O. sanctum* contained sitosterol and fatty acids A, B, and C. Three triterpenes and sitosterol are found in its roots (Baliga et al., 2013; Borah & Biswas, 2018). The evaluation and quantitative assessment of a plant extract's capacity to scavenge or reduce free radicals is essential since it sets standards for studies on human health, storage tests, and the application of the extracts as natural antioxidants. The DPPH technique is most commonly used to assess *in vitro* antioxidant activity (Rahmani et al., 2022).

In March, holy basil (*O. sanctum*) plant leaves were collected from G. B. Pantnagar University, Uttrakhand, India, and evaluation of its significant pharmacological characteristics was done using the ethanol

Table 1
Physical screening.

Factors	Physical properties
Mass of the dehydrated leaf powder	100 g
Yield	19.23 %
Extract's nature	Ethanol
Leaves colour	Green
Colour of extract	Greenish brown
Odor	Aromatic
Texture of leaves	Smooth

Fig. 1. The *O. sanctum* (Holy basil) plant shows leaves and seeds.

extract prepared from its leaves. Table 1 displays the physical properties of the ethanol extract of O. sanctum leaves. Furthermore, phenolic components were found to be 298.76 ± 0.25 mg gallic acid equivalent/100 g dry weight of extract. However, total flavonoids in this extract were 173.12 ± 0.58 quercetin equivalents/100 g dry weight of extract. Table 2 represents the qualitative screening of phytochemicals in the extract. The two most frequent antioxidants found in plants are phenolics and flavonoids. Therefore, the significant quantities of poluphenols and flavonoids might contribute to the antioxidant nature of holy basil.

4.1. H2O2 reduction potential

The hydroxyl radical causes cellular stress by reacting indiscriminately with every macromolecule it comes into contact with. In addition to causing direct oxidation of lipids, proteins, and DNA, H_2O_2 also causes necrotic cell death through mitochondrial-driven apoptosis Okoko and Ere (2012). Therefore, the reduction of these cellular impacts and the substantial enhancement of health and wellbeing could be achieved through hydrogen peroxide scavenging. Considering it, a H_2O_2-reducing assay of *O. sanctum* was conducted. The reducing capability of the extract changed according to the ethanol extract concentration (Fig. 2). A significant percentage of H_2O_2 was reduced at all of the extract doses, ranging from 5.42 % to 72.7 %.

4.2. DPPH assay

The DPPH assay is a useful indicator of the typical antioxidant profile since most natural antioxidants have reactive hydrogen atoms that act as a reductant. According to the findings of the current investigation, *O. sanctum*'s capacity to scavenge free radicals ranged from 6.98 to 71.3 % (Fig. 3). The methanol extract with 600 μg/ml had the maximum scavenging activity (71.3 %), whereas the extract with 50 μg/ml had the lowest (6.98 %). At 200 μg/ml, ascorbic acid showed 67.99 %. Given their exceptional ability to scavenge DPPH and their other established advantages, the extract shows promise for use as an antioxidant and therapeutics against oxidative stress-mediated diseases.

4.3. BSA denaturation inhibition

By inhibiting the activity of the cyclooxygenase enzyme, non-steroidal anti-inflammatory drugs (NSAIDs) reduce inflammation. On the other hand, ulceration, bleeding, perforation, and blockage are negative effects of these drugs. Denaturation of proteins induces the development of auto-antigens in illnesses such as rheumatic arthritis, cancer, and diabetes, which are situations of inflammation as indicated above. Therefore, it is possible to reduce inflammatory activity by inhibiting protein denaturation (Dharmadeva et al., 2018). Denaturation refers to how proteins alter their tertiary and secondary forms in reaction to extrinsic stresses like temperatures, chemicals, or their combination. Structural alteration of proteins is a widely accepted factor involved in chronic inflammation (Anwar et al., 2020a). According to current research, *O. sanctum* leaf extract effectively inhibits the denaturation of BSA caused by heat. The most significant inhibition (69.13 %) was found at 600 μg/ml (Fig. 4). Nevertheless, the maximum

Table 2
Phytochemical constituents and quantitative screening.

Phytochemical constituents	Presence in extract
Alkaloids	+
Saponins	+
Tannins	+
Flavonoids	+
Glycosides	+
Terpenoids	+
Phenolic compounds (FeCl3 test)	+

inhibition was 68 % when ibuprofen, a common anti-inflammatory drug, was administered at 200 μg/ml.

4.4. Anti-proteinase activity

Leukocyte proteinase has been shown to be crucial in the development of tissue damage during inflammatory reactions, and protease inhibitors, such as flavonoids, have been shown to offer a considerable degree of protection (Leelaprakash and Mohan, 2011). Proteinase inhibitors prevent tissue damage by preventing different proteases from acting on them. According to our research, leaf extract also markedly reduced proteinase activity. The highest proteinase inhibition activity of 67.27 % was seen in leaf ethanol extract (600 μg/ml) (Fig. 5). Nevertheless, diclofenac sodium demonstrated maximal inhibition (65.968 % at 200 μg/ml).

4.5. Egg albumin denaturation inhibition activity

Using the denaturation approach, the egg albumin method offers a less expensive alternative for evaluating the anti-inflammatory properties of herbal medicine (Dharmadeva et al., 2018). Auto-antigen formation might be caused by *in vivo* protein denaturation in patients with rheumatic issues. *O. sanctum* extract showed inhibition of heat-induced denaturation of proteins in a concentration-dependent way, based on the evidence presented (Fig. 6). However, the maximum inhibition for the popular medication diclofenac was 64.57 % (200 μg/ml).

4.6. Impact on browning intensity of glycated samples

To ascertain whether the extract suppresses the early glycated-BSA, we examined the extract's effect on browning. There was a noticeable variation in the absorbance at 420 nm between the glycated-BSA samples treated with leaf extract and the glycated-BSA samples (incubated without glucose). Intense brown pigments emerge during the first phase of the three-step chemical process producing AGEs. Browning intensity can be considered as one of the first signs of glycation. Our findings showed that the browning intensity of the glycated-BSA samples was considerably reduced due to the extract. The reduction of browning intensity was highest for BSA-glucose sample incubated with 600 μg/ml of leaf extract and it was 56.61 % inhibitory effect on browning intensity. 100 % browning, or the maximum browning intensity, was noticed for glucose incubated with BSA only (Fig. 7).

4.7. Protein aggregation index

Glycation occurs at a polypeptide's side chains or N-terminal group and it leads to alteration of the structure and functions of proteins. Furthermore, carbonyl groups attached to proteins trigger the production of clusters. Therefore, glycation causes protein aggregation. Numerous reports have been published regarding aggregation of proteins due to glycation (Sherwani et al., 2022; Anwar et al., 2020a). BSA-glucose incubated alone exhibited the highest degree of aggregation (red column). However, *O. sanctum* extract significantly decreased the glycation-induced aggregation of various BSA samples in a concentration dependent manner (Fig. 8).

4.8. Congo red (CR) assay

It has been reported that glucose modifies the secondary structure of BSA by increasing the solvent-accessible surface area of the glycation-prone regions. One well-known test for cross-structure in the glycated protein is the CR technique, which exhibits specific absorption at 540 nm following binding (Anwar et al., 2020a; Rubab et al., 2016). There was a rise in intensity at 540 nm specific to amyloid when the absorbance of solutions of CR-glucose samples was evaluated. Glycation-induced modification of the native BSA's canonical secondary
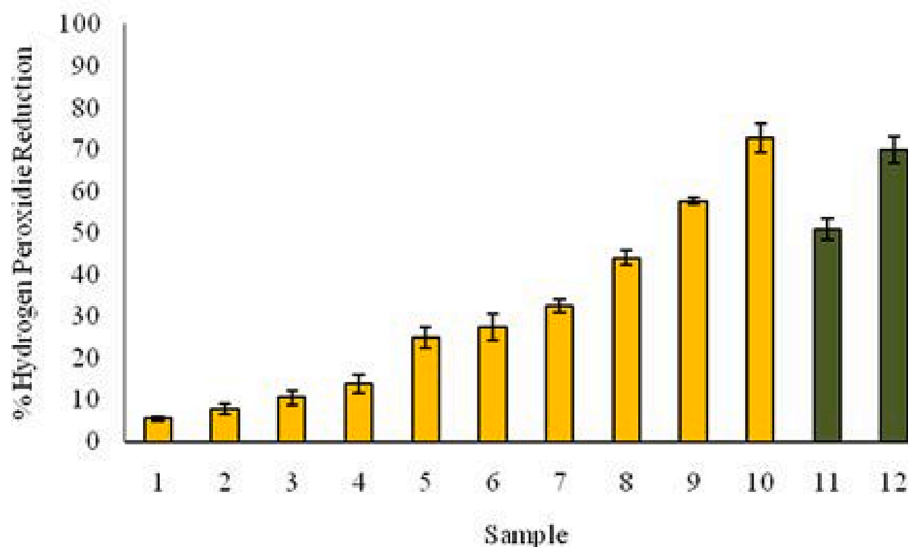
Fig. 2. The H_2O_2-reducing capability of an ethanol extract of *O. sanctum* leaves expressed as a percentage. Samples containing leaf ethanol extract (0.050, 0.075. 0.10, 0.15, 0.20. 0.25, 0.30, 0.40, 0.50, and 0.60 mg/ml) are displayed in yellow columns. In contrast, samples containing ascorbic acid (0.10 mg/ml and 0.20 mg/ml) are shown in the green column. For this figure, it was found that the p-value significance seemed under 0.05 (n = 3, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
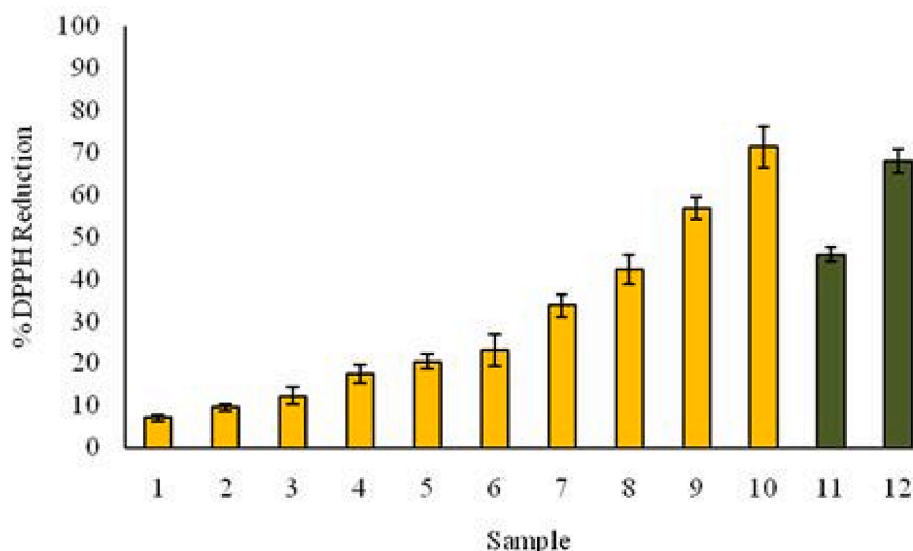

Fig. 3. Percentage DPPH reduction. Samples containing ethanol extract (0.050, 0.075. 0.10, 0.15, 0.20. 0.25, 0.30, 0.40, 0.50, and 0.60 mg/ml) are shown by yellow bars. Green bars reflect ascorbic acid (0.10 and 0.20 mg/ml). For this figure, it was found that the p-value significance seemed under 0.05 (n = 3, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

structure was lessened with the addition of extract. A concentration-dependent reduction in BSA fibrillation was observed in the glycated samples treated with *O. sanctum* extract (Fig. 9).

4.9. UV-absorption studies

Proteins undergo structural changes as a result of non-enzymatic glycation. Compared to native BSA (kept alone), glycated BSA (in the absence of extract) displayed a significant hyperchromicity or prominent peak at a wavelength of 280 nm. It has been suggested that alterations affecting the microenvironment of aromatic and amino acid residues may be the basis of the hyperchromicity (Anwar et al., 2014). As the extract concentration increases, there was a noticeable reduction in the hyperchromicity of those BSA samples incubated with both

extract and glucose (Fig. 10).

4.10. Fluorescent AGEs inhibition

BSA treated with glucose had a peak of fluorescence intensity at 450 nm (Anwar et al., 2014; Sherwani et al., 2022). In this experiment, BSA, kept with glucose, serves as a negative control. As the content of extract increased, BSA samples kept with glucose continuously displayed a reduction in the specific fluorescence of AGEs at λ450 (Fig. 11). The maximum inhibition of AGEs fluorescence was observed in a sample with a 600 μg/ml extract concentration. According to this study, *O. sanctum* extract protects against the formation of AGEs in a dose-dependent manner.
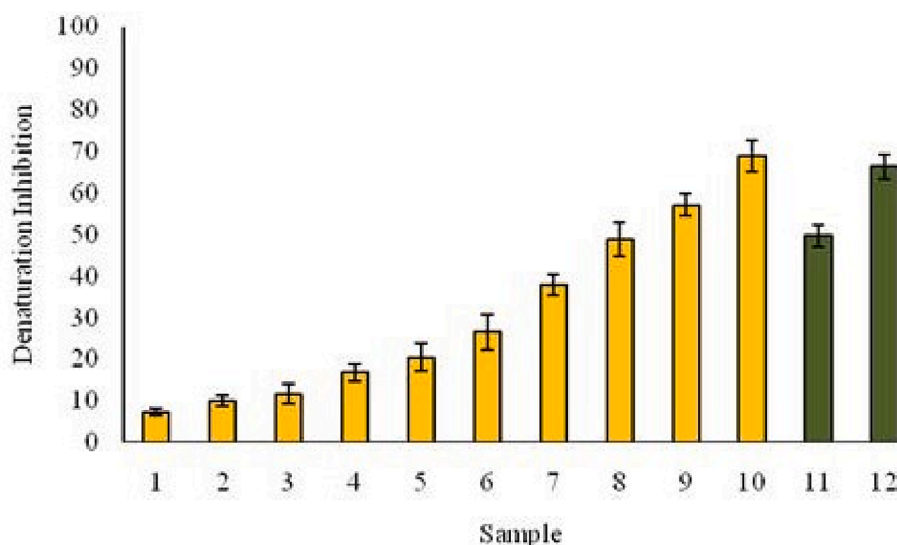
Fig. 4. BSA denaturation inhibition (%). Samples with concentrations (0.050, 0.075. 0.10, 0.15, 0.20. 0.25, 0.30, 0.40, 0.50, and 0.60 mg/ml) of leaf extract are shown by yellow bars (numbered 1–10) on the X-axis. Samples with ibuprofen concentrations (0.10 μg/ml and 0.20 mg/ml) are shown by bars in green columns. The findings are shown as means ± SEM (n = 3, p < 0.05). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
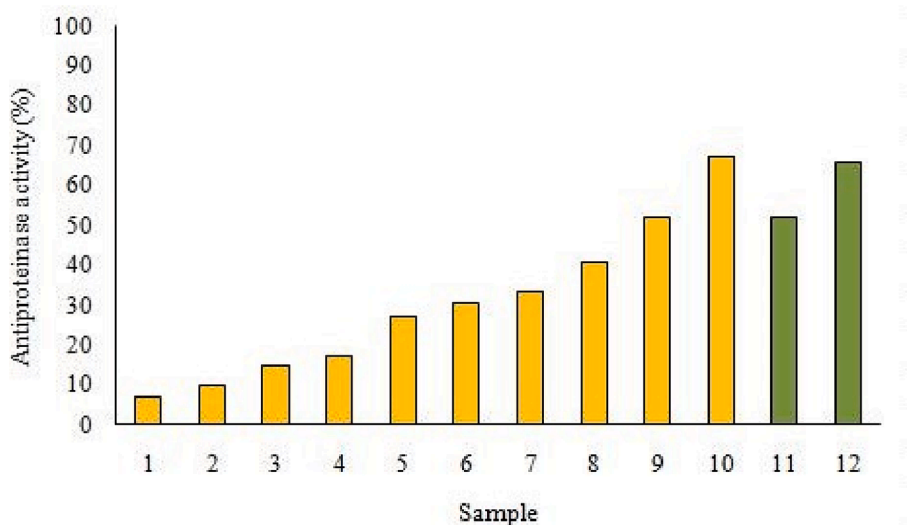

Fig. 5. Percentage anti-proteinase activity. Samples with varying quantities of ethanol extract (0.050 to 0.600 mg/ml) are shown in yellow columns. Green columns offer diclofenac sodium samples (0.10 mg/ml and 0.20 mg/ml). The means ± SEM (n = 3, p < 0.05) are displayed for the data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.11. Impact on fibrilization

When glucose glycates BSA, ThT-specific fluorescence (at 480 nm) increases. This indicates the formation of fibrils as a result of glycation. However, ThT-specific fluorescence at 480 nm declined in the BSA samples incubated with both glucose and extract with an increase in extract content (Fig. 12). The findings showed that BSA treated with glucose alone showed the highest ThT-specific fluorescence at 480 nm. According to the findings, leaf extract exhibited protection against the production of AGEs and fibrilization in a dose-dependent manner.

4.12. Antimicrobial potential

Over the past three decades, the pharmaceutical industry has created new antibiotics. However, these antibiotics have not been able to stop the spread of several bacteria that possess the genetic capacity to spread and develop drug resistance. Consequently, these bacterial infections are linked to a high rate of morbidity and mortality, particularly in individuals with weakened immune systems. Furthermore, numerous studies have demonstrated the adverse effects of antibiotic overuse and misuse, which can damage the immune system as well as important organs like the liver, kidneys, and certain cells like the spleen and pancreas. Therefore, the hunt for novel chemotherapeutic options to eradicate diseases brought on by drug-resistant bacteria and lessen the damage caused by antibiotics has been aided by the established efficacy of traditional therapy (Ababutain, 2011).

In our investigation, we looked into the antimicrobial actions of *O. sanctum* extract against both bacteria and fungus. The ethanol extract demonstrated a significant inhibitory zone against all tested microorganisms at 100 mg/ml (Table 3).
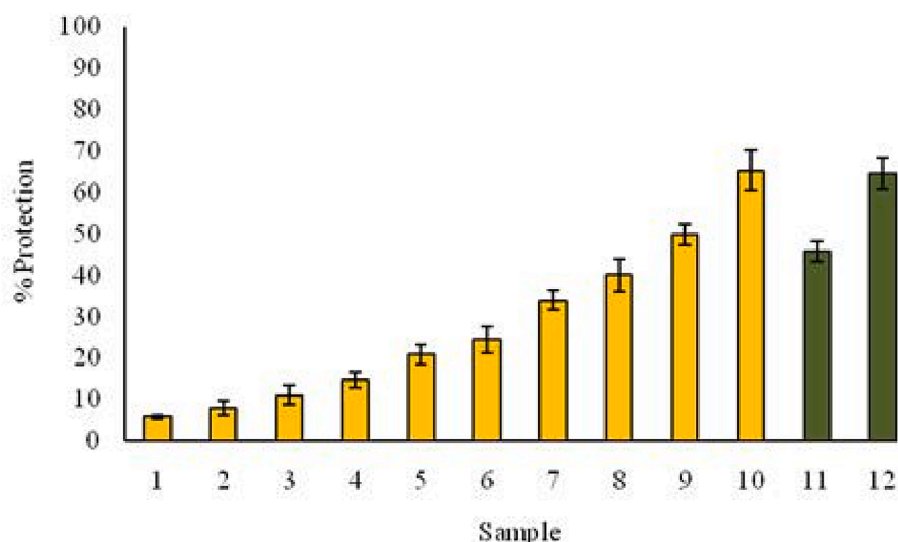
Fig. 6. The percentage of egg albumin that is protected from denaturation caused by heat. The yellow bars numbered 1 to 10 on the X axis indicate leaf extract samples (0.050 to 0.600 mg/ml). Samples containing different amounts of diclofenac (0.10 mg/ml and 0.20 mg/ml) are shown in green columns. The means $\pm$ SEM ($n = 3$, $p < 0.05$) present the results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
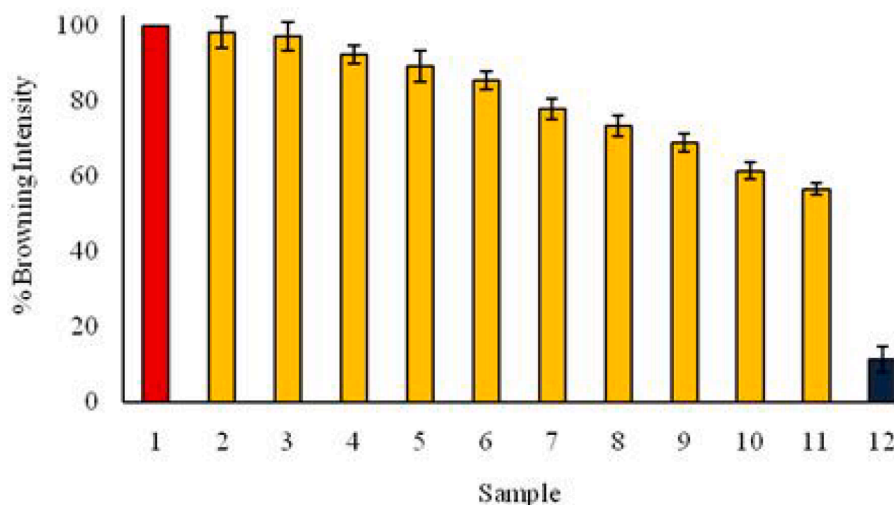

Fig. 7. This figure shows how the presence of *O. sanctum* leaf extract reduces the browning intensity. BSA is believed to have glycated to 100 % of its initial form after 15 days of incubation with glucose (100 % browning: Red column). Browning, or the degree of glycation, is demonstrated to be reducing as extract concentration increases in samples 2–11 (Yellow columns), which included 0.050 to 0.600 mg/ml of extract. Sample 12 (blue column) was incubated with BSA without extract or glucose for 15 days. The data is shown as means $\pm$ SEM ($n = 3$, $p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4.13. Molecular docking study

The present study examined the molecular interactions between protein/enzyme molecules, such as carbon anhydrase, catalase, and superoxide dismutase (Fig. 13) and active constituents of tulsi, including eugenol, beta-sitosterol, ursolic acid, 1-triacontanol, salvianolic acids, and rosmarinic acid (Fig. 14). Table 4 displays the study's details. Fig. 15 shows the molecular interactions that occur between ligands and enzymes.

The molecular dynamics analysis reveals that the superoxide dismutase enzyme interacts with Eugenol and Beta-Sitosterol ligands. Eugenol binds with a -4.4 kcal/mol binding energy and interacts with the amino acids Gly12, Pro13, Val14, and Leu144. Beta-sitosterol binds with a stronger binding energy of -6.2 kcal/mol and interacts with the amino acids Asp11, Pro13, and Leu144. The SBDSite_Sphere coordinates and radii provide information about the binding site locations and sizes

for these interactions.

The molecular dynamics analysis indicates that the catalase enzyme binds to two ligands – ursolic acid (PubChem CID 64945) and 1-triacontanol (PubChem CID 68972). Ursolic acid has a stronger binding energy of -6.1 kcal/mol and interacts with 12 amino acid residues, while 1-triacontanol has a binding energy of -4.7 kcal/mol and interacts with 15 amino acid residues. Both ligands bind within the same binding site defined by the SBDSite_Sphere coordinates and radius.

The molecular dynamics analysis reveals that the carbon anhydrase enzyme binds to two ligands – salvianolic acids (PubChem CID 86278266) and rosmarinic acid (PubChem CID 5281792). Salvianolic acids have a strong binding energy of -13.7 kcal/mol and interact with 9 amino acid residues, including Lys178, His94, and His64. Rosmarinic acid has a binding energy of -7.0 kcal/mol and interacts with 12 amino acid residues, including Gln92, His94, and His64. Both ligands bind within the same binding site defined by the SBDSite_Sphere coordinates
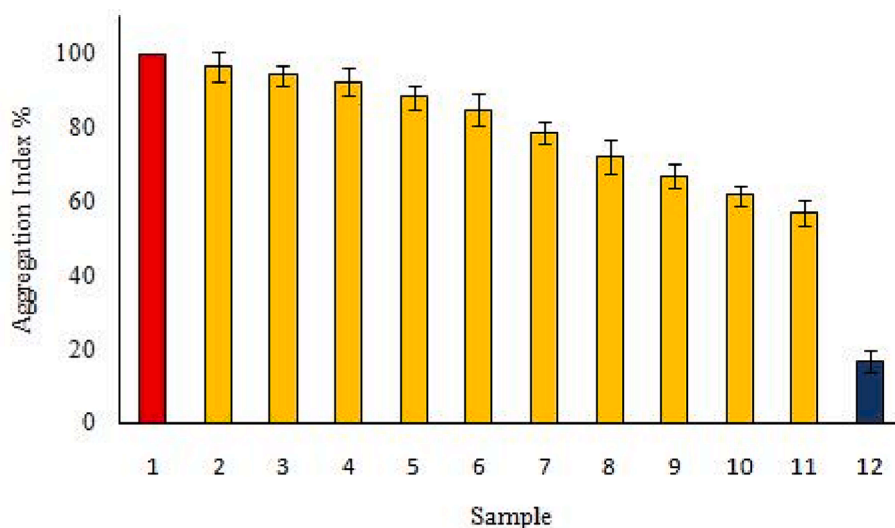
Fig. 8. Aggregation decreases due to leaf ethanol extract. BSA (sample 1) has been incubated with glucose (Red bar) for fifteen days. It is said to have the most excellent glycation aggregation index. Samples 2 through 11 contained leaf (Yellow columns) ethanol extract (0.050 mg/ml to 0.600 mg/ml). BSA in Sample 12 (Blue column), which had not been exposed to glucose or extract during incubation, exhibited the lowest aggregation index. The means $\pm$ SEM (n = 3, p < 0.05) are displayed for data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
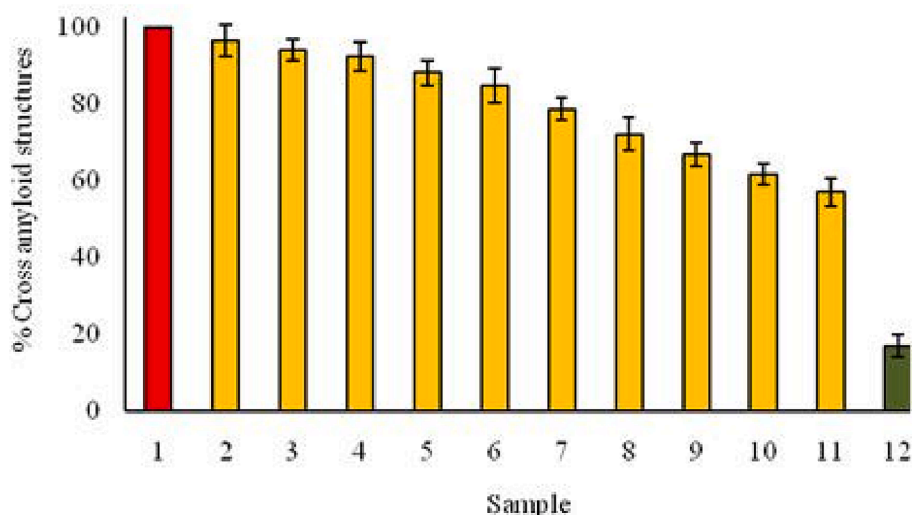

Fig. 9. Cross amyloid structures. Red column (1) represents BSA kept with glucose for 15 days, exhibits the most structural alterations and comprises 100 % amyloid fibrils. Yellow columns (2–11) represent extract (0.050 to 0.600 mg/ml). Data indicate a decrease in cross-amyloid formations as the concentration of leaf extract increases (Yellow columns). Sample 12 (green column) had the most miniature cross-amyloid formations and contained BSA that had been incubated alone for the same period. The means $\pm$ SEM (n = 3, p < 0.05) are displayed for data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

and radius.

The Ramachandran Plot analysis Ramachandran et al. (1963) (Fig. 16) used in the study showed that most residues were in advantageous positions, suggesting a suitable environment for molecular interactions. A-chain of enzymes was found to be involved in the interaction, as denoted by the letter "A" alongside the interacting amino acids. The identification of ligand-interacting amino acid types was color-coded, providing insights into the nature of molecular bonds: dark green for the conventional hydrogen bond, purple for Pi-Sigma, light purple for Alkyl, light green for van der Waals, orange for Pi-cation, pink for Pi-Pi stacked, light pink for Pi-Pi T-shaped, and red for the unfavorable bump (Fig. 15).

5. Discussion

Inflammation, glycation, and oxidative stress contribute to several chronic diseases. *O. sanctum* possesses anti-inflammatory, antioxidant, anti-microbial, anti-tumor, and antidiabetic qualities (Singh et al., 2010; Sharma et al., 2022; Sharma et al., 2021). Holy basil is pretty effective in humans and animal models due to its multiple health beneficial effects (Jamshidi & Cohen, 2017). Therefore, it is very necessary to understand the mechanism of *O. sanctum* and its implications for diseases in humans. The current study examined the anti-inflammatory, antioxidant, and anti-glycation characteristics of an ethanol-prepared *O. sanctum* leaf extract. The plant's leaf extract has undergone pharmacological analyses to determine its antibacterial, immunomodulatory, anti-stress, anti-inflammatory, antiglycating, and anti-AGE generation qualities.
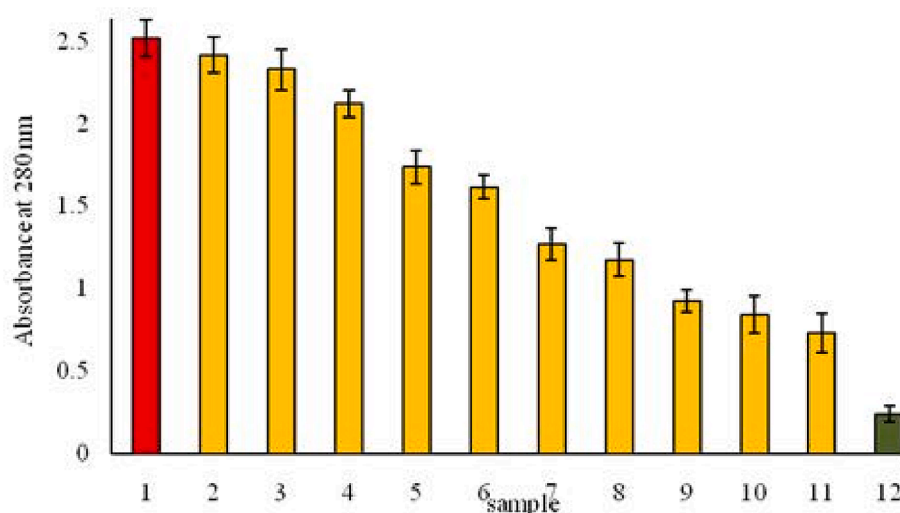
Fig. 10. Decrease in absorbance due to the presence of extract. Sample 1 is thought to have the most significant structural alterations and represents BSA having glucose placed for fortnights. Sample 2–11 had an extract concentration of 0.050 to 0.600 mg/ml (Yellow column). BSA has incubated alone in Sample 12 (Green column). The means ± SEM (n = 3, p < 0.05) are displayed for the results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
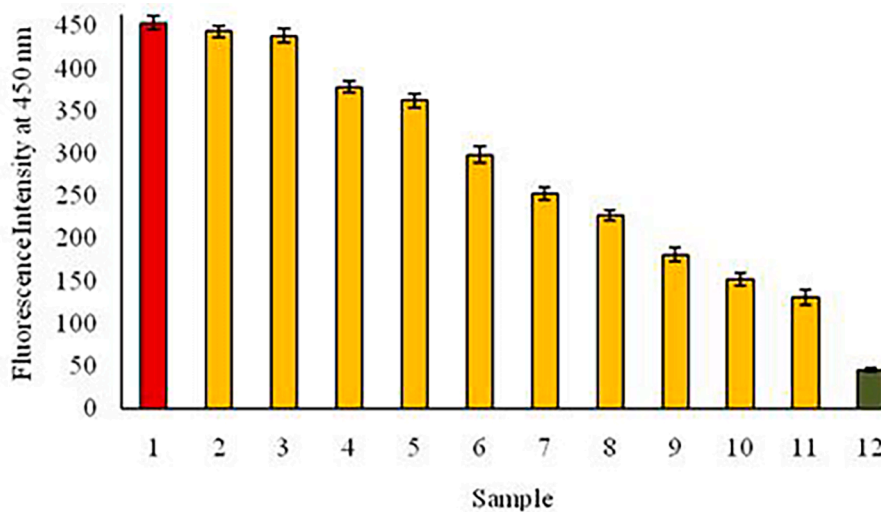

Fig. 11. Reduction in fluorescence intensity (at 450 nm). Sample 1 would have the most significant structural alterations and refers to the BSA sample kept with glucose for 15 days (Red column). Sample 2–11 had an extract concentration of 0.050 to 0.600 mg/ml (Yellow column). BSA has incubated alone in Sample 12 (green column). The means ± SEM (n = 3, p < 0.05) are displayed for data. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The leaf ethanol extract confirmed the presence of various phytochemicals. Polyphenols are helpful electron donors because their architectures feature an aromatic ring and a hydroxyl group. Phenolic compounds are, therefore, necessary for antioxidant activity (Rahmani et al., 2022). Calculating the total phenol concentration of plant extracts is a first step towards determining their antioxidant ability. Many factors, such as the plant material's harvest time, location, climate, and method of drying, might affect the amount of phenolic chemicals found in extracts made from it. A significant influence is also played by the way the extraction process is executed, including the duration, temperature, kind and polarity of the solvent, and the portion of the plant that is extracted. It is especially crucial to select a solvent that is inexpensive, non-toxic, safe, and capable of extracting phenolic chemicals as efficiently as possible. For the purpose of extracting flavonoids and phenolic acids, it is frequently more efficient to employ a combination of solvents (Kozłowska et al., 2022). Based on the results of our experiment, the extract contained the most significant amounts of phenolic and flavonoid components.

In 2020, antioxidant capability of methanol extract of dried leaf powder of *O. sanctum* was measured by Chaudhary and colleagues through *in vitro* assays (Chaudhary et al., 2020). The current study showed that ethanol leaf extract of *O. sanctum* had significant antioxidant activity through hydrogen peroxide and DPPH-reducing activities. Thus, our study supported the findings of Chaudhary and colleagues that *O. sanctum* had antioxidant potential. Chaudhary and colleagues have suggested that high antioxidant nature of this extract might be due to the presence of polyphenolic compounds (Chaudhary et al., 2020). We also found high overall polyphenolic content in the extract. Hence, our results support the idea that enormous levels of polyphenolic chemicals provide an antioxidant mode of action to *O. sanctum*.

Plant extract might prevent AGEs formation (Sherwani et al., 2022). The current study demonstrated that *O. sanctum* inhibited glycation and AGEs formation. Previous studies have suggested that the antioxidant nature and hence, the polyphenolic content of plant extract contribute to
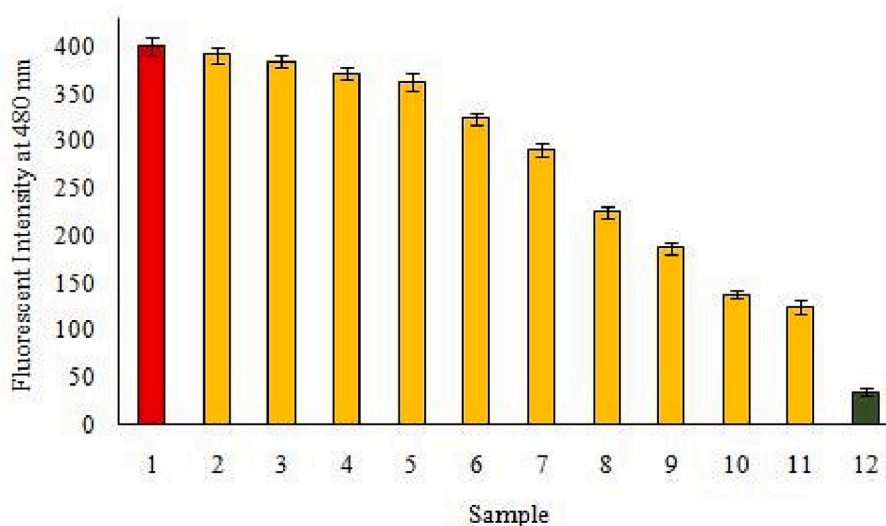
Fig. 12. Decline in fluorescence intensity (at 480 nm). Fluorescence intensity decreased (at 480 nm) when leaf extract was present in the samples. Sample 1 is thought to have the most significant structural alterations and is equivalent to BSA co-incubated with glucose (without extract) for 15 days (Red column). Sample 2–11 had an extract concentration of 0.050 to 0.600 mg/ml (Yellow column). BSA has incubated alone in Sample 12 (green column) without any extract or glucose. The means $\pm$ SEM (n = 3, p < 0.05) are displayed for the results. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Inhibitory zones against bacterial and fungal strains.

Test Organisms	*O. sanctum* extract (Diameter of zone of inhibition in mm)	
	For 50 mg/ml	For 100 mg/ml
Staphylococcus aureus	16	20
Escherichia coli	17	21
Klebsiella pneumoniae	18	22
Pseudomonas aeruginosa	12	16
Escherichia faecalis	12	16
Candida albicans	14	19

the antiglycation and AGEs formation inhibition activity (Sherwani et al., 2022; Anwar et al., 2020a). Thus, our results corroborated previous studies that demonstrated a strong relationship between the antiglycation and antioxidant properties of plant extracts. Hyperglycemia, or high hemoglobin-glucose adduct level, is a severe problem among people with diabetes. Elevated blood plasma sugar levels harm various organs, including the kidneys, blood vessels, and eye lenses. Hemoglobin and glucose form a covalent bond. Our findings suggest that *O. sanctum* may play a significant role in the therapeutic approach to prevent glucose from interacting with red blood cells in the blood, as demonstrated by the inhibition of *in vitro* BSA-glycation in this research.

Additionally, hydroxyl free radicals are created when H_2O_2 breaks down in the blood. Hydroxyl radicals cause DNA damage and activate oncogenes, such as C-Raf-1 and K-ras (Pourahmad et al., 2016). In diabetes patients, ROS and oxidative stress cause oxidative damage to various biomolecules and connective tissue macromolecules (Younus & Anwar, 2018). In the current study, the hydrogen peroxide reduction test and DPPH reducing activity were the two techniques employed to evaluate its fighting capacity against oxidants. It has been demonstrated that holy basil extract, for instance, scavenges DPPH radicals and hydrogen peroxide in a content-dependent manner. The hydrogen peroxide assay and DPPH findings indicate that the extract has a significant antioxidant capacity similar to that of ascorbic acid. Therefore,
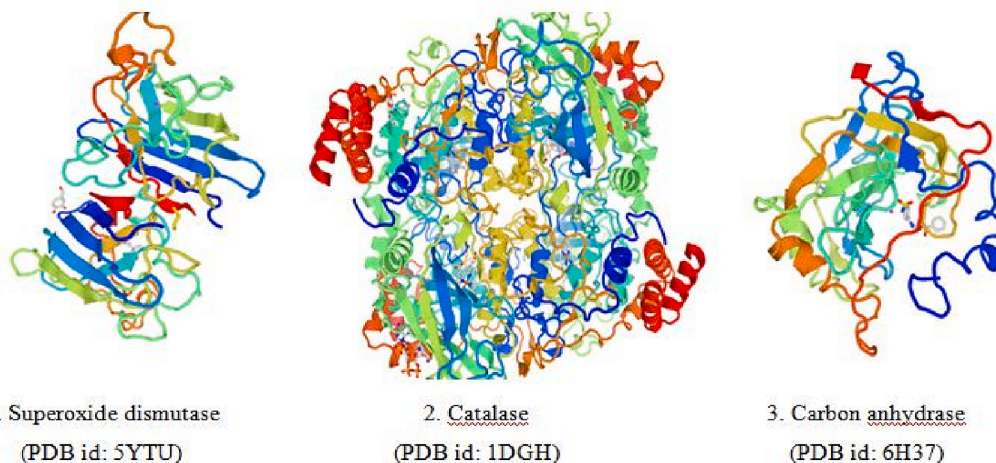

1. Superoxide dismutase (PDB id: 5YTU)

2. Catalase (PDB id: 1DGH)

3. Carbon anhydrase (PDB id: 6H37)

Fig. 13. The 3D structures of the selected enzymes.

1. Eugenol

2. Beta-Sitosterol

3. Ursolic acid

4. 1-Triacontanol
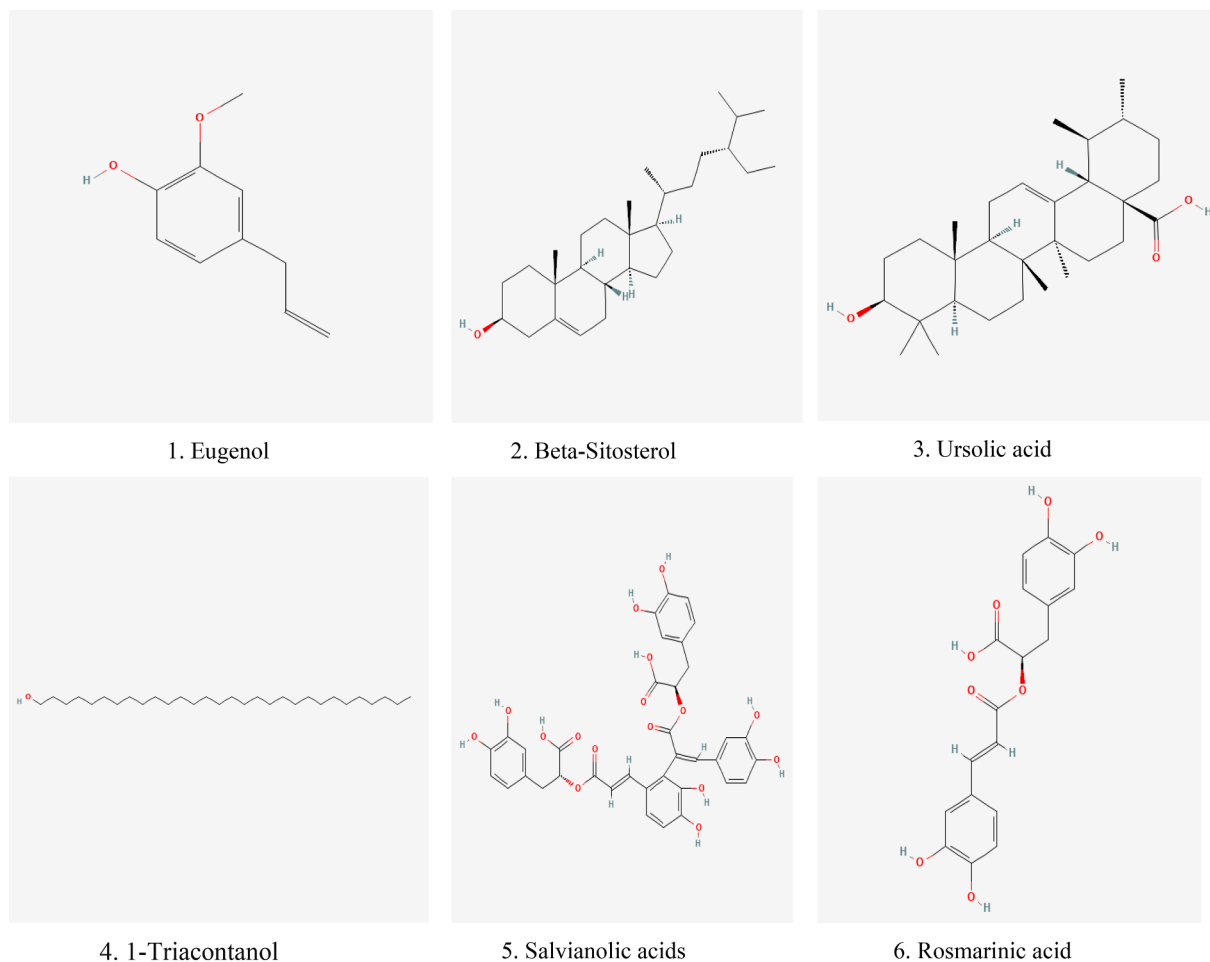
5. Salvianolic acids

6. Rosmarinic acid

Fig. 14. The 2D structures of the selected basil ligands.

Table 4
Information about molecular docking investigation.

1. Superoxide dismutase (PDB id: 5YTU)				
Ligands	PubChem CID	Binding Energy (kcal/mol)	Interacting Amino Acids	SBDSite_Sphere: XYZ coordinates, Radius
Eugenol	3314	−4.4	Gly12, Pro13, Val14, Leu144	−76.243300 6.146800 −3.292600, 13.00
Beta-Sitosterol	222,284	−6.2	Asp11, Pro13, Leu144	−76.243300 6.146800 −3.292600, 12.00
2. Catalase (PDB id: 1DGH)				
Ursolic acid	64,945	−6.1	Ala357, Pro162, Phe161, Ile159, Pro158, Gly353, Asp360, Phe356, Thr361, His364, Val73, His166	25.591047 40.102372 60.221023, 11.336446
1-Triacontanol	68,972	−4.7	Glu71, Pro70, Arg355, Arg72, His354, His155, Phe157, Leu155, Val74, Asp350, Pro152, Phe356, Ala357, Pro158, Gly353	25.591047 40.102372 60.221023, 11.336446
3. Carbon anhydrase (PDB id: 6H37)				
Salvianolic acids	86,278,266	−13.7	Lys178, Trp5, Gln67, His94, Ser65, Thr200, His96, His64, Asn62	18.291346 24.603231 4.360962, 11.019049
Rosmarinic acid	5,281,792	−7.0	Gln92, Ser65, His94, Tyr7, His96, Thr200, Pro201, Trp5, His64, Asp69, Thr60, Asn62	18.291346 24.603231 4.360962, 9.019049

the extract's potential to reduce DPPH and H_2O_2 may be contributed by various substances present in holy basil leaves and their involvement in the stoichiometric quenching reaction. Both studies show that *O. sanctum* extract has considerable antioxidant capacity. Oxidative stress is implicated in the pathophysiology of many diseases. Consequently, the extract's antioxidant capacity may be helpful in the fight against several illnesses.

According to Liu et al. (2017), inflammation is the body's primary defense against various stressors, infections, burns, hazardous substances, and allergies. According to Coussens and Werb (2002), approaches against inflammation can be therapeutic for preventing and treating tumors. As is often known, biological proteins that have undergone denaturation become inactivated, and this process also plays a role in the inflammatory response (Mizushima & Kobayashi, 1968).
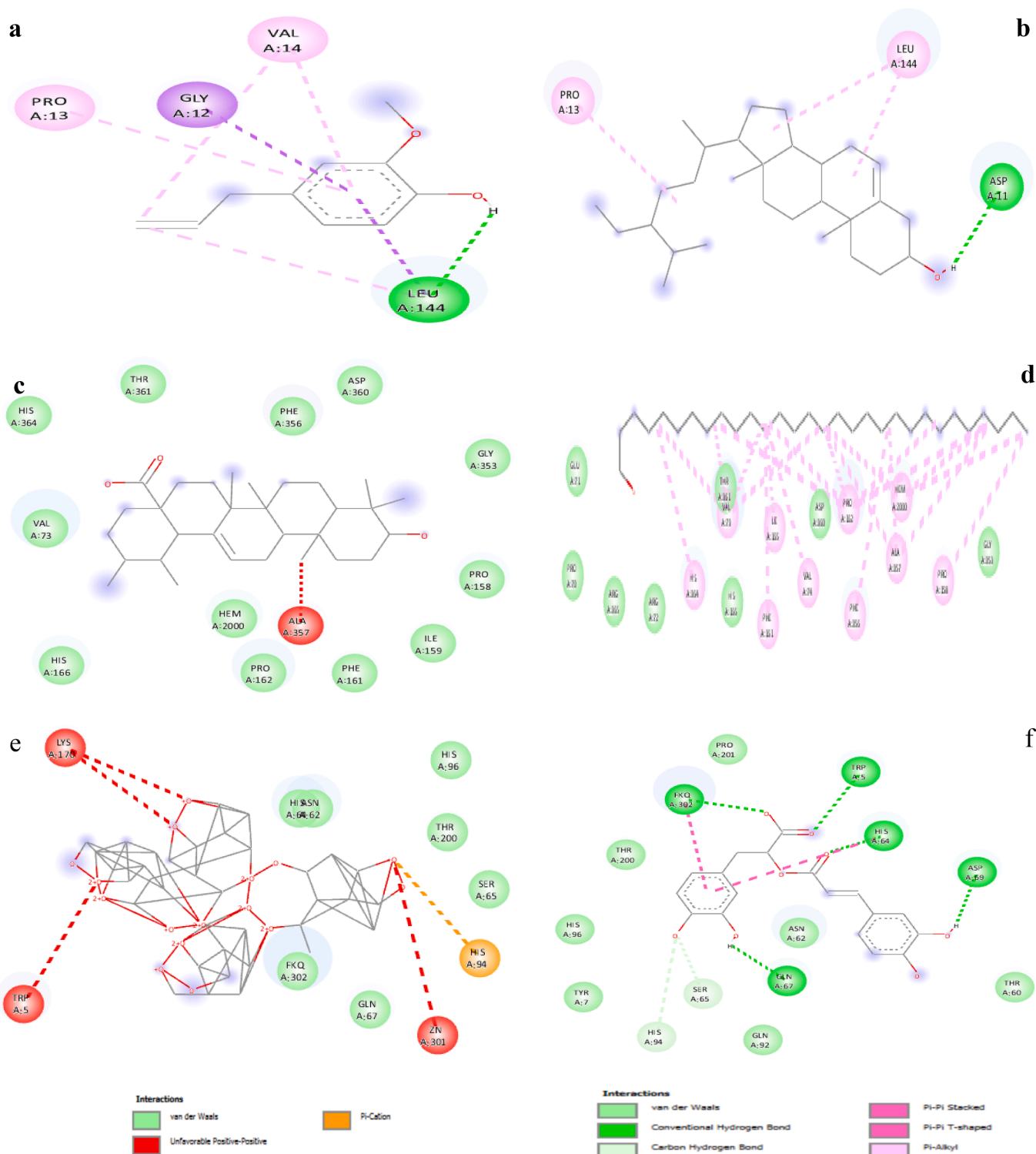
Fig. 15. Two-dimensional illustration exhibiting the interaction sites of the target and the docked ligand. (a) eugenol; (b) beta-sitosterol; (c) ursolic acid; (d) 1-tri-acontanol; (e) salvianolic acids; (f) rosmarinic acid.

Heat causes BSA to denaturate and release antigens linked to type-III hypersensitivity reactions, which are linked to illnesses such as systemic lupus erythematosus, glomerulonephritis, rheumatoid arthritis, and serum sickness (Elisha et al., 2016). The inflammation-inhibiting effects of the *O. sanctum* leaf ethanol extract were investigated through protein denaturation assay and a proteinase action inhibition test. The results showed that, as previously reported in research, the plant extract successfully prevented protein denaturation because it significantly improved the percentage of inhibition of denaturation with an increase in extract concentration (Anwar et al., 2020a). Denaturation most likely occurs through changes to hydrophobic, disulfide, electrostatic, and hydrogen bonding (Elisha et al., 2016). Because a variety of distinct bioactive components are present in a plant extract, the phytochemical extracts exhibit significant heterogeneity. Hence, it can be assumed that multiple bioactive substances present in holy basil leaf extract may combine and interact to protect through inhibition of changes to
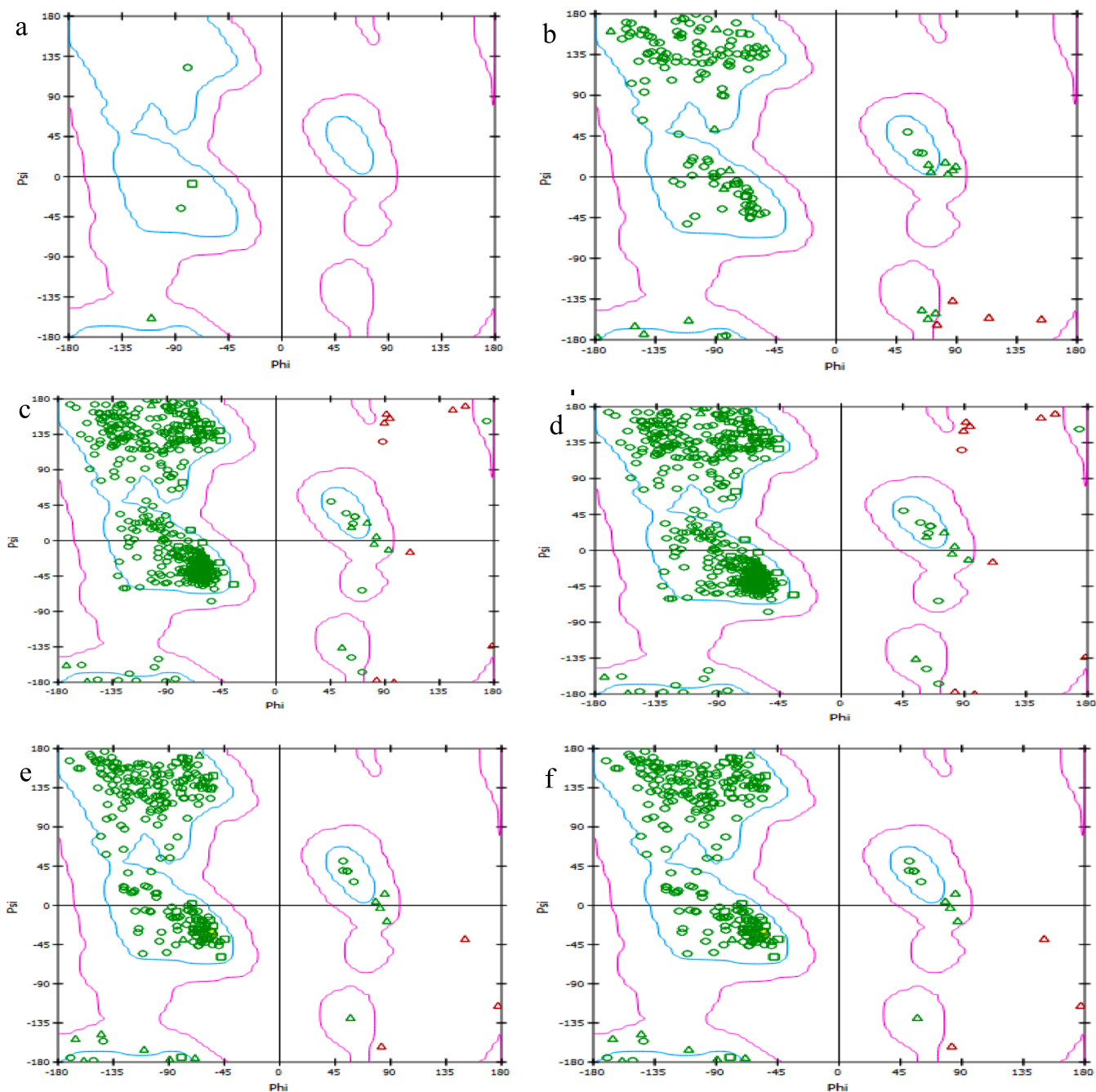
Fig. 16. Ramachandran plots of the docked ligand with the targeted enzymes, superoxide dismutase with (a) eugenol and (b) beta-sitosterol; catalase with (c) ursolic acid and (d) 1-triacontanol; carbon anhydrase with (e) salvianolic acids and (f) rosmarinic acid.

hydrophobic, disulfide, electrostatic, and hydrogen bonding involved in the protein denaturation. Therefore, we can assume that holy basil protects from alterations in the secondary structures of BSA due to its active molecules.

ROS-reducing action might be helpful in the prevention of the denaturation of egg albumin, which would subsequently stop autoantibodies from developing. This suggests that the extract from *O. sanctum* may prevent the development of autoantibodies linked to RA and CVD. Additionally, it has been revealed that AGEs have a crucial role in genotoxicity by promoting tumor cells' survival, proliferation, invasion, and metastasis (Lin et al., 2016). Therefore, blocking the production of AGEs might be an alternative therapeutic strategy for the management of cancer (Turner, 2015), CVD (Lamprea-Montealegre et al., 2022), and diabetes complications (Younus & Anwar, 2018).

In the current investigation, *O. sanctum*'s extract successfully reduced AGEs and glycation. Numerous scholarly investigations have demonstrated a robust association between polyphenolic content, antioxidant activity, and the suppression of AGE formation. Many plant extracts and purified metabolites with antioxidant characteristics have been shown to exhibit anti-AGE activity frequently (Anwar et al., 2020; Anwar et al., 2014). However, we can suggest that other mechanisms, such as the trapping of dicarbonyl species or AGE-breaking, may also be involved in the antioxidant extracts' anti-AGE activity of *O. sanctum*'s extract. In our previous research, a noticeable reduction in hyperchromicity was reported for those specimens that underwent incubation with extract and glucose, as the extract concentration was to be increased (Anwar et al., 2020a). Moreover, flavonoids and other polyphenolic compounds have been noted to potentially aid in preventing

the growth of cancer (Liu, 2004).

Aging-related AGE buildup causes alterations in the cardiovascular system, such as atherosclerosis, vascular stiffening, endothelial dysfunction, and decreased central compliance of the arteries (Donato et al., 2018). Several reports depicted a correlation between AGE levels and the severity and onset of myocardial infractions. Furthermore, according to a few studies, AGE-targeting therapy may benefit individuals with heart failure. The activation of transcription factor NF-κB appears to be a trait that distinguishes AGE-induced activation of several cell types (Bacher et al., 2021).

AGEs trigger a number of autophagy-related cell signaling cascades, including NF-κB, PKC, ERK, and MAPK (Verma et al., 2016). The decrease of active dicarbonyl compounds, suppression of ROS generation, preservation of the protein structure, and breakdown of AGEs are the primary processes that prevent the formation of AGEs. By scavenging free radicals, chelating metal ions, trapping active carbonyl compounds, covering protein glycation sites, and reducing blood glucose levels, these substances prevent the development of AGEs (Song et al., 2021). Therefore, it might be possible that *O. sanctum* extract may inhibit AGEs formation through either of these mechanisms or a combination of these mechanisms. Thus, AGEs formation inhibition activity of this extract inhibits autophagy-related cell signaling cascades, including NF-κB, PKC, ERK, and MAPK.

Numerous T-cells of the synovial membrane induce the development of osteoclasts, macrophages, and chondrocytes using cytokines. These cells then create metalloproteinases and other cytotoxins, breaking cartilage and bone (Jang et al., 2021). NF-κB was shown to be significantly activated in primary cardiac fibroblasts. In addition to being required for pathologic alterations in diseases, AGEs and RAGEs are also linked to the advancement of numerous life-threatening diseases like diabetes and atherosclerosis. Inflammation-protecting substances that can block AGEs are shown to be effective modes of treatment for degenerative diseases and conditions. When AGEs binds to cell receptors, it activates NF-kB, which increases the synthesis of proinflammatory cytokines. Furthermore, the formation of new, immunologically important epitopes in synovial proteins may result from the production of AGEs. According to Guo et al. (2018), both systems might be implicated in starting and controlling the inflammatory and damaging processes in RA. Current data showed that *O. sanctum* extracts significantly and concentration-dependently declined browning intensity, AGEs development, and cross-amyloid structures across all studies relating to the suppression of glycation and AGEs creation. Thus, the current investigation suggests that *O. sanctum* extract may have therapeutic value in preventing the development of RA under hyperglycemic circumstances.

According to our research, *O. sanctum* extract exhibits significant antimicrobial actions. Many phytoconstituents present in plant extracts have been suggested to target membrane-bound enzymes, cell wall polypeptides, and surface-exposed adhesins present in the microbial cell. In addition, they have the ability to make complexes with extracellular and soluble proteins (Cowan, 1999). Therefore, *O. sanctum* extract could have shown antimicrobial activity due to any or a combination of these mechanisms of phytoconstituents present in it.

Antioxidant enzymes are a vital line of defense against the pathophysiology of many diseases. Nevertheless, these enzymes may become inactivated because they are glycated (Anwar et al., 2014). Although the exact processes by which *O. sanctum* extract prevents diseases are unknown, they most likely have something to do with their unique structure. In order to explore the workings of the phytochemicals of *O. sanctum* towards suppressing oxidative stress and restoration of antioxidant molecules, as well as to establish a connection between the chemical characteristics of the promising molecules of *O. sanctum*, a docking study was conducted.

Using molecular docking research, it was possible to ascertain how successfully specific proteins and bioactive compounds created a stable protein–ligand complex posture throughout time by confirming the interactions of superoxide dismutase, catalase, and carbon anhydrase with the bioactive components of basil. The bioactive compounds in basil can interact with the target proteins in our molecular docking investigation. Thus, the current computational analysis opens up new possibilities for synthesizing proinflammatory polyphenols and oxidative stress inhibitors.

According to the molecular docking data, every ligand that was looked at could bind with the antioxidant enzymes. Given that efficient binding with these enzymes might enhance antioxidant activity—an essential component in the fight against oxidative stress—this suggests possible therapeutic ramifications. By evaluating the spatial arrangement of amino acid residues, the Ramachandran Plot analysis—a technique frequently employed in structural bioinformatics—helped validate the molecular relationships. The plot's favorable positions point to a persistent and energetically favorable binding arrangement between the ligands and the enzymes, bolstering the interactions' possible effectiveness. The current research can be used to develop an appropriate alternative therapy because oxidative stress, inflammation, and the development of AGEs in diabetic patients are associated with rheumatoid arthritis and CVD. Since rheumatoid arthritis and CVD are linked to oxidative stress, inflammation, and AGEs in diabetic patients, the current research might be very beneficial for the creation of an effective alternative medication for RA in the future through clinical and *in vivo* studies. In addition, safety analysis and cytotoxicity concerning the dose are compulsory.

There are many controversies in studies that are related to our topic of the manuscript. Challenges about the use of herbal medications for disease management include toxicity, overdose and its harmful effects, herb-drug interaction, and incorrect indication, adulteration, misidentification, harmful metal contamination, or poorly processing (Ekor, 2014). Pharmacovigilance and a regulatory framework, global management standards and quality standards for radical sources of herbs, seed and seedling breeding, planting, harvesting, and storing, are crucial in order to guarantee the safety and quality of herbal medicines (Kumar et al., 2016). The WHO Collaborating Centre for International Drug Monitoring-Uppsala Monitoring Centre (WHO-UMC) is tasked with coordinating the global ADRs data and searching it for indications of new ADRs (Olsson, 1998). The Governmental Register of Medicinal Preparations contains information on approximately 600 herbal medicinal products (HMP) that have been approved for use as pharmaceuticals. Federal Law No. 61 FZ (dated 12.04.2010), "Regarding the circulation of drugs," governs all aspects of the development, preclinical and clinical studies, evaluation, state registration, standardization and quality control, manufacturing, preparation, storing, transporting, importing and exporting, advertising, releasing, selling, using, and disposing of pharmaceutical preparations (including HMPs) (Ekor, 2014).

6. Conclusion

In a nutshell, the ethanol extract of leaf of *O. sanctum* exhibited a substantial phenolic and flavonoid content, outstanding antioxidant capacity, and free radical elimination action. Additionally, the findings shed light on anti-inflammatory, anti-oxidant, and anti-AGEs formation effects of *O. sanctum*. Using an integrated method of molecular docking investigations, we obtained structural insights into the putative binding mechanisms of drug-like bioactive ligands in *O. sanctum* against important molecular targets that are crucial to the pathophysiology of chronic inflammation. In addition, the positive binding capabilities signify potential therapeutic avenues for combating oxidative stress. The noticed strong link between AGEs and protein denaturation inhibition assays, total phenolic content, and antioxidant capacity indicates that compounds with high antioxidant potential are present in *O. sanctum*, which could be responsible for extract's significant inhibitory activity against inflammation, glycation and AGEs formation. In summary, *O. sanctum* have a variety of medicinal uses and can be a source of active

chemicals that combat glycation-associated problems in diabetes and certain cancers in addition to having anti-aging qualities. These findings could pave the route for additional investigation of these potentials in the development of antioxidant-based therapies in treating diabetic people with CVD and rheumatoid arthritis. Therefore, more phytochemical and pharmacological research is required in this regard. In addition, it is necessary to take into account factors like efficacy, toxicity, side effects associated with overdose and its negative effects, herb-drug interaction, and improper indication.

CRediT authorship contribution statement

Shehwaz Anwar: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Ravindra Raut:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. **Fahad A. Alhumaydhi:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Acknowledgment

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