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Original article

Green Synthesis of Fragaria Vesca Loaded Zinc Oxide Nanoparticles and Their Biomedical Applications: An In-Vitro and In-Silico Study

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Abstract

Goal: Zinc oxide nanoparticles, or ZnO NPs, are extensively employed in biomedical applications, including antibacterial goods, cancer prevention, and wound healing. One fruit that is widely accessible and beneficial for renal disorders is the wild strawberry, or *Fragaria vesca*. Using *Fragaria vesca* extract, this study investigates the environmentally friendly synthesis of ZnO NPs & evaluates their excellent therapeutic applications in conjunction with in silico molecular docking.

Methods: Zinc Oxide Nano particles are synthesised with fruit extract from *F. vesca* and were examined using TEM, SEM & UV visible Spectroscopy. The biomedical assays included DPPH for antioxidant activity and albumin denaturation for anti-inflammatory effects. The key phytoconstituents (ellagic acid, capsaicin, and quercetin) were assessed using Cresset Flare molecular docking against xanthine oxidase (PDB: 1FIQ) and COX-2 (PDB: 3LN1).

Results: NPs had a UV peak at 430 nm and a spherical/rod shape (10-100 nm). At 10 µg/mL, DPPH inhibition was 76%; at 50 µL, and anti-inflammatory inhibition was 76.2%. Docking affinities: processes were confirmed by the superior performance of ellagic acid (−13.586 kcal/mol to XO, −12.384 to COX-2).

Keywords: Anti-inflammatory, Antioxidant, *Fragaria vesca*, Molecular docking, Zinc oxide nanoparticles.

Introduction

In the twenty-first century, the innovative technique known as nanotechnology is revolutionising the field and generating enthusiasm all around the world. However, a lack of knowledge about its possible consequences and the lack of regulations to control new concerns have impeded its advancement. [1] Researchers continue to advance despite obstacles in finance, administration, production, legislation, and technology. Green knowledge, or "green nanotechnology," is a discipline that combines the impressions of “green chemistry” & green engineering” where green means using goods derived from plants. [2] This method uses fewer materials and renewable resources while consuming less energy and gasoline. By lowering greenhouse gas emissions, potentially hazardous waste, and the consumption of energy, water, and raw materials, nanotechnology has enormous potential to protect the environment and the climate. Some of the main benefits of green nanotechnology are lowering waste and greenhouse gas emissions, increasing energy efficiency, and utilising fewer non-renewable raw resources. It offers a fantastic chance to step in and stop negative effects before they happen. [3]

Zinc oxide nanoparticles (ZnO NPs) highly sought-after metal oxide nanoparticle, widely used due to their exceptional physical and chemical properties.[4] Initially employed in rubber industry, ZnO NPs offer benefits such as increased wear resistance, improved toughness and strength, and anti-aging properties.[5] Their strong UV absorption possessions make them progressively popular in special care products, including cosmetics & sunscreens.[6] In adding to their UV-blocking capabilities, ZnO NPs possess exceptional antibacterial and antimicrobial properties. As a result, they are utilized in the textile industry to enhance the performance of finished fabrics with functional characteristics that include resistance to UV and visible light, antimicrobial activities, and deodorizing qualities[7] ZnO NPs have a diverse range of applications, not limited to the industries, but also including concrete production, photocatalysis, electronics, and electrotechnology, among others[8].

Eating fruits and fruit-based foods improves overall health by lowering low-density lipoprotein (LDL), which in turn decreases the risk of developing diseases such as cancer, cardiovascular disease, cataracts, immune system impairment, gastrointestinal issues, high blood pressure, elevated cholesterol, and age-related macular degeneration.[9] Fruit and vegetables should make up half of a person's plate in order to support a healthy lifestyle because they are rich in nutritional fibre, minerals (calcium, iron magnesium and Potassium), Vitamnins (Vit.C, flic acid& Vitamin A precursors) other advantageous plant chemicals by antioxidant benefits. The Food and Agriculture Organization of the United Nation and the World Health Organizations (FAO/WHO) recommend that people consume at least 400g of fruits and vegetables everyday (avoid potatoes & other carbohydrate roots) to decrease the risk of chronic medical conditions (such as heart disease, malignancy, diabetes & being overweight) and address issues of micronutrient deficiencies.[10] Fruits can be further divided into tropical, sub-tropical, and temperate categories according to their place of origin and the local climate.[11]

Zinc oxide nanoparticles were created in the present study using an aqueous extract of *Fragaria vesca,* or wild strawberry. [12] Scanning electron microscopy, Transmission electron microscopy and UV- visible spectroscopy. Have been applied to analyse the produced nanoparticles. The anti-inflammatory & antioxidant properties of the green produced zinc oxide nanoparticles. were evaluated. Furthermore, by comparing important phytoconstituents to pertinent protein targets, in silico molecular docking was carried out using Cresset Flare to validate processes. It was hypothesised that *Fragaria Vesca* phytoconstituents improve NP bioactivity by targeted inhibition/activation.

Materials and Methods

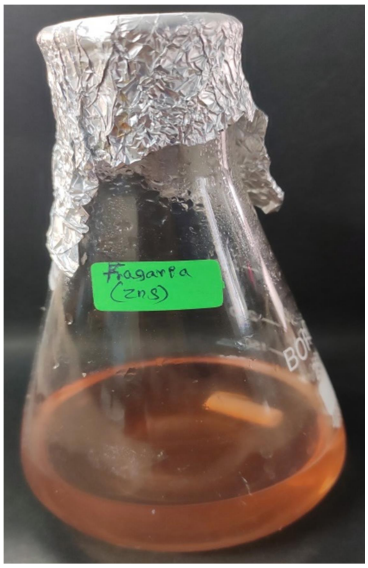
Collection and Preparation of *Fragaria vesca* Extract

To eliminate harmful materials, distilled water had been utilized to wash the freshly harvested *Fragaria vesca* fruits. The extract of fruit was prepared from 10 g of *Fragaria vesca*, which was slashed into minor pieces and ground with a mortal pistol. After mixing the pulverized fruit with 100 ml of distilled water and resulting solution is boiled for 15 minutes at 50 to 60°C The heated extracts were filtered through a muslin cloth. The fruit extract was saved for future use.

Green Synthesis of Fragaria vesca Mediated Zinc Nanoparticles

20 mM of zinc nitrate was dissolved in 50 ml of distilled water. 50 millilitres of filtered Fragaria vesca extarct have been added. The reaction mixture was then maintained at 600 rpm on magnetic stirrer for 48 hours. In meantime, the creation of zinc nanoparticles detected using UV-visible spectroscopy. After that, the pellet and supernatant separated using centrifugation. The pellet kept in an airtight Eppendorf tube for later use in characterisation and biological applications, while the supernatant was disposed of. [13]

Fig 1: Final colour change image of *Fragaria vesca* mediated zinc oxide nanoparticles.



Characterization

A double-beam spectrophotometer (ESICO) was employed at designated time intervals to characterise the zinc nanoparticles synthesised through a green method. A scanning electron microscope (SEM) employed to inspect

the dimensions & morphology (SEM). The physical parameters of green zinc nanoparticle synthesis were elucidated through the application of transmission electron microscope (TEM) techniques.

Biomedical Applications

Antioxidant Action - The DPPH Method

The antioxidant properties of biogenetically synthesised zinc oxide nanoparticles have been assessed using the DPPH assay. Several doses (2–10µg/mL) of Fragaria vesca extract-mediated zinc oxide nanoparticles dissolved in 450µl of 50mM Tris HCL Bufferr (pH 7.4) and mixed with 1 mLof 0.1 mM DPPH in methanol, followed by an additional 1mL of 0.1mM DPPHin methanol for a 30-minute incubation period. Subsequently, absorbance measurements at 517 nm were utilised to assess the decrease in DPPH free radicals. Butylated hydroxytoluene was employed as a control. The formula used to calculate the proportion of inhibition is presented below:[14]

$$\% \text{ inhibition} = \frac{\text{(Absorbance of control – Absorbance of test sample) X 100}}{\text{Absorbance of control}}$$

Anti-Inflammatory Properties – Albumin Denaturation assay

The anti- inflammatory properties of biosynthesised zinc nanoparticles were assessed through albumin denaturation analysis. A 1% aqueous solution of bovine serum albumin was mixed with 0.05mL of zinc nanoparticles at varying concentrations (10 µl, 20 µl, 30µl ,40 µl and 50µl). A small quantity of 1N HCL added to adjust the pH of the mixture to 6.3. The samples were incubated at ambient temperature for 20 minutes , followed by a 30-minute boling period at 55°C in a water bath. Following cooling of the samples, the absorbance was restrained spectrophotometrically at wavelength of 660nm. Diclofenac sodium was utilised as a benchmark. The control substance utilised was dimethyl sulphoxide (DMSO). The following formula was utilised to determine the percentage of denaturation of proteins:[15]

$$\% \text{ inhibition} = \frac{\text{(Absorbance of control - Absorbance of sample)} \times 100}{\text{Absorbance of control}}$$

***In-Silico* Molecular Docking**

Molecular docking was conducted utilising Cresset Flare software to predict the binding modes and affinities of key phytoconstituents from the Fragaria vesca extract against specific protein targets associated with the observed activities. Standard reference drugs were utilised as controls in the docking process. Selected Proteins are Xanthine oxidase — 1FIQ (antioxidant)[16] and COX-2 — 3LN1 (anti-inflammatory) [17].

Selected phytoconstituents: Ellagic acid, capsaicin, and quercetin are significant phytoconstituents found in Fragaria vesca.[18] Proteins were prepared through the removal of water and non-essential ligands, the addition of hydrogens, and minimisation. Docking employed the Lead Finder module utilising the default settings. Grid boxes were positioned at the centre of active sites. Interactions were visualised and analysed through poses[19].

Statistical Analysis

Data analysed using GraphPad Prism (v10.3.0) as mean ± SEM . One–way ANOVA with Tukey's test: $P < 0.05$ is significant.

Results

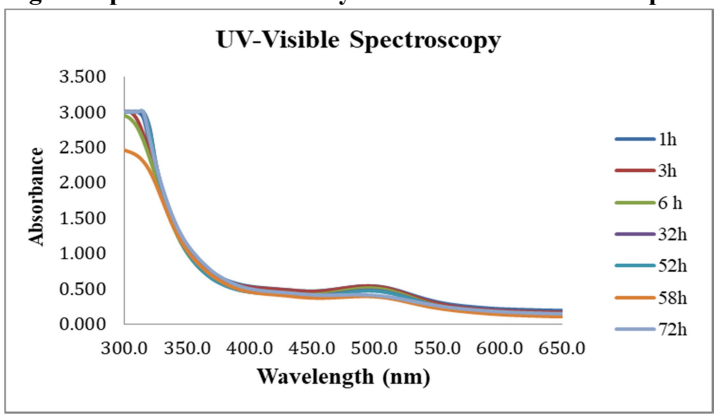
Visual Observation

Visual observation is the initial method employed to validate the synthesis of nanoparticles through the colorimetric alterations in the solution. In the present study , the fruit-mediated zinc oxide nanoparticles showed colour changes from pale pink to pale brown . This first shows that the fruit extract can reduce and cap.

UV- Visible Spectroscopy

The synthesis of zinc oxide nanoparticles was visualised with a UV-visible spectrophotometer. *Fragaria vesca* fruit extract acts as a reducing & stabilising agent. Because of the biological molecules in the fruit extract, it converts zinc sulfate nanoparticles to zinc sulfide nanoparticles. As a result of the adding of fruit extract, the colour changes from white to light brown, and then to dark brown as ZnONPs form. The colour of the nanoparticle changes during the ZnONPs synthesis. UV-visible spectroscopy was performed from 0 to 72 hours. Zinc nanoparticle surface plasmon resonance produced a maximum peak at 430nm.[20] Finally, the nanoparticles were centrifused at 8000rpm for 10minutes to analyse their synthesis. the zinc oxide nanoparticles in Fragaria vesca extract pellet and powder were collected for future use.

Fig 2: Representation of the synthesis of Zinc oxide nanoparticles from *Fragaria vesca* fruit extract



Morphological Characterisation - SEM & TEM

The morphology, crystal size and surface morphology of zinc oxide nanoparticles in *Fragaria vesca* are clearly shown by a scanning electron microscope micrograph.[21] Comprehensive morphology analyses show that the outputs produced were in cubical and spherical shapes for pleasing appearances. The diameter was 100 nm.

TEM is an effective tool for obtaining quantitative measurements such as particle size, distribution, and morphology. An electron beam is used in TEM, and it interacts with a test sample to form an image on a photographic plate [22]. TEM analysis was used to look for Fragaria vesca-ZnONPs. The Fragaria vesca-ZnONPs were rod-shaped, with some particles being spherical. The normal particle size was discovered to be 10-45 nm.

Fig 3: Represented Scanning electron microscope (SEM) of Zinc oxide nanoparticles *Fragaria vesca* fruit extract

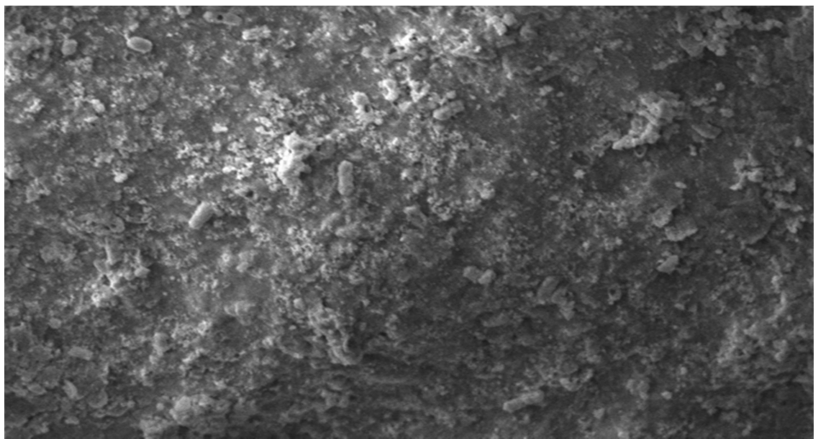
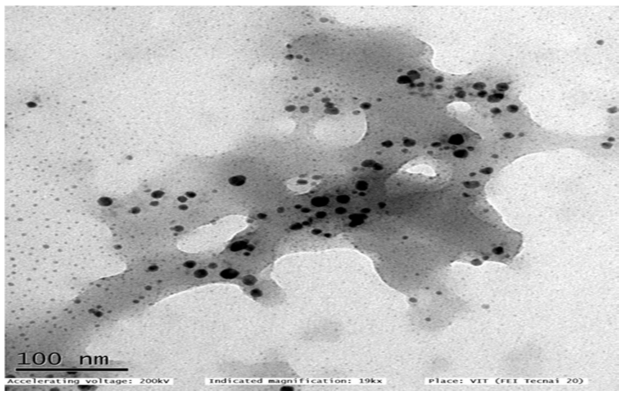


Fig 4: Represented Transmission electron microscope (TEM) of Zinc Oxide nanoparticles *Fragaria vesca* fruit extract



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Antioxidant Activity

Antioxidants protect people from a number of degenerative diseases and conditions. Phenolic compounds are prevalent antioxidants present in numerous natural products. The antioxidant activity of zinc oxide nanoparticles made with green synthesis was tested against the DPPH radical. Ascorbic acid is the standard for comparison. The percentage of inhibition of green synthesis of zinc oxide nanoparticles was found to vary by different concentrations. The inhibition was found to change the colour from yellow to dark brown[23]. As the concentration of nanoparticles goes up, so does the percentage of inhibition. Their inhibition rate is similar to that of the standard and ZnO nanoparticles.

Fig 5: Graphical representation of Antioxidant activity - DPPH assay of Zinc oxide nanoparticles *Fragaria vesca* fruit extract

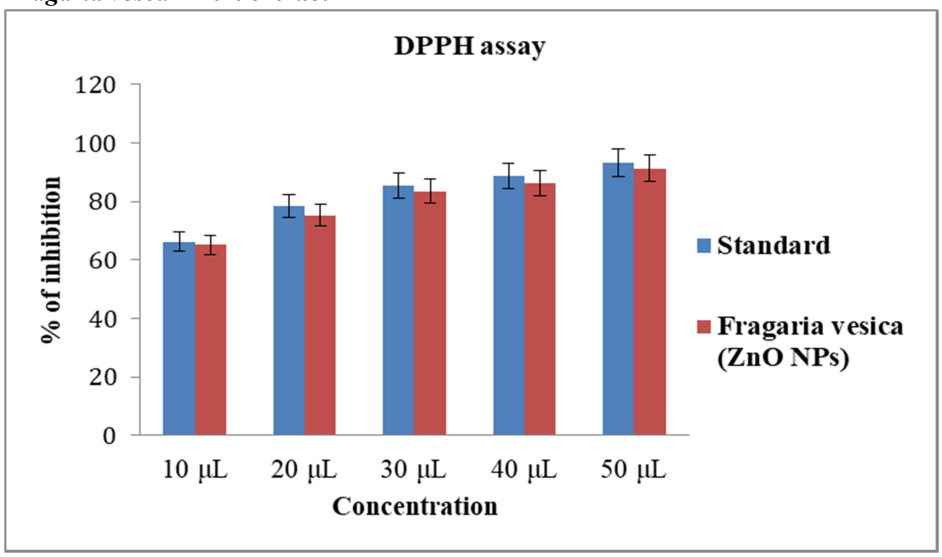


Table I. Antioxidant Activity (% Inhibition by DPPH Assay, mean ± SEM, n=3).

Concentration (µg/mL)	ZnO NPs*	Ascorbic Acid (Standard)
2	32.1 ± 1.2	35.4 ± 1.5
4	45.6 ± 2.1	48.2 ± 1.8
6	58.3 ± 1.9	62.1 ± 2.0
8	67.4 ± 2.3	71.5 ± 1.7
10	76.2 ± 1.6	80.3 ± 2.2

***P < 0.05 vs. standard.**

Anti-Inflammatory Properties

When the anti-inflammatory effect of nanoparticles of zinc oxide in an extract of Fragaria vesca fruit was measured with the bovine serum albumin denaturation analysis, the percentage of inhibition was 43.11% at 10 µl , 54.6% at 20 µl , 68.73% at 30µl, 71.08% at 40µl, and 76.2% at 50µl. Diclofenac sodium has been tested at different levels (10µL, 20µL, 30µL, 40µL, and 50µL) and is very effective at reducing inflammation. This study demonstrated that ZnO nanoparticles significantly mitigated inflammation by preventing protein denaturation. [24]

Figure 6: Graphical representation in the Anti-inflammatory activity – bovine serum albumin denaturation assay of Zinc oxide nanoparticles

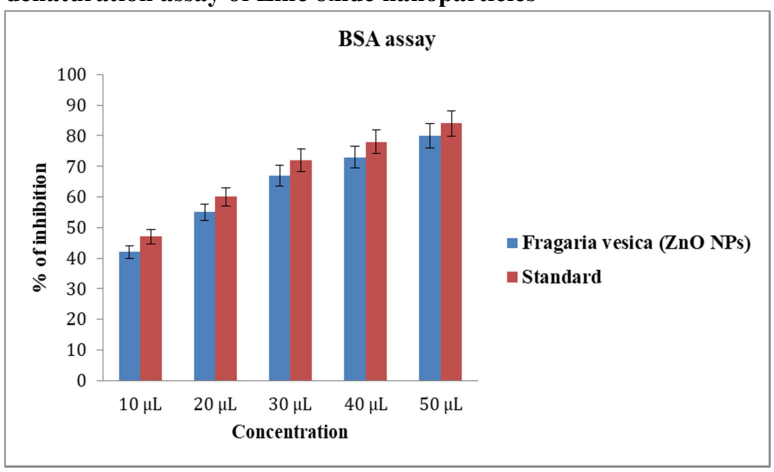


Table II. Anti-Inflammatory Activity (% Inhibition, mean ± SEM, n=3).

Concentration (µL)	ZnO NPs*	Diclofenac (Standard)
10	43.1 ± 1.8	50.2 ± 2.1
20	54.6 ± 2.0	62.4 ± 1.9
30	68.7 ± 2.3	75.1 ± 2.2
40	71.1 ± 1.7	78.3 ± 2.0
50	76.2 ± 2.1	82.5 ± 1.8

***P < 0.05 vs. standard.**

In Silico Molecular Docking Results

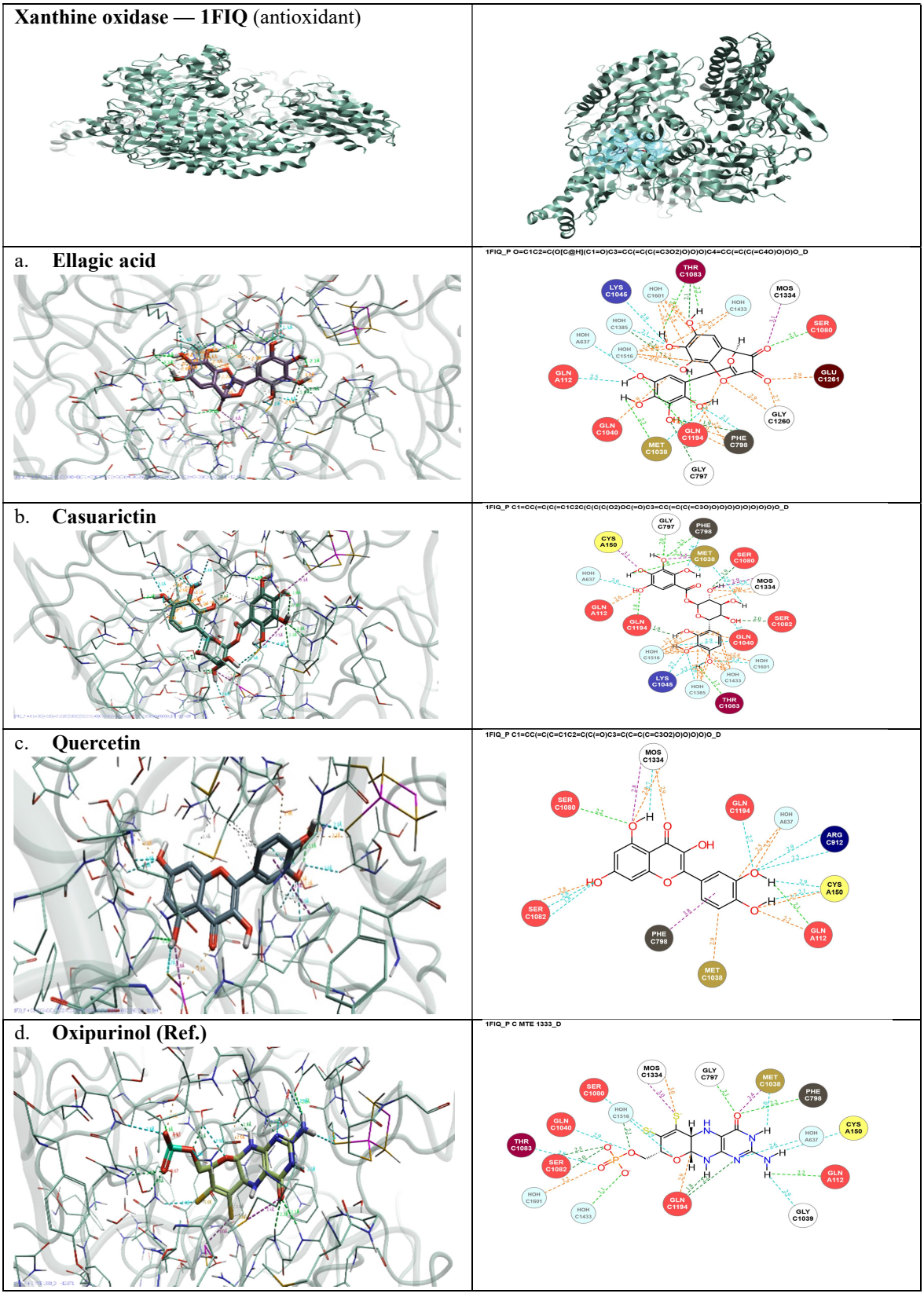
In silico molecular docking was performed to predict binding modes and affinities of major phytoconstituents from the *F. vesca* extract against selected protein targets relevant to those activities. Standard reference drugs were docked as controls.

1. Xanthine Oxidase — 1FIQ (Antioxidant)

Evaluation of Phytoconstituents from Fragaria vesca Extract as Potential Inhibitors of Bovine Milk Xanthine Oxidase (XO, PDB ID: 1FIQ).

Docking studies revealed strong binding affinities: ellagic acid (−13.586 kcal/mol), casuarictin (−12.556 kcal/mol), quercetin (-9.132 kcal/mol), compared with oxipurinol (−8.5 kcal/mol). Ellagic acid showed the best binding, ~5 kcal/mol stronger than oxipurinol, suggesting significantly higher potency. These results highlight ellagic acid and casuarictin as promising natural XO inhibitors that could support *F. vesca*-based nutraceutical applications in gout and oxidative stress management

Fig 7: Docking poses for XO (1FIQ): (a) Ellagic acid, (b) Casuarictin, (c) Quercetin, (d) Oxipurinol. conclusion: Superior phytoconstituent binding.



2. COX-2 — 3LN1 (Anti-Inflammatory)

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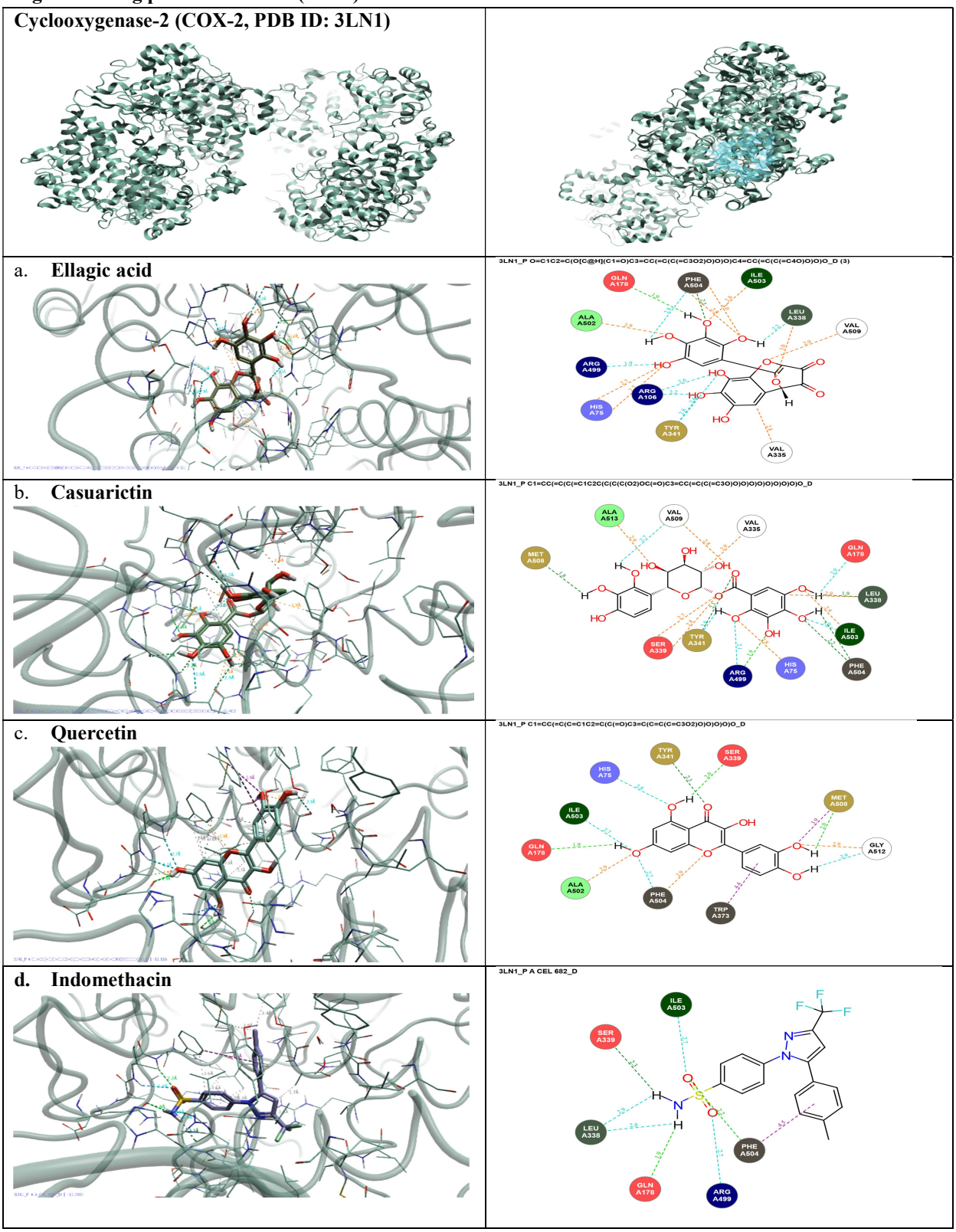
Evaluation of Phytoconstituents from Fragaria vesca Extract as Potential Inhibitors of Human Cyclooxygenase-2 (COX-2, PDB ID: 3LN1). Binding affinities: ellagic acid (−12.384 kcal/mol), casuarictin (−11.822 kcal/mol), quercetin (−10.563 kcal/mol), indomethacin (−9.815 kcal/mol). Ellagic acid outperforms with deep channel binding and multiple interactions

Fig 8: Docking poses for COX-2 (3LN1). Conclusion: Channel blockade for anti-inflammation



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Discussion

Fragaria vesca fruit extract was used to make zinc oxide nanoparticles is a green way to do it that has intriguing medicinal uses. Characterisation validated the presence of spherical and rod-shaped nanoparticles (10-100 nm) with a UV peak at 430 nm. In vitro assays demonstrated dose-dependent antioxidant (DPPH inhibition up to 76%), anti-inflammatory (protein denaturation inhibition up to 76.2%), aligning with existing data on plant-mediated nanoparticles. In silico docking confirmed mechanisms: Ellagic acid showed stronger affinities across targets (for example, −13.586 kcal/mol to XO vs. −8.5 for oxipurinol), which suggests that it is a key bioactive in F. vesca that causes the observed effects by blocking XO (an antioxidant) and COX-2 (an anti-inflammatory), Casuarictin and quercetin exhibited synergistic binding, augmenting total effectiveness. The combination of green-synthesized ZnO nanoparticles and F. vesca phytoconstituents increases bioactivity. Docking shows how molecules interact with each other, such as H-bonds and π-stacking in active sites. This corroborates the idea of phytoconstituent-mediated enhancement. Limitations: In vitro focus; difficulties in scaling up; possible toxicity at large doses. Future: anti-bacterial and cytotoxic activities and in vivo investigations, clinical trials, and NP-phytoconstituent formulations for targeted distribution.

Conclusion

Fragaria vesca mediated ZnO nanoparticles exhibit significant biological potential through antioxidant and anti-inflammatory property, mechanistically established by *in-silico* docking & *in-vitro* methods, emphasising the importance of ellagic acid.

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