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Original Research

Antiproliferative Activity of Lignans from *Olea ferruginea*: *In Vitro* Evidence Supported by Docking Studies

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Abstract

Background: The aim of the current study was to investigate the anticancer potential of bioactive compounds isolated from the leaves of *Olea ferruginea* (*O. ferruginea*). Lignans from *O. ferruginea* were previously described to possess antibacterial, antileishmanial, and antioxidant properties. Nevertheless, the antiproliferative activity of cycloolivil (1), ferruginan (2), and ferruginan A (3) have not been investigated in depth. **Methods**: The compounds were isolated from the ethyl acetate fraction of the leaves extract of *O. ferruginea*. The isolated molecules were evaluated for their anticancer activity against U-87 MG malignant glioma cells. In parallel, molecular docking studies were also performed to investigate the interaction of the compounds with a duplex DNA sequence and epidermal growth factor receptor (EGFR). **Results**: *In vitro* tests showed that all three compounds inhibit U-87 MG malignant glioma cell proliferation dose-dependently in the µM range, and ferruginan A (3) was highlighted as the most promising compound of the set. Molecular docking studies showed that the compounds could interfere with double stranded DNA possessing a cisplatin 1,2-d(GpG) intrastrand cross-link and EGFR. **Conclusions**: Overall, the findings suggest that the tested compounds from *O. ferruginea* may represent a starting point for the identification of novel tools to inhibit glioma cell proliferation.

Keywords: *Olea ferruginea*; cycloolivil; ferruginan; glioma; EGFR; docking

1. Introduction

Plants are a rich source of bioactive compounds of crucial relevance in the field of medicinal chemistry, and modern drug discovery tools help in rationalizing the bioactivity evidence of extracts and molecules used in traditional medicine. Computational tools and high-throughput screening (HTS) accelerate the transition from natural therapeutic agents to drug candidates [1,2].

The *Olea* genus comprises forty common species belonging to the Oleaceae family, among which *Olea ferruginea* is one of the most widespread species. *O. ferruginea* is found in Afghanistan, Pakistan, and Kashmir but also in the Mediterranean region [3].

Different parts of these plants have traditional uses in the treatment of several diseases. Antipyretic, antiseptic, antimicrobial, and antioxidant properties related to the use of parts of the plant or of their extract [4].

Through the years, flavonoids and other phenolic compounds were isolated from this plant, characterized, and studied for their potential therapeutic applications [5]. More in detail, molecules from *O. ferruginea* were previously described as anti-inflammatory and antidiabetic agents [6], as well as antibacterial and antileishmanial compounds [7]. Moreover, flavonoids and phenolic compounds from leaves and stem bark showed antioxidant properties [8]. On the other hand, only a few reports describing the antiproliferative and anticancer activity of *O. ferruginea* or its extracts can be retrieved in the literature, and they are related to the effects on MCF-7 breast cancer cell line, HeLa and Vero cells, and HepG2 human liver cancer cell line [9–11].

The aim of the current study is to investigate a new, potential role of three compounds isolated from the leaves of *O. ferruginea*: cycloolivil (1), ferruginan (2), and ferruginan A (3). In the current study, the molecules were

tested against the U-87 MG malignant glioma cell line, a model for the tumor affecting the central nervous system (CNS). Computational techniques, as well as *in vitro* experiments, were enrolled to assess the antiproliferative potential of such compounds and to better understand the possible underlying mechanisms. More in detail, cell survival upon treatment with the compounds was evaluated in comparison to cisplatin. Additionally, molecular modeling was enrolled to investigate the binding of the studied compounds towards a model sequence of duplex DNA targeted by cisplatin [12] and epidermal growth factor receptor (EGFR), a protein target involved in glioma progression [13,14].

2. Materials and Methods

2.1 Plant Collection and Isolation of Compounds

Leaves of *O. ferruginea*, *i.e.*, *Olea europaea* var. *ferruginea* (Royle), were collected from Tarnab, District Peshawar, Pakistan, during the summer season. The leaves were identified as *O. ferruginea* leaves by Dr. Muhammad Ilayas (University of Swabi). The voucher specimen number UOS/Bot104 was deposited in the herbarium of the Department of Botany of the University of Swabi, Pakistan. The extraction was carried out through modification of a protocol reported previously [6]. The leaves of *O. ferruginea* were dried in the shade at room temperature and then ground into a fine powder using a grinding machine; 5 kg of powder was extracted with 5 L of a H_2O/methanol (3:7) mixture for 15 days at room temperature. The resulting extract was filtered and concentrated using a rotavapor. This process was repeated twice more to achieve complete extraction; 1.5 kg of crude extract was then suspended in 2 L of distilled water for extraction with *n*-hexane, dichloromethane, and ethyl acetate. The ethyl acetate fraction, after evaporation, which provided 170 g of extract, was loaded into a silica gel chromatographic column. The elution was performed with a gradient of dichloromethane and methanol (from 100:0 to 60:40), and 6 fractions were collected. Fraction 4 was rechromatographed with *n*-hexane and ethyl acetate as the mobile phase, increasing solvent polarity from 100:0 to 20:80, giving 5 subfractions. Subfraction 3 provided cycloolivil ((1) 60 mg) and ferruginan ((2) 15 mg), which were obtained as white solids after further purification by column chromatography with *n*-hexane and ethyl acetate (100:0–30:70). On the other hand, ferruginan A ((3) 35 mg) was obtained from subfraction 4, which was processed similarly. The compounds were fully characterized, and analytical profiles were in agreement with previously reported data [7,9].

2.2 Antiproliferative Activity

The compounds extracted from *O. ferruginea* were evaluated for their antiproliferative activity on the U-87 MG malignant glioma cells. We profoundly acknowledge Prof. Norah Defamie, Pôle Biologie Sante Université de Poitiers, France, for kindly providing the cells that were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and that are identified by the ATCC code HTB-14. Cells were cultured in the Cancer Cell Culture & Precision Oncomedicine Lab, Institute of Basic Medical Sciences, Khyber Medical University Peshawar, Pakistan, and the cell line was used in agreement with previous studies from our group [15,16]. Authentication of cell lines was performed by the authors, and PCR analysis was carried out [15,17]. The cells were cultured in DMEM media (Gibco, USA) supplemented with 10% FBS (Cegrogen biotech, Germany) and 1% penicillin-streptomycin (Gibco, USA) at 37 °C in a 5% CO_2 humidified incubator (Humanlablne, Korea). Upon reaching confluency, the cells were seeded in a 96-well plate, and a growth inhibition assay was performed following a procedure reported previously [18]. Briefly, 5000 cells were seeded into each well of a 96-well plate and allowed to grow overnight at 37 °C with 5% CO_2 supply. Stock solutions of cycloolivil (1), ferruginan (2), and ferruginan A (3) were prepared in DMSO (3 mg/mL). The cells were then treated in triplicate with different dilutions of the compounds (400, 200, 100, 50, 25, 12.5, and 6.25 µM), and an equivalent volume of vehicle was used as a negative control. In addition, cisplatin was used as the positive control, utilizing the same dilutions as the test compounds. Then, the cells were incubated in a CO_2 incubator at 37 °C for 24 hours. Afterward, cells were fixed with 4% formalin (Scharlab, Spain) for 10 minutes at room temperature. Following fixation, 100 µL of 0.1% crystal violet (Daejung, Korea) was added to each well, and then a 10 min incubation at room temperature was performed. Cells were washed three times with PBS (Sigma Aldrich, St. Louis, MO, USA), and the plates were allowed to dry overnight. Finally, 200 µL of acetic acid (Merck, Germany) was added to each well to solubilize the crystal violet stain, and absorption spectra were recorded at 590 nm using a spectrophotometer (Biotek-ELISA, Santa Clara, CA, USA). IC_{50} values were calculated for each compound based on linear regression analysis.

2.3 Molecular Docking

Molecular docking studies were performed using Molecular Operating Environment (MOE 2016.0802). X-ray crystal structure of duplex DNA containing a cisplatin intrastrand cross-link was obtained from the Protein Data Bank (PDB ID 3LPV) [19,20], as also the crystal structure of EGFR (PDB ID 3POZ). The downloaded crystal structures were prepared and 3D protonated by using the "Prepare" module of MOE [21,22]. For docking simulations, docking grids were determined within 10 Å from the binding site. In the case of EGFR, the docking procedure was validated by using the re-docking method. Cognate ligand 03P, also known as TAK-285, was re-docked in the binding site. The pose showed a root-mean-square deviation (RMSD) of 2.0 Å with respect to the cognate, and interac-

tions with key amino acid residues were maintained. For all the ligands, ten docking poses were generated, and the top-ranked conformations based on docking score were selected. Furthermore, we also performed docking studies by using AutoDock 4.2 [23] to validate the results using the same parameters. The docking studies were carried out following our previously reported method [21]. Ligand interaction and visualization studies were carried out using Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, Waltham, MA, USA), while 3D artworks were processed using UCSF Chimera [24].

3. Results

O. ferruginea is a source of natural bioactive compounds [18]. The tested molecules, depicted in Fig. 1, were extracted from the leaves of the plant following a previously reported procedure with minor modifications. A scheme resuming the extraction process, which is based on fractionation and subsequent purifications through silica gel column chromatography of the extract, is depicted in **Supplementary Fig. 1**. Lignans from O. ferruginea were previously studied for their several potential biological applications. Our research group reported the *in vitro* anti-inflammatory and antidiabetic activity of ferruginan (2), and the results were supported by ligand-based and structure-based computational studies [6]. Cycloolivil (1) and ferruginan (2) are also endowed with antibacterial and antileishmanial activity [7]. Moreover, Sharma *et al.* [8] highlighted the antioxidant properties of extracts from several parts of O. ferruginea, which were related to the presence of flavonoids and phenolic compounds. The authors identified leaves and stem bark as the ideal sources of antioxidants to be extracted with methanol.

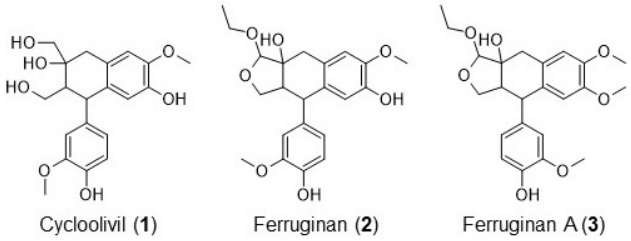

Fig. 1. Chemical structures of the isolated compounds.

3.1 Antiproliferative Activity

Concerning the studies focused on the antiproliferative activity of compounds from *O. ferruginea*, only a few reports can be retrieved in the literature. Ferruginan (2) was previously described as a cytotoxic agent in the MCF-7 breast cancer cell line (IC_{50} = 10.41 µg/mL) [9]. Hashmi *et al.* [10] tested quercetin, β-amyrin, oleuropein, and ligstroside isolated from *O. ferruginea* against HeLa and Vero cells, while Liaqat *et al.* [11] evaluated the cytotoxicity on HepG2 human liver cancer cell line of hexane, chloroform, ethanol, methanol, and water extracts of *O. ferruginea* barks, where the ethanol extract was highlighted as the most promising.

Table 1. Antiproliferative activity of the tested compounds reported as IC_{50} values.

Compound	IC_{50}
Cycloolivil (1)	225.0 ± 0.1 µM
Ferruginan (2)	320.0 ± 0.1 µM
Ferruginan A (3)	216.4 ± 0.1 µM
Cisplatin	124.3 ± 0.1 µM

The treatment of gliomas, tumors affecting the CNS, is very challenging. Despite new surgery protocols and advances in treatments and radiation techniques, the survival rate struggles to improve, mainly due to recurrence [25]. Thus, the search for novel therapeutic tools of natural and synthetic origin is wide open [26,27]. In this work, to evaluate the anticancer effects of cycloolivil (1), ferruginan (2), and ferruginan A (3), U-87 MG malignant glioma cells were treated with the compounds (6.25–400 µM), and the concentration range was selected in agreement with previous studies [9–11]. Cell survival was plotted against drug concentration, and IC_{50} values were calculated. The result indicated that the cell survival decreased upon the increase of compound concentration (**Supplementary Fig. 2**), and the IC_{50} values are reported in Table 1. Overall, the compounds showed mild cytotoxic activity, with IC_{50} values in the µM range. In particular, in the current experimental model, ferruginan A (3) was highlighted as the most promising compound of the set, as an IC_{50} value of 216.4 ± 0.1 µM was calculated for this molecule against U-87 MG cells. For reference, cisplatin was tested, and an IC_{50} value of 124.3 ± 0.1 µM was obtained for this compound under the mentioned experimental conditions.

3.2 Computational Studies

Computational drug discovery tools were enrolled in the current study to assist in the interpretation of experimental data. Double stranded DNA (dsDNA) is one of the potential targets of compounds developed for the treatment of glioma, and it also represents the macromolecular interactor of cisplatin. In particular, cisplatin binds the N7-purine bases, resulting in cell division block and apoptotic cell death, and several recent studies report the efficacy of therapies based on the combination of cisplatin and other cytotoxic drugs [12]. Thus, the first molecular modeling simulation was performed by docking the isolated compounds to the structure of a dsDNA possessing a cisplatin 1,2-d(GpG) intrastrand cross-link (PDB ID 3LPV). The results of the docking study are depicted in Fig. 2, in which the 2D interaction maps, as well as the 3D models, are reported for all the compounds. Cycloolivil (1) interacts through hydrogen bonds with adenine 17 and guanine

16 and with guanine 14 through a hydrophobic interaction. Ferruginan (2) binds thymine 8, adenine 17, and adenine 15 through hydrogen bonds thanks to the hydroxyl groups. The interaction pattern is slightly different for ferruginan A (3). Hydrogen bonds are formed with adenine 15, cytosine 17, and cytosine 18, but π-π interactions with adenine 15 and guanine 17 can also be observed. The calculated binding energy values computed for cycloolivil (1), ferruginan (2), and ferruginan A (3) are –6.8, –5.9, and –7.0 kcal/mol, respectively. The compounds showed the same trend when AutoDock was used. In this case, the values computed for cycloolivil (1), ferruginan (2), and ferruginan A (3) are –6.1, –5.6, and –6.6 kcal/mol, respectively.

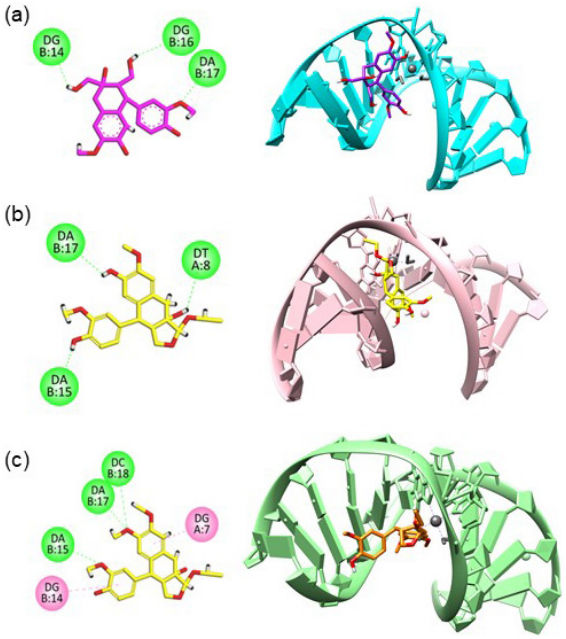

Fig. 2. Molecular docking study for the interaction of cycloolivil (1, a), ferruginan (2, b), and ferruginan A (3, c) with ds-DNA. 2D interaction maps and 3D models are depicted for the tested compounds.

The compounds could potentially exert their antiproliferative activity through several molecular mechanisms. Among these, it must be noted that EGFR is a target of primary relevance in the context of the treatment of glioma, as amplification and mutation of the EGFR gene were observed in these tumors [13,14]. The EGFR gene encodes for a transmembrane glycoprotein from the protein kinase superfamily. This protein works as a receptor for the epidermal growth factor (EGF): on the cell surface, EGFR binds EGF, promoting receptor dimerization and tyrosine autophosphorylation, resulting in enhanced cell growth [13]. Thus, in view of a potential multi-target mechanism involving the isolated compounds in their antiproliferative activity against glioma cells, another molecular docking study was performed considering the 3D structure of EGFR (PDB ID 3POZ). The results of the docking study are depicted in Fig. 3, in which the 2D interaction maps, as well as the 3D models, are reported for all the compounds. Cycloolivil (1) interacts with Lys745, Asp885, Cys797, and Asn842 through hydrogen bonds. Similarly, ferruginan (2) binds Arg841, Met793, Leu788, and Gln791, and interactions are mainly mediated by hydroxyl groups. Ferruginan A (3) interacts with the protein through a pattern that is intermediate between the two previous compounds. More specifically, Cys797 and Lys745 are targeted like in the case of cycloolivil (1), while the compound also binds Gln791 as in the case of ferruginan (2). Overall, in this case, the docking study suggests that the -OH groups represent the main groups mediating the interaction with the macromolecular target. The calculated binding energy values computed for cycloolivil (1), ferruginan (2), and ferruginan A (3) are –7.0, –8.0, and –7.4 kcal/mol, respectively. Also, in this case, the compounds showed the same trend when AutoDock was used. In this case, the values computed for cycloolivil (1), ferruginan (2), and ferruginan A (3) are –7.5, –8.3, and –7.8 kcal/mol, respectively. Additionally, the cognate ligand was re-docked for reference with both software and values of –10.4 kcal/mol and –10.9 kcal/mol were obtained by using MOE and AutoDock, respectively.

4. Discussion

The present research work aimed at contributing to the emerging and fast-growing body of research exploring the potential of natural products as sources of anticancer agents. The efficient isolation of cycloolivil (1), ferruginan (2), and ferruginan A (3) from the leaves of *O. ferruginea* once again confirms that this plant is a promising source of bioactive compounds.

The potential of natural sources of anticancer agents has been the subject of extensive research throughout the years, and several molecules have been identified as potential lead compounds for the development of new anticancer therapies. The results of the current study are of relevance since, while lignans were previously investigated as antiproliferative agents, they represent the first report concerning the activity of *O. ferruginea* components against glioma, a form of tumor for which effective treatment is still very challenging. Moreover, in this paper, we provided a preliminary mechanistic investigation of the observed effects.

Cycloolivil (1), ferruginan (2), and ferruginan A (3) showed modest growth inhibitory activity on the tested U-87 MG malignant glioma cell line, with ferruginan A (3) being the most promising compound of the set. Nevertheless, IC_{50} is still in the µM range for this compound, suggesting that the isolated molecules could serve as a starting point for future modifications and derivatizations of the scaffold to produce optimized derivatives with better antiproliferative activity.

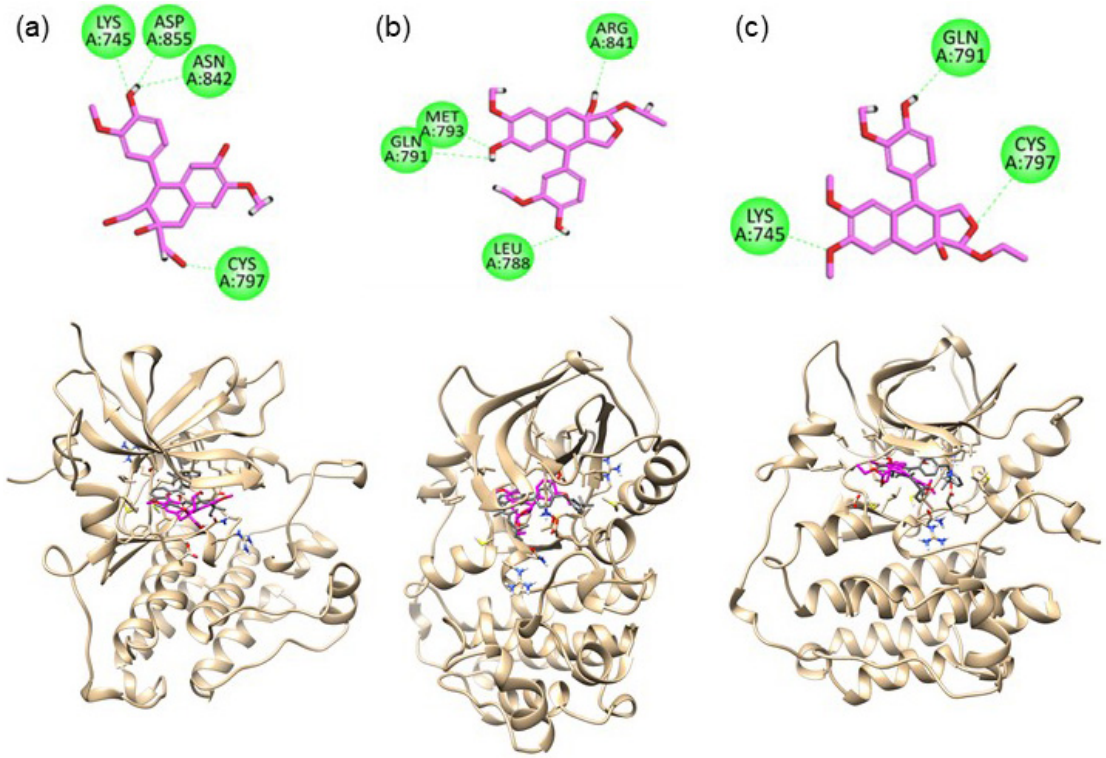

Fig. 3. Molecular docking study for the interaction of cycloolivil (1, a), ferruginan (2, b), and ferruginan A (3, c) with EGFR. 2D interaction maps and 3D models are depicted for the tested compounds.

The molecular docking studies carried out in this work provide insight into two potential and previously documented molecular mechanisms underlying the anticancer activity of compounds targeting gliomas. Docking to a ds-DNA sequence highlighted that the studied lignans may act synergically with cisplatin, as they efficiently target a site close to the d(GpG) intrastrand cross-link. In this simulation, ferruginan A was highlighted as the most promising compound of the set, in agreement with *in vitro* data. The results of the second part of the study suggest that the compounds may exert their anticancer activity by inhibiting EGFR through interactions with relevant residues of the protein and that the molecules share a similar interaction pattern. In this case, calculated binding energy values highlighted ferruginan (2) as the best binder, even if this compound was the one showing the highest IC_{50}, while the more promising derivative ferruginan A (3), according to *in vitro* data, ranked second in terms of docking score. Thus, these findings are partially consistent with previous studies that have demonstrated the importance of EGFR in glioma development and progression.

5. Conclusions

The present study provides valuable insights into the potential anticancer activity of bioactive compounds isolated from the leaves of *O. ferruginea*.

The results of the growth inhibition assay indicated that all three isolated compounds exhibited growth inhibitory activity against U-87 MG malignant glioma cells. More specifically, ferruginan A (3) showed the most potent anticancer activity.

Experimental results were paired by computational studies aiming at investigating two putative mechanisms involved in gliomas by which these potentially multi-target antiproliferative agents may act. All the tested compounds were predicted to interact with the major groove of dsDNA, suggesting a role in the interference with cellular processes. Additionally, the compounds target the binding pocket of EGFR, sharing a similar interaction pattern. Computational results, in terms of docking score, were partially consistent with *in vitro* data.

It must be pointed out that the potency of the studied molecules, for which IC_{50} is in the micromolar range, is not optimal, and further development would be needed. In particular, the rational design of semi-synthetic derivatives, also based on the results of the current study, would be desirable.

Overall, the current study paves the way for the future optimization of lignans from *O. ferruginea* and for the experimental assessment of the molecular mechanisms underlying the biological activity of such compounds.

Availability of Data and Materials

Data present within the article and **Supplementary Materials**.

Author Contributions

AR and GR contributed to the design of the work. AA, IK, ZAS, BM, AJ, NA, SN and ZA acquired, analyzed, or interpretated data for the work. UR and TSA interpreted the data for the work and wrote the draft of the manuscript. All authors contributed to writing and editing. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Given the role as Guest Editor, Giovanni Ribaudo had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Jen-Tsung Chen.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbl2809216.

References

[1] Mushtaq S, Abbasi BH, Uzair B, Abbasi R. Natural products as reservoirs of novel therapeutic agents. EXCLI Journal. 2018; 17: 420–451.

[2] Camp D, Davis RA, Evans-Illidge EA, Quinn RJ. Guiding principles for natural product drug discovery. Future Medicinal Chemistry. 2012; 4: 1067–1084.

[3] Sarker SD, Nahar L. An introduction to natural products isolation. Methods in Molecular Biology. 2012; 864: 1–25.

[4] Geyid A, Abebe D, Debella A, Makonnen Z, Aberra F, Teka F, *et al*. Screening of some medicinal plants of Ethiopia for their anti-microbial properties and chemical profiles. Journal of Ethnopharmacology. 2005; 97: 421–427.

[5] Ayeleso TB, Matumba MG, Mukwevho E. Oleanolic Acid and Its Derivatives: Biological Activities and Therapeutic Potential in Chronic Diseases. Molecules. 2017; 22: 1915.

[6] Rauf A, Rashid U, Shah ZA, Rehman G, Bashir K, Jamil J, *et al*. Anti-Inflammatory and Anti-Diabetic Activity of Ferruginan, a Natural Compound from Olea Ferruginea. Processes. 2023; 11: 545.

[7] Zafar S, -Ur-Rehman F, Shah ZA, Rauf A, Khan A, Humayun Khan M, *et al*. Potent leishmanicidal and antibacterial metabolites from *Olea ferruginea*. Journal of Asian Natural Products Research. 2019; 21: 679–687.

[8] Sharma RK, Sharma N, Kumar U, Samant SS. Antioxidant properties, phenolics and flavonoids content of some economically important plants from North-west Indian Himalaya. Natural Product Research. 2022; 36: 1565–1569.

[9] Shah ZA, Mujawah AAH, Ullah I, Rauf A, Rashid U, Khalil AA, *et al*. Antioxidant and Cytotoxic Activity of a New Ferruginan A from *Olea ferruginea*: *In Vitro* and *In Silico* Studies. Oxidative Medicine and Cellular Longevity. 2022; 2022: 8519250.

[10] Hashmi MA, Shah HS, Khan A, Farooq U, Iqbal J, Ahmad VU, *et al*. Anticancer and Alkaline Phosphatase Inhibitory Effects of Compounds Isolated from the Leaves of Olea Ferruginea Royle. Records of Natural Products. 2015; 9: 164–168.

[11] Liaqat S, Islam M, Saeed H, Iqtedar M, Mehmood A. Investigation of Olea ferruginea Roylebark extracts for potential in vitroantidiabetic and anticancer effects. Turkish Journal of Chemistry. 2021; 45: 92–103.

[12] Macieja A, Kopa P, Galita G, Pastwa E, Majsterek I, Poplawski T. Comparison of the effect of three different topoisomerase II inhibitors combined with cisplatin in human glioblastoma cells sensitized with double strand break repair inhibitors. Molecular Biology Reports. 2019; 46: 3625–3636.

[13] Taylor TE, Furnari FB, Cavenee WK. Targeting EGFR for treatment of glioblastoma: molecular basis to overcome resistance. Current Cancer Drug Targets. 2012; 12: 197–209.

[14] Lin B, Ziebro J, Smithberger E, Skinner KR, Zhao E, Cloughesy TF, *et al*. EGFR, the Lazarus target for precision oncology in glioblastoma. Neuro-Oncology. 2022; 24: 2035–2062.

[15] Nasir S, Nazir S, Hanif R, Javed A. Glioblastoma Multiforme: Probing Solutions to Systemic Toxicity towards High-Dose Chemotherapy and Inflammatory Influence in Resistance against Temozolomide. Pharmaceutics. 2023; 15: 687.

[16] Raja SA, Andleeb S, Javed A, Sabahat S, Parvaiz F, Mureed H, *et al*. Green synthesised AuNps using Ajuga Bracteosa extract and AuNps-Free supernatant exhibited equivalent antibacterial and anticancerous efficacies. PLoS ONE. 2023; 18: e0282485.

[17] Konain K, Faheem M, Ullah K, Ayub S, Ahmed J, Huma Z, *et al*. Biomarker-Guided Drug Repurposing and Molecular Validation of Angiotensin-2 Receptor Type-1 in Brain Tumor. Precision Medicine Communications. 2023; 3: 27–42.

[18] Khan I, Baeesa S, Bangash M, Schulten HJ, Alghamdi F, Qashqari H, *et al*. Pleomorphism and drug resistant cancer stem cells are characteristic of aggressive primary meningioma cell lines. Cancer Cell International. 2017; 17: 72.

[19] Rehman G, Hamayun M, Iqbal A, Ul Islam S, Arshad S, Zaman K, *et al*. In Vitro Antidiabetic Effects and Antioxidant Potential of *Cassia nemophila* Pods. BioMed Research International. 2018; 2018: 1824790.

[20] Nawaz H, Waseem A, Nafees M, Arshad MN, Rashid U. Synthesis, Characterization, Cytotoxicity and Computational Studies of New Phosphine- and Carbodithioate-based Palladium(II) Complexes. Applied Organometallic Chemistry. 2017; 31: e3771.

[21] Farooq U, Naz S, Shams A, Raza Y, Ahmed A, Rashid U, *et al*. Isolation of dihydrobenzofuran derivatives from ethnomedicinal species Polygonum barbatum as anticancer compounds. Biological Research. 2019; 52: 1.

[22] Sadiq A, Mahnashi MH, Alyami BA, Alqahtani YS, Alqarni AO, Rashid U. Tailoring the substitution pattern of Pyrrolidine-2,5-dione for discovery of new structural template for dual COX/LOX inhibition. Bioorganic Chemistry. 2021; 112: 104969.

[23] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, *et al*. AutoDock4 and AutoDockTools4: Auto-

mated docking with selective receptor flexibility. Journal of Computational Chemistry. 2009; 30: 2785–2791.

[24] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, *et al*. UCSF Chimera–a visualization system for exploratory research and analysis. Journal of Computational Chemistry. 2004; 25: 1605–1612.

[25] You F, Zhang C, Liu X, Ji D, Zhang T, Yu R, *et al*. Drug repositioning: Using psychotropic drugs for the treatment of glioma. Cancer Letters. 2022; 527: 140–149.

[26] Sharifzad F, Ghavami S, Verdi J, Mardpour S, Mollapour Sisakht M, Azizi Z, *et al*. Glioblastoma cancer stem cell biology: Potential theranostic targets. Drug Resistance Updates: Reviews and Commentaries in Antimicrobial and Anticancer Chemotherapy. 2019; 42: 35–45.

[27] Redaelli M, Mucignat-Caretta C, Isse AA, Gennaro A, Pezzani R, Pasquale R, *et al*. New naphthoquinone derivatives against glioma cells. European Journal of Medicinal Chemistry. 2015; 96: 458–466.