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# In vitro anti-inflammatory, antidiabetic, antibacterial, and in silico studies of Ferruginan A isolated from *Olea ferruginea* Royle (Oleaceae)

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ABSTRACT

*Objective:* Traditionally, *Olea ferruginea* Royle (Oleaceae) has been used as a painkiller and antidiabetic in various ailments. To provide a scientific background to this folklore the current study was designed to anti-inflammatory and antidiabetic effects of one of the isolated compound from this plant.
*Methods:* Ferruginan A was isolated from the ethyl acetate extract of *Olea ferruginea* bark. This isolated molecule was subjected to *in-vitro* anti-inflammatory and antidiabetic effects using HRBCs and glucose uptake tests. The compound was also tested for molecular docking and ADMET study.
*Results:* Regarding the anti-inflammatory effect, the tested compound demonstrated a 69.82 % inhibition at a concentration of 100 μg/mL, while the Ferruginan A (100 μl/mL) increased the uptake of glucose (3.79–71.86 %) in the yeast cell. Similarly, the zone of inhibition values of Ferruginan A (24.98 mm) against Escherichia coli were found to be comparable to standard (Imipenem: 31.09 mm). The mechanism of antidiabetic and anti-inflammatory effects was explored by using docking simulations performed on four molecular targets related to diabetes and inflammation. The results showed that the isolated compound may act as an antidiabetic agent by inhibiting the 5′ Adenosine monophosphate-activated protein kinase (AMPK). While it also showed inhibition of anti-inflammatory targets COX-1, COX-2, and Tumor necrosis factor alpha (TNF-α). The ADMET prediction study revealed that isolated compound possesses favorable ADMET profile.
*Conclusion:* It was concluded that Ferruginan A might be a significant anti-inflammatory and antidiabetic molecule.

## 1. Introduction

Since last decade, natural compounds derived from medicinal plants are extensively investigated throughout the world owing to their health promoting benefits in treating various ailments (Hussain et al., 2018). Medicinal plants are possessed cache of bioactive compounds, which have been used in galenical products that are considered safe and effective in treating different metabolic syndromes in primary health care facilities (Farnsworth, 1988). Evidently, these medicinal plants have been consumed significantly either directly or as bioactive components isolated from them (Fabricant and Farnsworth, 2001). To date, approximately 50,000 + medicinal plants have been a source of health promotion for nearly 80 % of the world's residents (Gewali and Awale, 2008). Traditionally, various ancient practitioners prescribed natural products derived from either marine or terrestrial plants owing to their associated disease-modulating properties. World Health Organization has stated that developed countries' health care mostly relies on natural products. Various plant-derived natural products (lovastatin, Taxol, cyclosporine-A, etc.) have been successfully formulated by the pharmaceutical industry for the development of drugs that are used in

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treating several ailments. In this context, nearly 40 % of recently developed drugs are of natural origin (Dias et al., 2012). Bioactive compounds present in herbal and medicinal plants have attained significant attention of researchers owing to their wide-ranged application in the treatment of different diseases like diabetes, cancer, inflammation, obesity, hyperlipidemia, cardiovascular diseases, etc. Around the globe, these natural constituents also act as antioxidants, neuroprotective and hepatoprotective agents (Liu et al., 2019). The demand for these compounds is increasing day by day owing to their associated health benefits and the shift in consumer preferences from synthetic to natural products. Therefore, the pharmaceutical industries and scientists are more focused on research and development in the field of drug development (Wojdylo et al., 2019).

*Olea ferruginea* R belongs to the family Oleaceae and the genus Olea. This is an evergreen plant with having height ranging from 10 to 50 feet. This plant is medicinally important and is cultivated in hilly areas of Pakistan, Kashmir, and Afghanistan (Hashmi et al., 2014). In Pakistan, it is widely distributed in different regions like Dir, Swat Valley, Azad Kashmir, Murree, and Chitral Ginai, 1968). Since ancient times, different parts (fresh leaves and stem bark) of *Olea ferruginea* R have been used for treating several ailments and therefore have been utilized by local people for the treatment of fever, dental problems, bleeding gums, skin rashes, toothache, and muscular problems (Haq et al., 2011; Murad et al., 2011). Additionally, the fruit of this medicinal plant is well known industrially and pharmaceutically for its rich oil content that is used as an ointment to significantly relieve pain in rheumatism and dislocated bones (Yousaf et al., 2004). Moreover, it is evident from the literature that different parts of *Olea ferruginea* R possess antiasthmatic, astringent, antimalarial, antidiabetic, antihyperlipidemic, anti-inflammatory, and antileprosy properties (Haq and Hussain, 1995).

Around the globe, the genus Olea comprises of nearly 30 to 40 species and is extensively cultivated for their oil production (Abbasi et al., 2010). Olea plant extracts comprise of several polyphenols such as flavonoids, secoiridoid, and lignin. This plant has shown antimicrobial, anticancer, and antioxidant properties owing to its polyphenolic contents *i.e.* 17 to 23 % (Bendini et al., 2009; Ray et al., 2019). Natural phenolic compounds isolated from Olea species have been reported to be involved in the reduction of cancer cell progression through various mechanisms like cell cycle arrest and inhibition of signaling pathways Zorić et al., 2016). Moreover, to cope with the escalating demand for olive oil worldwide, agriculturists are focused on promoting the cultivation of Olea europea and exploring alternative plants in the genus Olea for meeting the production requirements of olive oil (Gorzynik-Debicka et al., 2018). Purposely, underutilized wild-type olives (*Olea ferruginea* R) are being considered as a potential alternative source for the production of sufficient content of olive oils. Extraction of olive oils from *Olea Ferruginea* R will not only aid in establishing a balance in the supply chain, however, but it will also help in improving economics and human health (Anwar et al., 2013). In this milieu, scientific investigations need time to enhance the production, oil recovery rate, and health-promoting benefits associated with wild olives (Knoops et al., 2004). Even though various studies have been conducted to enhance its oil production and recovery rate but its isolated phytochemical constituents and associated health benefits have been less explored (Gorzynik-Debicka et al., 2018; Liaqat et al., 2021). Recent work by our research group has reported that ferruginan and cycloolivil isolated from *Olea Ferruginea* R possess anti-leishmanial and anti-bacterial properties (Joshi, 2012; Zafar et al., 2018). Similarly, we have further reported in another study that ferruginan A has shown significant antioxidant and moderate anticancer properties (Shah et al., 2022). Considering our earlier findings regarding the health-promoting benefits of wild-type olive, we further designed the present study to investigate its potent antidiabetic and antiinflammatory properties of ferruginan A.

## 2. Material and methods

### 2.1. Chemicals and reagents

All the chemicals and solvents used in this study were analytical grade. Purposely, Diclofenac Sodium, EDTA, distilled water, PBS (phosphate buffer saline) solution, and commercial Baker's yeast were procured from Sigma Aldrich. While, *n*-Hexane, ethyl acetate, silica gel column, methanol, butanol, and dichloromethane was purchased from Merck.

### 2.2. Plant material collection

The stems of the *Olea ferruginea* R plant were procured from Agriculture Research Institute (ARI), Tarnab, Peshawar. Different parts such as leaves, twigs, and stems were separated and later dried under shade.

### 2.3. Extraction, fractionation, and isolation

Stems of *Olea ferruginea* R dried in shade (3 kg) were ground using a commercial grinder and soaked in methanol: water (7:3) at 25 °C. This soaking process was carried out for five days and repeated three times. Each time the mixture was filtered, and resultant extract was pooled followed by drying under a vacuum to collect 700 g of crude extract. This obtained crude extract was further dissolved in 2 L of distilled water and fractioned through ethyl acetate, n-hexane, butanol, dichloromethane, and water. Upon drying the resultant fractions were ethyl acetate (200 g), n-hexane (155 g), butanol (25 g), aqueous (75 g), and dichloromethane (90 g). For the isolation of Ferruginan A (Fig. 1), ethyl acetate extract (200 g) was eluted through a silica gel column 60 (0.0062–0.200 mm; Merck). It was eluted using a mobile phase comprising of *n*-hexane: ethyl acetate (100: 0–0: 100). Mobile phase having composition of *n*-hexane: ethyl acetate (70: 30) was used for obtaining Ferruginan A. *Ferruginan A.* was isolated as a white amorphous powder (Shah et al., 2022).

### 2.4. In-vitro anti-inflammatory activity

For the assessment of the *in-vitro* anti-inflammatory potential of isolated compound, heat-induced hemolysis activity assays were performed in this study at different concentrations (10, 20, 30, 40, 50, 80 and 100 µl/mL). Ferruginan A in hypotonic saline solution on HRBC (hemolysis of human red blood cells) were determined by adopting the methods elaborated by Anosike et al. (2012). For this, 5 mL of blood sample was collected from healthy male volunteers who have not been receiving any anti-inflammatory drug for the last 10 days and placed in a

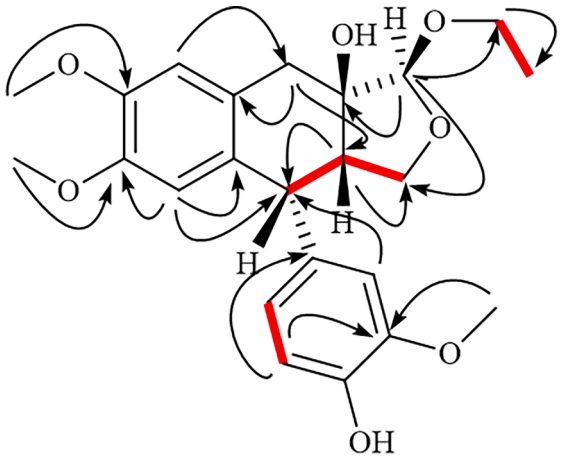


**Fig. 1.** Chemical structure of Ferruginan A.

flacon tube containing EDTA as an anticoagulant. This was further centrifuged for 5 min at 3000 rpm. Afterward, the supernatant was separated and the remaining content was washed with an isosaline solution. This centrifugation and washing process was carried out thrice until the appearance of clear supernatant. The pellet containing HRBCs was used for the preparation of 10 % suspension in an isotonic solution. The control (1 mL) reaction mixture prepared in this study comprised of 10 % blood suspension (100 µl) along with distilled water (20 µl) and PBS solution (880 µl). Similarly, standard reaction mixture (1 mL) consisted of 10 % blood suspension (100 µl) along with different diclofenac sodium (DS) concentrations plus PBS solutions (10–80 µl DS + 890–820 µl PBS). Moreover, test samples reaction mixture (1 mL) consisted of 10 % blood suspension (100 µl) along with different concentrations of Ferruginan A plus PBS solutions (10–80 µl Ferruginan A + 890–820 µl PBS). All these prepared mixtures were incubated for a period of 30 min at a temperature of 54 °C. Later these mixtures were centrifuged for 5 min at 54000 rpm. In this experiment, all the analysis were performed in triplicate and their absorbance were noted at 560 nm through a spectrophotometer.

Following formula was utilized for the calculation of percentage of inhibition (H) of HRBC lysis:

$$\%inhibition(H) = [(ControlAbs - SampleAbs)/ControlAbs] \times 100$$

### 2.5. In-vitro antidiabetic activity

In order to determine the effect of different experimented concentrations of (5 to 100 µl/mL) Ferruginan A and Metronidazole (reference standard) on glucose uptake by Baker's yeast (*S. cerevisiae*) cells, the method documented by Bhutkar and Bhise (2013) was followed. Commercially available baker's yeast underwent a thorough washing process using distilled water through centrifugation for 5 min at 4200 rpm until a clear supernatant as an endpoint. Afterward, suspension (10 % v/v) of baker's yeast was prepared in distilled water. Varied concentrations (5 to 100 µl/mL) of Ferruginan A and reference standard were mixed with glucose solution (1 mL; 5 mmol/L) and incubated for 10 min at 37 °C. After centrifugation of 10 min, yeast solution (0.1 mL) was added and again incubated at 37 °C for another 60 min. Lastly, the sample tubes were subjected to centrifugation for 5 min at 3000 rpm, and the glucose content was subsequently determined in the supernatant. The experiment was performed in triplicate. Following formula was used to analyze the increase in uptake of glucose in yeast cells:

$$\%glucoseuptake = \left[\frac{ControlAbs - SampleAbs}{ControlAbs}\right] \times 100$$

### 2.6. In vitro antibacterial activity

In this study, Ferruginan A was tested for its antibacterial property through disc diffusion method. The bacterial strain used in this study were Klebsiella pneumonia, Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Following the reported procedure by Zafar et al. (2018), a sterile petri dish containing 10 mL of sterile nutrient agar medium was incubated at 37 °C for 24 h to assess its sterility. After the agar plates were allowed to solidify, evenly spaced wells were created in the plates. The test samples were prepared using sterile dimethyl sulfoxide (DMSO) by dissolving fractions (2 mg/ml) and compounds (1 mg/ml). Imipenem was used as standard in this study. 0.1 mL solutions of the extracts and compounds were carefully introduced into their corresponding wells. After 2 h of refrigeration, the plates were subsequently incubated at 37 °C for 24 h. The resulting zones of inhibition were observed and measured in millimeters (mm).

### 2.7. Docking studies

The plant extract's three-dimensional structure was docked against COX-1, COX-2, and TNFα to investigate their anti-inflammatory activities and AMPK for anti-diabetic activity. Using alphanumeric codes, crystal structures of the target proteins were acquired from the Protein Data Bank (PDB). Using the Molecular Operating Environment (MOE), the compound's 3D structure was docked into the active sites of proteins COX-1 (PDB = 1EQG), COX-2 (PDB = 1CX2), diabetes AMPK (PDB = 3AQV), and TNFα (PDB = 2AZ5). The standard procedure was followed for compound and protein minimization, docking calculation, and analysis (Sadiq et al., 2021; Nadeem et al., 2021; Javed et al., 2021, Shah et al., 2022). The optimal orientation was selected based on the S-score and its deep interaction within the receptor site, as illustrated in the figure. Following our previously published procedure for preparation, the extracted compounds were protonated and minimized in the proper manner. The redock approach was used prior to molecular docking to adapt to the docking process. The binding interactions of freshly extracted compounds were compared to those of the native ligand of the enzyme after docking was completed. For evaluating 2D and 3D interaction plots, the Discovery Studio (DS-2021) tool was employed. Only validated docking procedures (RMSD > 2.0) were used in subsequent research.

### 2.8. ADMET study

Drug discovery is one of the expensive research projects, therefore, the in-silico study not only saves time but also saves research expenses. The tested compound was subjected to ADMET (absorption, distribution, metabolism, and toxicity) study. This study was performed using online software (SwissADMET and pkCSM).

## 3. Results

### 3.1. Effect of Ferruginan A on Heat-Induced hemolysis

Ferruginan A significantly inhibited the heat-induced lysis of the HRBCs membrane in a dose-dependent manner. Ferruginan A (10–100 µl/mL) showed 8.82 %, 17.67 %, 30.84 %, 46.81 %, 54.95 %, 62.81 %, and 69.82 % of inhibition, respectively. Whereas, for the standard group, the diclofenac sodium (10–100 µl/mL) showed a percentage inhibition ranging from 32.66 to 85.71 %, respectively (Fig. 2).

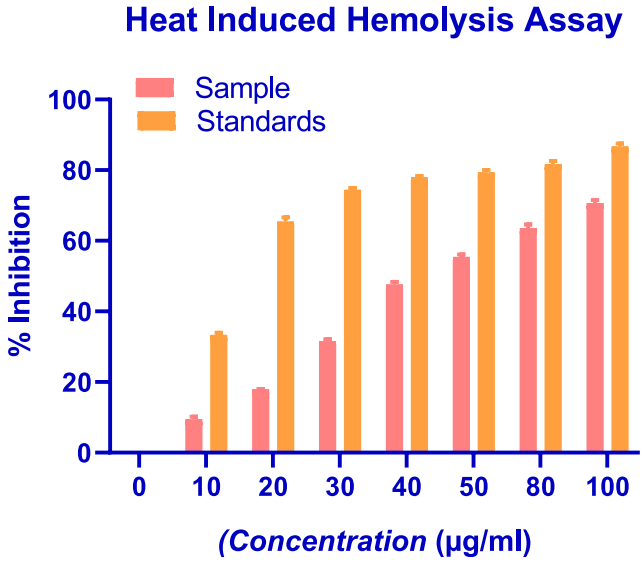


**Fig. 2.** Effect of Ferruginan A on Heat induced hemolysis assay.

### 3.2. Effect of different concentration of Ferruginan A on glucose uptake by yeast cells in glucose solution

**Ferruginan A** at 05 µl/ml and 100 µl/ml increased the uptake of glucose from 3.79 % to 71.86 % in the yeast cell, respectively. This increase in uptake by baker's yeast was noticed to be in a concentration-dependent manner. While, Metronidazole (standard drug) were used for comparison purpose and showed significant effect on uptake of glucose by the yeast cells. According to assay, there is an increase from 17.09 % and 85.71 % in glucose uptake at 05 µl/ml and 100 µl/ml concentration of metronidazole, respectively. Fig. 3 shows that the activity of **Ferruginan A** and the standard.

### 3.3. Antibacterial activity

The antibacterial activity of Ferruginan A is given in Table 1. The compound 1 was screened for its antibacterial properties (zone of inhibition: mm) against selected bacterial strain *i.e.,* Klebsiella pneumonia., Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Zone of inhibition values of Ferruginan A (24.98 mm) against Escherichia coli was found to be comparable to standard (Imipenem: 31.09 mm).

### 3.4. Docking study

The PDB accession codes of the target proteins are COX-1 (1EQG), COX-2 (1CX2), diabetes AMPK (3AQV), and TNF-α (2AZ5). The isolated compound was docked into the active sites of COX-1 (PDB ID = 1EQG), COX-2 (PDB ID = 1CX2), and TNF-α (PDB ID = 2AZ5) to investigate their anti-inflammatory potential. Following the docking simulation, all orientations were examined, and the best pose was chosen based on the S-score and interaction with key amino acids in the target proteins' active sites. Fig. 4 depicts the generated 2D interaction plot. Hydroxy groups form conventional hydrogen bond interactions with Tyr385, Tyr355, and Arg120 in the active site of COX-1 (Fig. 4a). In the active site of COX-2, the hydroxyl and alkoxy groups of compounds respectively show hydrogen bonding interactions with important residues Arg513 and Tyr385. The aryl ring shows π-sigma interaction with Ser353 (Fig. 4b). Compound alkoxy and hydroxy groups form conventional hydrogen bonding interactions with Gly121 and Tyr151 in TNF-a active site residues. Aryl ring show π-π stacked interaction Tyr119 (Fig. 4c).

Lastly, the isolated compound was docked with AMPK protein (PDB ID = 3AQV) to explore its potential anti-diabetic activity. After the docking computation was accomplished, all poses were analyzed, and the best orientation was chosen based on their S-score and interaction. Fig. 5 depicts the interaction 2D plot. The compound's alkoxy and hydroxy groups exhibit conventional hydrogen bonding interactions with Tyr95, Glu100, and Asp103, as well as π-alky interactions with the essential amino acid Leu22.

**Table 1**
Antibacterial activity of Ferruginan A isolated from *Olea ferruginea.*

| Strain | Zone of inhibition (mm) | | |
|---|---|---|---|
| | Control | Ferruginan A | Imipenem |
| Klebsiella pneumonia | 0 | 8.65 ± 1.54 | 28.98 ± 1.87 |
| Bacillus subtilis | 0 | 15.87 ± 1.00 | 30.55 ± 1.23 |
| Staphylococcus aureus | 0 | 18.65 ± 1.65 | 29.09 ± 1.66 |
| Escherichia coli | 0 | 24.98 ± 0.98 | 31.09 ± 1.09 |

### 3.5. ADMET study

The **Ferruginan A** was subjected to an ADMET toxicity study. This study demonstrated that the drug likeliness of the compound is very good and obeys the rule of five, *i.e.* a compound-like drug must not have H-bond acceptors not more than 10 and H-bond donner must be not more than 5. The study also exhibited a good ADMET study as shown in Table 2. The intestinal absorption was 100 % with no AMES toxicity. The compound was also resulted free of hepatotoxic and skin sensitization effects.

## 4. Discussion

Medicinal plants possess health-promoting benefits owing to the

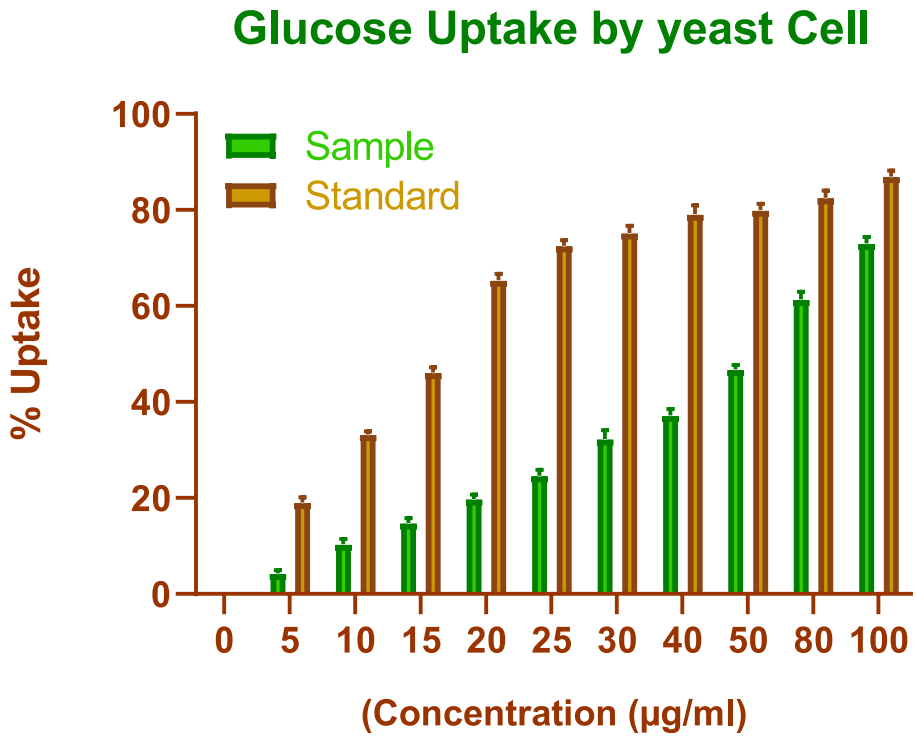


**Fig. 3.** Effect of Ferruginan A on glucose uptake by yeast cells in glucose solution (Glucose Yeast Uptake Assay/5mM).

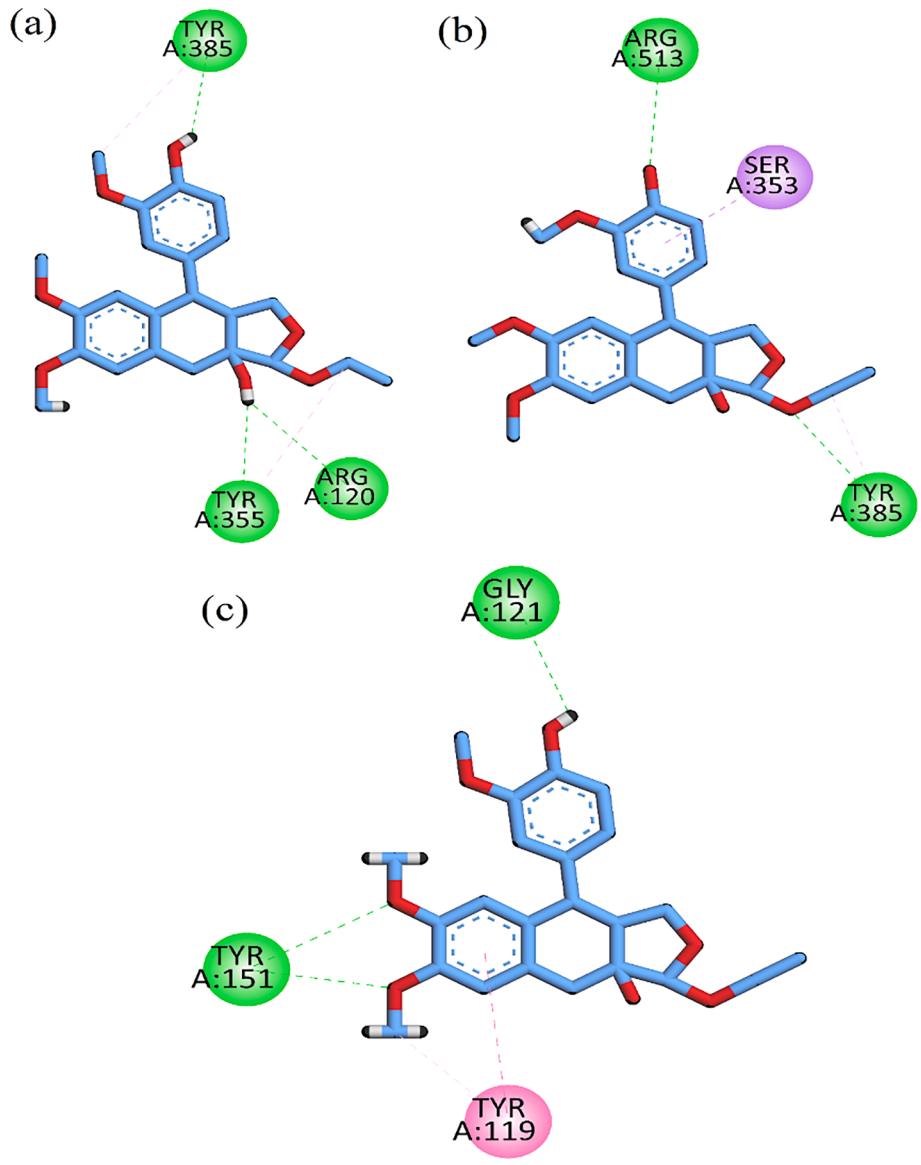


**Fig. 4.** 2D plot of interaction of (**a**) COX-1(PDB ID = 1EQG), (**b**) COX-2 (PDB ID = 1CX2) and (**c**) TNF-a (PDB ID = 2AZ5).

presence of biologically active components in their matrices. Similarly, different bioactive properties like antidiabetic, anticancer, antioxidant, antibacterial, and antileishmanial associated with used of *Olea Ferruginea* R might be attributed to the occurrence of bioactive compounds. Olea plant extract encompasses several polyphenols such as lignin, phenolic acids, flavonoids, and secoiridoid. Recently, various studies have revealed different bioactive components present in *Olea Ferruginea* R like cycloolivil and ferruginan A possessing various bioactivities (Zafar et al., 2018).

Therefore, presence of phenolic compound in extract of *Olea Ferruginea* R may assist in increasing the uptake of glucose. Purposely, in this study, **Ferruginan A** was isolated from *Olea Ferruginea* R and was assessed for its *in-vitro* antidiabetic properties by performing glucose uptake by yeast cells assay. Results of this study revealed that **Ferruginan A** increased the uptake of glucose to yeast cells. Generally, in skeletal muscles the uptake of glucose occurs owing to aggregation of glucose transporting molecule across the cell membrane within the cell. These molecules are regulated through myocytes/leptocytes in response to elevated content of insulin secreted in blood (Rajeswari and Sridevi, 2014). Glucose uptake by yeast cells assay is effectively being employed as in-vitro method for diabetes as the yeast cells have affinity for glucose. In this study, **Ferruginan A** (isolated compound) and Metronidazole (standard drug) enhanced the uptake of glucose by yeast cells, which may be of the reason that they enhance the glucose uptake across peripheral cells (Bhutkar and Bhise, 2013). Mechanistically, metronidazole, the standard drug, increases the uptake of glucose by hepatic and skeletal cells. **Ferruginan A**, the isolated compound, may also have same mechanism of action as that of metronidazole. Across the yeast membrane, the transportation of glucose may also occur through facilitated diffusion. Similarly, increase in glucose uptake by yeast cells in the presence of **Ferruginan A** may be owing to both increased glucose metabolism and facilitated diffusion. Further, in-vivo studies are suggested to validate the binding of **Ferruginan A** with glucose and transportation across cell membrane.

Several scientists to assess the *in-vitro* anti-inflammatory properties of plant extracts, isolated compounds, and drugs have performed membrane stabilization tests like heat-induced hemolysis and HRBC membrane stabilization assays. For this purpose, in this study, *in-vitro* anti-inflammatory properties of **Ferruginan A** were assessed through heat-induced hemolysis assay. Results as discussed in earlier section showed that **Ferruginan A** inhibited heat-induced HRBCs membrane lysis in a dose-dependent manner with maximum inhibition of 69.82 %

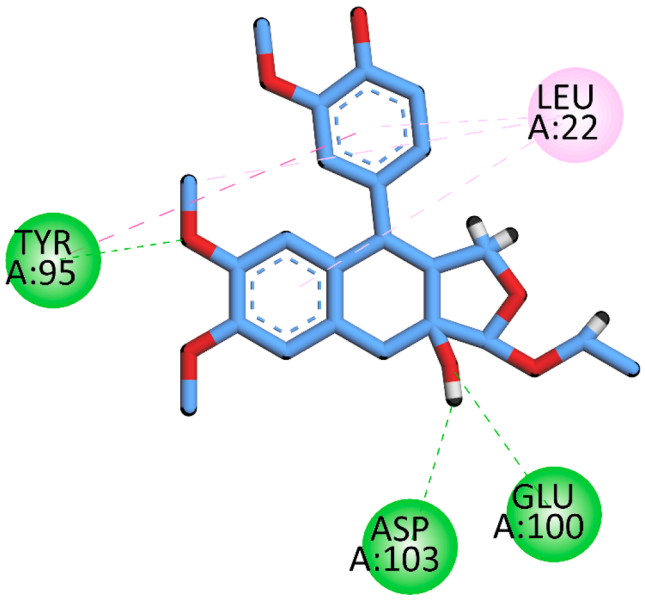


**Fig. 5.** 2D plot of interaction of Diabetes AMPK protein (PDB ID = 3AQV).

**Table 2**
ADMET study of Ferruginan A.

SMILS of tested **Ferruginan A**, CCOC1OCC2C(C3 = CC = C(O)C(OC) = C3)C3 = C(CC12O)C = C(OC)C(OC) = C3

| Category | ADMET in units | **Ferruginan A** |
|---|---|---|
| Physicochemical property | No of H-bond acceptor | **7** |
| | No of H-bond donner | **2** |
| Absorption | Water solubility (log mol/L) | −4.731 |
| | Caco2 permeability (log Papp in $10^{-6}$cm/s) | 1.311 |
| | Intestinal absorption (%) | 100 |
| | Skin permeability (log Kp) | −2.747 |
| Distribution | VDss human (log L/kg) | 0.033 |
| | CNS permeability (log PS) | −3.323 |
| | BBB permeability (log BB) | −0.27 |
| Metabolism | CYP2D6 substrate | NO |
| | CYP3A4 substrate | Yes |
| | CYP1A2 Inhibitor | NO |
| | CYP2C19 Inhibitor | Yes |
| | CYP2D6 Inhibitor | No |
| | CYP3A4 Inhibitor | Yes |
| Excretion | Total clearance (log ml/min/kg) | 0.389 |
| | Renal OCT2 substrate | No |
| Toxicity | AMES toxicity | NO |
| | Max. tolerated dose human (log mg/kg/day) | −0.158 |
| | Oral rat acute toxicity (LD50, Mol/kg) | 2.535 |
| | Oral rat chronic toxicity (LOAEL, log mg/kg/bw/day | 2.394 |
| | Hepatotoxicity | No |
| | Skin sensitization | No |

Link: https://biosig.lab.uq.edu.au/pkcsm/prediction_single/adme_1677478135.7, https://www.swissadme.ch/index.php.

at 100 μg/mL. While, diclofenac sodium (standard drug) revealed 85.71 % of inhibition at 100 μg/mL. These results suggest the anti-inflammatory properties of isolated compound *i.e.* **Ferruginan A. Ferruginan A** may helped in stabilizing the HRBC membrane via inhibiting the rupturing and release of lysosomal enzymes (Shams et al., 2019). Inflammation causes damaging of tissues that lead to different types of diseases. Chronic and acute inflammation may lead to membrane destabilization (Anosike et al., 2012). While, the inflammation that results in membrane destabilizing can be inhibited through drugs. Synthetic drugs have side effects therefore scientists are more focused on natural products. In recent times, it has been found that natural products are effective in relieving the inflammation and pains (Shenoy et al., 2010; Babu et al., 2011; Anosike et al., 2012; Zhang and Tsao, 2016). Heat-induced hemolysis assay is performed to assess the in-vitro anti-inflammatory properties as it resembles lysosomal membrane (Shenoy et al., 2010). This test gives an overview regarding stabilization of lysosomal membrane, which are key in regulating inflammatory reactions (Babu et al., 2011; Kuropka et al., 2017). Elevated temperature increases the permeability of membrane and release of hemoglobin that are inhibited by anti-inflammatory candidates. Anti-inflammatory constituents bind with cyclooxygenases (lysosomal enzyme) and hence inhibits the lysis of HRBC membrane (Boniface et al., 2014). Results of this study reveals that isolated **Ferruginan A** significantly possesses in-vitro antidiabetic and anti-inflammatory properties in a concentration-dependent manner.

Various studies have reported the antibacterial and antifungal properties of olives. The zone of inhibition is a crucial concept in assessing the antibacterial activity of antimicrobial agents, such as antibiotics or disinfectants. It refers to the clear area surrounding an antimicrobial disk or agent on a culture plate where bacterial growth is inhibited. For this purpose, in this study Ferruginan A was screened for its antibacterial properties (zone of inhibition: mm) against selected bacterial strain *i.e.*, Klebsiella pneumonia., Bacillus subtilis, Staphylococcus aureus, and Escherichia coli. Zone of inhibition values of Ferruginan A (24.98 mm) against Escherichia coli was found to be comparable to standard (Imipenem: 31.09 mm). Results of our study were in accordance with earlier study conducted by Mehmood and Murtaza (2018). They revealed that methanolic extract of O. ferruginea had significant inhibitory potential against *E. coli, B. subtilis, and S. Aureus.* On the other hand, Amin et al. (2013) also stated promising antifungal and antibacterial properties of different fractions of O. ferruginea against both gram negative and gram-positive microbes.

Four target enzymes associated with anti-inflammatory and antidiabetic pathways were selected to perform the docking simulations. The purpose was to explore the possible mechanism. The isolated compound was docked into the binding sites of these four selected enzymes. Isolated compound showed hydrogen bond interaction with deeply located Arg513 in the selective COX-2 binding site. Moreover, it showed good interactions in the binding site of Tumor necrosis factor alpha (TNF-α), which is considered as a key target for the development of drugs for many inflammatory diseases such as rheumatoid arthritis.

## 5. Conclusions

Conclusively, results of this study demonstrated that **Ferruginan A** isolated from *Olea Ferruginea* R possessed noteworthy in-vitro antidiabetic, antiinflammatory and antibacterial properties that validate the use of this plant in folk medical health systems. Further, in-vivo studies must be conducted to authenticate the anti-inflammatory and antidiabetic properties associated with isolated compound. Docking studies on various molecular targets of the disease showed the isolated compound may act by inhibiting the AMPK, COX-1 and COX-2. Moreover, it showed good interactions in the binding site of Tumor necrosis factor alpha (TNF-α), which is considered as a key target for the development of drugs for many inflammatory diseases such as rheumatoid arthritis.

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## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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