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Isosaponarin from *Eutrema japonicum* as a Potential Therapeutic Agent for Irritable Bowel Syndrome: Quantitative Analysis, ADME Profiling, Network Pharmacology, and Molecular Docking Insights

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Eutrema japonicum (commonly known as wasabi) is a perennial plant within the Brassicaceae family, recognized for its culinary and potential therapeutic properties. Isosaponarin (ISP), a di-glycosylated flavone extracted from *E. japonicum*, has demonstrated significant antioxidant and anti-inflammatory effects, which may be beneficial for irritable bowel syndrome (IBS). This study explores the potential of isosaponarin (ISP) as a therapeutic agent for irritable bowel syndrome (IBS) symptoms by modulating inflammatory cytokines and related pathways. The ISP content in *E. japonicum* extracts was quantified using high-performance liquid chromatography (HPLC). Network pharmacology was employed to identify key protein targets related to IBS, and molecular docking simulations were conducted to assess the binding affinities of these proteins to ISP. The findings showed that the *E. japonicum* extract contained 12.08 mg/g DW of ISP. It further indicated that ISP possesses strong binding affinities for epidermal growth factor receptor (–10.6 kcal/mol) and tumor necrosis factor- α (–11.8 kcal/mol). The network pharmacology analysis identified these proteins as pivotal targets within IBS-associated inflammatory processes. This study suggests that ISP from *E. japonicum* may serve as a promising complementary therapy for IBS. Further investigation through *in vivo* studies and clinical trials is required to confirm its efficacy and explore its therapeutic potential in human subjects.

Key words: irritable bowel diseases, inflammation, flavonoids, molecular docking, HPLC

INTRODUCTION

Irritable bowel syndrome (IBS) is a common functional gastrointestinal disorder characterized by persistent abdominal pain or discomfort accompanied by changes in bowel habits (Ng *et al.*, 2018). This condition involves excessive and sustained immune responses targeting the intestinal microbiota in genetically predisposed individuals. Although the exact etiology of IBS remains unclear, it is widely accepted that the disease pathogenesis results from the multifactorial interplay of genetic predisposition, environmental triggers such as dietary habits, smoking, and antibiotic exposure, dysbiosis of the gut microbiota, and dysregulation of immune function (Shaikh *et al.*, 2023). Clinically, IBS patients typically exhibit bloating, chronic diarrhea, abdominal pain, and rectal bleeding (Simrén *et al.*, 2017). IBS treatment is complex and usually involves a combination of pharmacological and non-pharmacological interventions aimed at inducing and maintaining remission, preventing complications, and improving quality of life (Weaver *et al.*, 2017). Pharmacologic management for mild cases

includes the use of over-the-counter antidiarrheals to reduce stool frequency, antispasmodics for cramping, and lifestyle modifications such as adherence to a low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAP) diet and caffeine avoidance (Lucak *et al.*, 2016). In moderate to severe cases, more targeted pharmacologic therapies are required.

Isosaponarin (ISP), a di-glycosylated flavone characterized as apigenin-6-C-glucoside-7-O-glucoside, is a polyphenolic compound found naturally in various plant species, including medicinal herbs and barley (Hashimoto *et al.*, 2023; Cho *et al.*, 2024). As part of the flavone subclass, ISP is noted for its antioxidant and anti-inflammatory activities, which have garnered interest for its potential therapeutic application in chronic inflammatory diseases such as IBS (Chagas *et al.*, 2022). In IBS, excessive production of inflammatory cytokines and reactive oxygen species perpetuates mucosal injury and immune dysregulation (Muro *et al.*, 2024). Flavonoids such as ISP can regulate major inflammatory signaling pathways, including nuclear factor kappa B (NF- κ B), mitogen-activated protein kinase (MAPK), and Janus kinase/signal transducer and activator of transcription (JAK/STAT) cascades, thereby suppressing the expression of cytokines such as tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and interleukin-1 β (IL-1 β) (Han *et al.*, 2021). The glycosylation of ISP may further enhance its solubility and stability, improving its pharmacokinetic profile and bioavailability compared to its aglycone counterpart, apigenin.

Eutrema japonicum (Miq.) Koidz., commonly

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known as wasabi, is a perennial plant in the Brassicaceae family native to Japan and the Russian Far East (Park *et al.*, 2017). Cultivated for its pungent underground storage rhizome, which is traditionally used as a condiment in Japanese cuisine, *E. japonicum* requires specific and demanding growing conditions, including cool temperatures (8–20 °C), high humidity, low light, and continuous access to clean, flowing water, all of which are conditions typically found in mountain stream environments (Sultana and Savage, 1970). This plant features large, heart-shaped leaves and small white flowers and can be grown in semi-aquatic environments (known as Sawa wasabi) or under field conditions (Oka wasabi), although Sawa wasabi is considered to be of superior quality. Due to its slow growth cycle and sensitivity to environmental conditions, authentic wasabi commands a high market price, with most production centered in regions such as Shizuoka, Nagano, and the Izu Peninsula (Yamane *et al.*, 2023). Beyond its culinary value, *E. japonicum* has attracted scientific interest for its bioactive compounds, such as isothiocyanates, which have demonstrated antimicrobial and chemopreventive activities (Szewczyk *et al.*, 2023).

In this study, we explored the potential of *E. japonicum* as a source of bioactive flavonoids relevant to IBS treatment. Building on the known anti-inflammatory and antioxidant activities of ISP, we investigated the presence of ISP in *E. japonicum* extracts using a high-performance liquid chromatography (HPLC) and nuclear magnetic resonance spectroscopy (NMR) methods. Furthermore, molecular docking experiments were conducted to assess the binding affinity of ISP to key protein targets involved in IBS-associated inflammatory pathways. Through this integrated approach, we aim to provide preliminary evidence supporting the pharmacological potential of *E. japonicum* and identify new plant-derived compounds for complementary IBS therapy.

MATERIALS AND METHODS

Plant materials

Eutrema japonicum extracts were supplied by Saemtong Wasabi Co. Ltd. (Cheorwon, Republic of Korea). The aerial parts of *E. japonicum* were harvested on July 28, 2022. After harvesting, the material was lyophilized and subsequently extracted using a 3-h shaking method developed by one of the authors. A voucher specimen was deposited in the Herbarium of the Department of Plant Science and Technology, Chung-Ang University, Anseong, Republic of Korea.

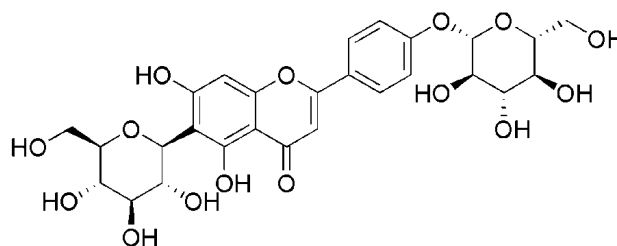


Fig. 1. Chemical structure of ISP.

Instruments and reagents

HPLC was performed using the Waters Alliance 2695 Separations Module (Milford, MA, USA), equipped with a pump, autosampler, and a Waters 996 Photodiode Array (PDA) detector. Data acquisition and processing were conducted using Empower 3 Chromatography Software (Milford, MA, USA). Chromatographic separation was achieved using a YMC Pack Pro C18 column (4.6 × 250 mm, 5 µm) supplied by YMC Korea Co., Ltd. (Seongnam, Gyeonggi, Republic of Korea). Ultrasonic treatment of samples was carried out using a POWERSONIC 410 ultrasonic bath (Hwashin Instrument Co., Seoul, Republic of Korea). Sample weights were measured using an Ohaus Pioneer analytical balance (Merck KGaA, Darmstadt, Germany), and centrifugation was performed using an N-Biotek MICRO-CENVAC centrifuge (Clayton, Victoria, Australia). HPLC-grade solvents, including water, trifluoroacetic acid (TFA), and acetonitrile (ACN), were purchased from J.T. Baker (Radnor, PA, USA). The reference standard, ISP, was provided by the Natural Product Institute of Science and Technology (Anseong, Republic of Korea) (Fig. 1).

HPLC analysis and calibration

Extracts and standard compounds were first dissolved in HPLC-grade methanol to concentrations of 30 mg/mL and 1 mg/mL, respectively. The solutions were sonicated for 10–20 min and filtered through 0.45 µm PVDF membrane filters. The standard was further diluted to appropriate concentrations for calibration. Each sample (10 µL) was injected into the system at a flow rate of 1.0 mL/min. Detection was performed at 270 nm, maintaining the column temperature at 40 °C. The mobile phase consisted of 0.1% TFA in water (solvent A) and ACN (solvent B), applied using a gradient elution program: 90% A from 0 to 10 min, 40% A at 40 min, 0% A at 45 min, and returned to 90% A from 50 to 60 min. A five-point calibration curve was generated using various concentrations of the standard solution. Linearity was assessed by determining the coefficient of determination

Table 1. Calibration data of ISP.

Compound	^a t _R (min)	^b Calibration equation	R ²	Content (mg/g FD)
ISP	15.6	y = 21017x + 73290	0.9997	12.08 ± 0.01

^a retention time in minutes

^b Y = peak area, X = concentration of standards (µg/mL)

^c Coefficient of determination

(R^2). Quantification of the compound in the samples was based on the calibration curve equation. For each concentration, the mean peak area ($n = 3$) $\pm$ standard deviation (SD) was plotted, with concentration ($\mu\text{g/mL}$) on the x-axis and peak area on the y-axis (Table 1).

Structure elucidation and confirmation

The NMR spectrum of ISP was recorded with an ADVANCE III HD 500 NMR (Bruker, Germany) spectrometer operating at 500 MHz with a 5 mm BBFO (plus) probe head, and tetramethylsilane (TMS) was used as an internal standard. ^1H NMR ($\text{DMSO}-d_6$) δ : 13.48 (1H, brs, OH-5), 8.03 (2H, d, $J = 8.9$ Hz, H-2', 6'), 7.19 (2H, d, $J = 8.9$ Hz, H-3', 5'), 6.86 (1H, s, H-3), 6.47 (1H, s, H-8), 5.03 (1H, d, $J = 7.3$ Hz, H-1''), 4.58 (1H, d, $J = 9.8$ Hz, H-1'''). ISP was confirmed by comparing these values with a reference (Czarnomska *et al.*, 2024).

Identification of target genes and overlap with disease targets

Potential protein targets for molecular docking were predicted using the SwissTargetPrediction tool (<http://www.swisstargetprediction.ch>) (Daina *et al.*, 2019) and the SuperPred web server (<https://prediction.charite.de>) (Nickel *et al.*, 2014), in combination with the GeneCards database (<https://www.genecards.org>) (Stelzer *et al.*, 2016). SwissTargetPrediction utilized structural similarity based on SMILES input, while SuperPred applied machine learning to predict protein targets and disease associations. The GeneCards database was queried using the keyword "IBS" to retrieve disease-associated genes. Predicted protein targets were compared with IBS-related genes using Venny 2.1 (<https://bioinfogp.cnb.csic.es/tools/venny/>) (Oliveros, 2015) to identify overlapping, disease-relevant targets.

Protein-protein interaction network analysis

Interactions among the identified targets were examined using the STRING database (<https://string-db.org/>) (Szklarczyk *et al.*, 2023), with *Homo sapiens* selected as the species. Protein-protein interaction (PPI) data were filtered using a high-confidence threshold (≥ 0.4). Both experimental and predicted interactions, including co-expression and curated sources, were considered. Network analysis focused on identifying hub proteins by evaluating network centrality metrics such as degree, betweenness, and clustering coefficients.

Key target screening

For visualization and further analysis, the interaction networks were imported into the Cytoscape software (Shannon *et al.*, 2003) using the STRING plugin. A confidence threshold of 0.4 was applied. The NetworkAnalyzer tool in Cytoscape was used to compute centrality metrics, including degree, betweenness, and closeness. Proteins with the highest scores were selected as key regulatory nodes within the network.

In silico ADME prediction

To evaluate the pharmacokinetic properties of ISP, absorption, distribution, metabolism, and excretion (ADME) predictions were conducted using the SwissADME web tool (<http://www.swissadme.ch/>) (Daina *et al.*, 2017). The SMILES notation of ISP was obtained from the PubChem database and submitted to SwissADME to predict parameters related to physico-chemical properties, lipophilicity, water solubility, oral bioavailability, and drug-likeness. Key parameters assessed included gastrointestinal (GI) absorption, blood-brain barrier (BBB) permeability, P-glycoprotein (P-gp) substrate likelihood, and compliance with Lipinski's rule of five.

Molecular docking simulation

Two protein targets, tumor necrosis factor alpha (TNF- α) and epidermal growth factor receptor (EGFR), were selected for docking based on prior analyses. Their 3D structures were downloaded in PDB format from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (<https://www.rcsb.org/>) (Berman, 2000). The ligand ISP was sourced from PubChem (<https://pubchem.ncbi.nlm.nih.gov/>) (Kim *et al.*, 2024) and converted into PDB format using the SwissTargetPrediction tool. Pre-docking preparations, including the removal of water molecules, addition of hydrogen atoms, and charge assignment, were performed using the PyMOL software (Schrödinger and De Lano, 2020) and AutoDock Tools (Morris *et al.*, 2009). Docking simulations were executed using the Vina module in the AMDock suite (Eberhardt *et al.*, 2021). Biovia Discovery Studio Visualizer (Version 2019, Dassault Systèmes BIOVIA) was used to illustrate the interactions between ISP and the target proteins.

RESULTS

Quantitative analysis of ISP

The HPLC results summarized in Table 1 provide key calibration data for the detection of ISP in *E. japonicum*. The retention time for ISP was recorded at 15.6 min, indicating the time required for the compound to elute from the HPLC column. The calibration equation, $y = 21017x + 73290$, was used to correlate the detected peak area to the concentration of ISP (in mg/g freeze-dried material), with a coefficient of determination (R^2) of 0.9997, demonstrating excellent linearity and precision. The content of ISP in the extract was quantified at 12.08 mg/g of freeze-dried material, indicating that 1 g of the freeze-dried extract contains 12.08 mg of ISP.

Identification of associated targets

Potential targets were identified by querying the keyword "irritable bowel syndrome" against the GeneCards database, resulting in 5711 IBS-related targets. Additionally, 190 targets associated with ISP were predicted using the SwissTargetPrediction tool and SuperPred web server. After standardization, 148 over-

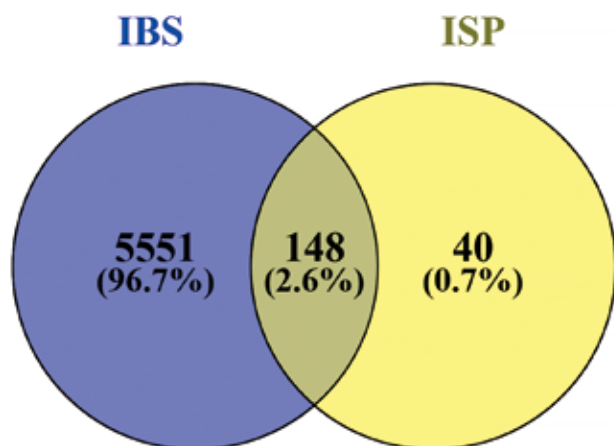


Fig. 2. Intersection targets of IBS and ISP.

lapping targets were identified (Fig. 2). The discovery of these 148 common protein targets suggests that ISP may influence multiple pathways relevant to IBS.

Protein–protein interaction network visualization

To explore the biological interactions among the 148 overlapping proteins, a PPI network was constructed using the STRING database (Fig. 3). The resulting network was imported into Cytoscape for topological analysis. Centrality parameters, particularly betweenness centrality, were calculated to identify key regulatory proteins. This analysis highlighted three core targets with the highest centrality values: epidermal growth factor receptor (EGFR), tumor necrosis factor alpha (TNF-

α), and RAC- α serine/threonine-protein kinase (AKT1) (Fig. 4). Mapping ISP targets within the PPI network provided insights into the biological significance of these proteins. These core targets were subsequently prioritized for molecular docking studies based on their central roles in the network and their relevance to IBS pathophysiology.

Physical properties and ADME profiling

The computed physicochemical, lipophilicity, and water solubility parameters of ISP are summarized in Table 2. ISP has a high molecular weight of 594.52 g/mol and a topological polar surface area (TPSA) of 260.20 Å². ISP also possesses 15 hydrogen bond acceptors and 10 hydrogen bond donors, with a consensus log *P*-value of -1.64. Predictive models also indicated good aqueous solubility, with estimated values ranging between 1.5 and 2.3 mg/mL across different tools.

The pharmacokinetic parameters revealed notable limitations. ISP exceeded the optimal thresholds defined by drug-likeness rules, reflecting its highly polar nature and limited potential for passive diffusion through lipid membranes (Zhang *et al.*, 2022). The lipophilicity analysis further supported this observation, as the low consensus log *P*-value confirmed that ISP is relatively hydrophilic and unlikely to be membrane-permeant. While this limits its ability to access intracellular targets such as AKT1, it supports a strategic focus on extracellular or membrane-bound proteins like TNF- α and EGFR. Nevertheless, the favorable water solubility of ISP (~1.5–2.3 mg/mL) suggests good suitability for aqueous-based biological assays and docking simulations.

ADME predictions also indicated low gastrointesti-

Table 2. Predicted ADME properties of ISP.

Physicochemical properties		Water solubility	
Formula	C ₂₇ H ₃₀ O ₁₅	Log S (ESOL)	-2.40
Molecular weight	594.52 g/mol	Solubility	2.35 mg/mL; 3.95 × 10 ⁻³ mol/L
No. of heavy atoms	42	Class	Soluble
No. of aromatic heavy atoms	16	Log S (Ali)	-3.36
Fraction Csp ³	0.44	Solubility	2.62 × 10 ⁻¹ mg/mL; 4.41 × 10 ⁻⁴ mol/L
No. of rotatable bonds	6	Class	Soluble
No. of H bond acceptors	15	Log S (SILICOS-IT)	-0.59
No. of H bond donors	10	Solubility	1.54 × 10 ² mg/mL; 2.59 × 10 ⁻¹ mol/L
Molar refractivity	138.73	Class	Soluble
TPSA	260.20		
Lipophilicity		Drug likeness	
Log <i>P</i> _{ow} (iLOGP)	2.06	Lipinski	No; 3 violations
Log <i>P</i> _{ow} (XLOGP3)	-1.60	Ghose	No; 4 violations
Log <i>P</i> _{ow} (WLOGP)	-2.76	Veber	No; 1 violation
Log <i>P</i> _{ow} (MLOGP)	-4.10	Egan	No; 1 violation
Log <i>P</i> _{ow} (SILICOS-IT)	-1.79	Muegge	No; 3 violations
Consensus Log <i>P</i> _{ow}	-1.64	Bioavailability score	0.17
Pharmacokinetics			
GI absorption	Low	CYP2C9 inhibitor	No
BBB permeant	No	CYP2D6 inhibitor	No
P-gp substrate	Yes	CYP3A4 inhibitor	No
CYPA12 inhibitor	No	Log <i>K</i> _p (skin permeation)	-11.06 cm/s
CYP2C19 inhibitor	No		

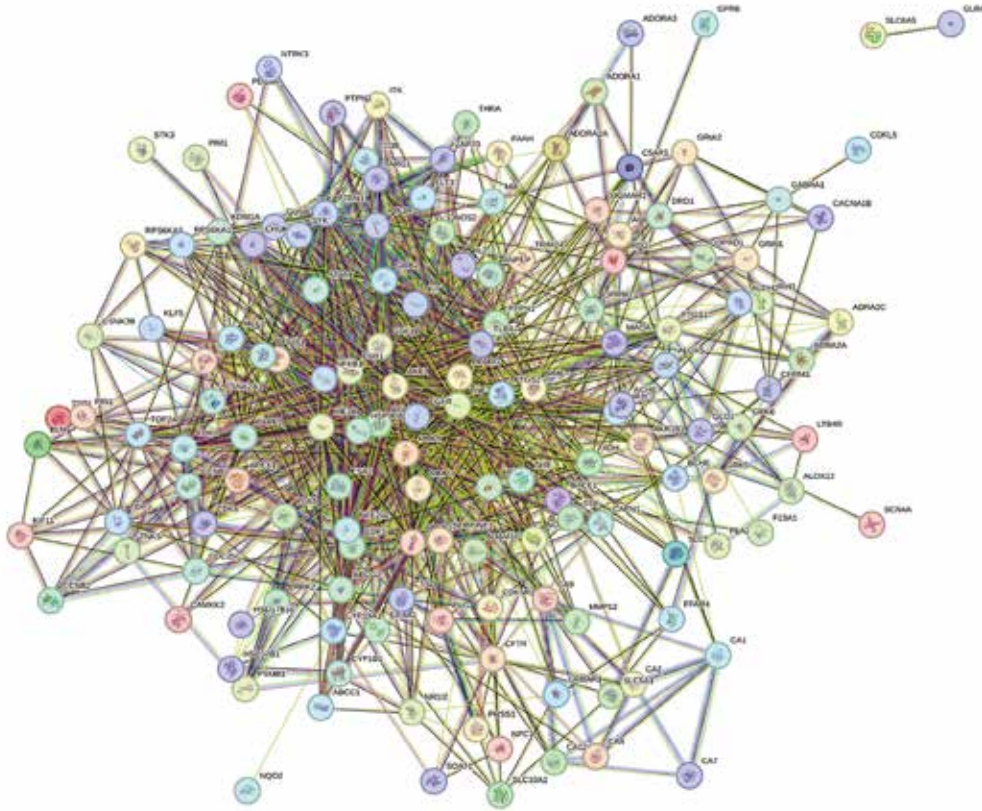


Fig. 3. STRING network of the intersected targets.

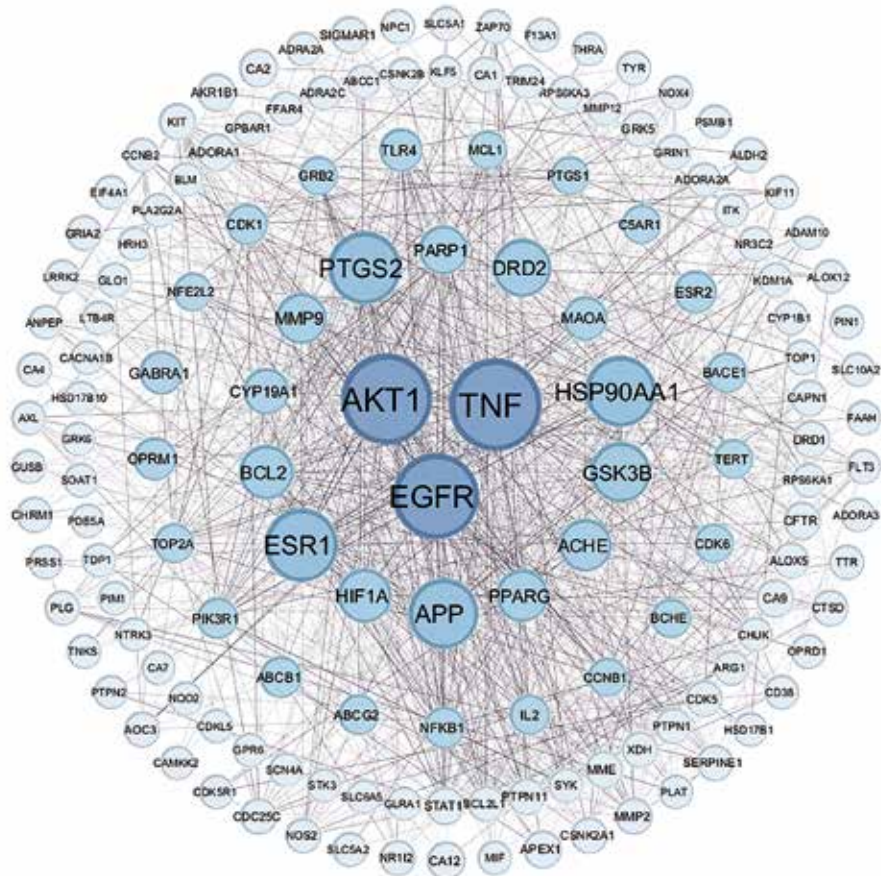


Fig. 4. Merged network of IBS-associated and ISP-related intersecting genes. The blue color intensity and the size of the node correspond to the degree value, indicating the significance of each target.

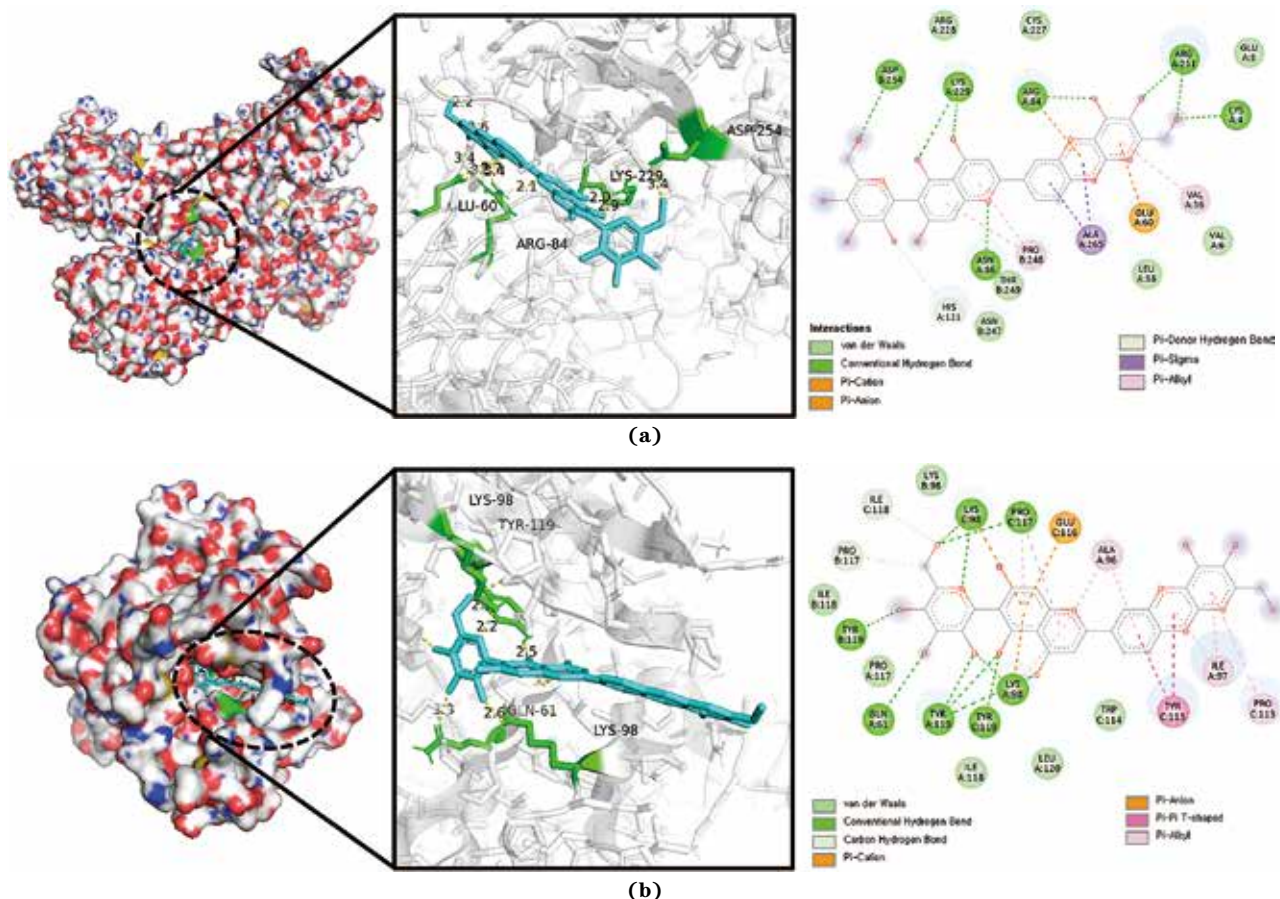


Fig. 5. Molecular docking prediction of ISP binding to EGFR (a) and TNF- α (b). The interactions with key amino acid residues of the target proteins are shown in both 3D and 2D representations.

nal (GI) absorption, lack of blood–brain barrier (BBB) permeability, and classification as a P-glycoprotein (P-gp) substrate, suggesting potential active efflux and poor oral bioavailability. ISP violated several drug-likeness filters, including Lipinski, Ghose, Veber, Egan, and Muegge criteria, largely due to its high molecular weight and excessive TPSA ($>140 \text{ \AA}^2$). The compound also received a low oral bioavailability score of 0.17. Based on these findings, molecular docking against the intracellular kinase AKT1 was not pursued, as ISP's physicochemical properties strongly suggest limited cellular penetration. Instead, docking efforts were focused on more accessible targets, such as the secreted cytokine TNF- α and the membrane-bound receptor EGFR, which align better with the predicted distribution profile of ISP.

Molecular docking simulation

Molecular docking simulations were conducted on two target proteins, EGFR and TNF- α . The visualized docking poses revealed strong potential binding affinities of ISP to these key inflammatory proteins, supporting its proposed anti-inflammatory properties and supporting its potential use in early-phase drug discovery (Fig. 5). The binding affinities of ISP for EGFR and TNF- α were calculated to be -11.8 kcal/mol and -10.6 kcal/mol , respectively. These results suggest that ISP may effectively interact with inflammation-associated targets at

the molecular level.

DISCUSSION

In this study, the content of ISP in *E. japonicum* was quantitatively analyzed by HPLC. This approach enables the standardization of extracts for further research, ensuring consistent therapeutic applications and effective dosage determination (Nasim *et al.*, 2022). Moreover, precisely quantifying the concentrations of an active compound provides key insights into its bioavailability and pharmacokinetics. Both of these factors are critical for investigating the anti-inflammatory and antioxidant properties of ISP, particularly in conditions such as IBS (Wang *et al.*, 2023).

IBS is characterized by a complex interplay of gastrointestinal motility dysfunction, visceral hypersensitivity, and immune dysregulation (Saha, 2014). Proteins such as AKT1, TNF- α , and EGFR play key roles in various signaling pathways associated with inflammation and cellular survival (Nitulescu *et al.*, 2018; Parameswaran and Patial, 2010; Wee and Wang, 2017). This suggests that ISP may not only alleviate inflammation but also promote mucosal healing and integrity by influencing proliferative pathways. By targeting several proteins involved in inflammatory responses, ISP could offer a synergistic approach to treating IBS symptoms, address-

ing not only inflammation but also other contributing factors, such as gut motility and pain sensitivity (Kumar *et al.*, 2022). This interconnectedness suggests that therapies derived from ISP could have broader applications, potentially extending beyond IBS to other inflammatory bowel diseases such as Crohn's disease and ulcerative colitis, where similar inflammatory mechanisms are at play.

ADME profiling was conducted to determine whether ISP has the characteristics of a suitable orally administered drug (Abera *et al.*, 2024). Despite its lack of predicted inhibition of major cytochrome P450 enzymes, which reduces the risk of metabolic drug–drug interactions, the poor permeability profile suggests that ISP is unlikely to access intracellular targets (Faris *et al.*, 2024). While ISP may face challenges as a systemically administered drug, its bioavailability could be improved through structural modifications, such as deglycosylation, where the sugar moiety is removed (Hu *et al.*, 2023). Furthermore, ISP still holds promise as a locally acting agent in the gut, especially in the context of inflammatory bowel conditions like IBS.

A previous study found that administration of *E. japonicum* or *Wasabia japonica* potentially prevents colitis by interfering with the NF- κ B signaling pathway through both *in vitro* and *in vivo* analyses (Kang *et al.*, 2017). Another study on a distant relative found that the oral delivery of *W. koreana* improves the symptoms of IBS by upregulating the expression of FosB and c-Fos antibodies in Zymosan-induced mice (Park *et al.*, 2021).

Out of the two targets examined in the present study, ISP showed the strongest binding affinity to TNF- α (Table 3). TNF- α is a cytokine known to play a key role in the pathophysiology of several inflammatory and autoimmune diseases, and it is recognized as a central regulator of inflammatory responses (Jang *et al.*, 2021). Commercial drugs targeting this cytokine, such as Certolizumab and Golimumab, form complexes with the TNF- α dimer, preventing the activation of downstream signaling complexes that trigger inflammation and other signaling pathways (Zia *et al.*, 2020). These large, protein-based agents differ fundamentally from ISP, a small-molecule flavone glycoside derived from plants. Unlike biologics, ISP does not share structural similarities with antibodies. It possesses a polyphenolic aromatic scaffold with a sugar moiety, characteristic of flavonoids, and lacks the peptide-based tertiary structure of monoclonal antibodies. While ISP is unlikely to bind TNF- α directly in the same way as antibodies, it may modulate TNF- α -mediated inflammation through an indirect mechanism, such as antioxidant activity or inhibition of cytokine signaling pathways. This functional conver-

gence, despite structural divergence, suggests that ISP could serve as a small-molecule alternative or complementary modulator in inflammatory conditions like IBS, where TNF- α plays a central role.

The ability to simulate and evaluate binding affinities can accelerate the preclinical phase for new therapies (Vijayan *et al.*, 2021). Hence, future research could include high-throughput molecular docking assays to explore other bioactive compounds that might enhance or synergize with the effects of ISP, creating a library of potential combinatorial therapies. The versatility of ISP and its potential to act on various targets suggest its role in personalized medicine approaches for managing IBS. Genetic predispositions influencing individual responses to treatment could be considered, and combining ISP with lifestyle interventions (e.g., diet modifications) might lead to tailored treatments that enhance patient outcomes. Further research into pharmacogenomics could help identify which IBS patients might benefit most from ISP-based therapies.

Beyond its individual therapeutic potential, exploring ISP in combination with other phytochemicals could yield potent synergistic effects (Eawasakul and Bunluepuech, 2024). For example, combining ISP with other known anti-inflammatory flavonoids or isothiocyanates may enhance bioavailability and efficacy while leveraging their combined mechanisms of action. Future research on ISP should focus on several critical directions. First, there is a need to transition from *in silico* models to *in vivo* studies and human clinical trials. This shift would enable the determination of ISP's actual therapeutic efficacy, safety profile, dosage adjustments, and pharmacodynamics in real-world scenarios. The results from computer simulations should be experimentally validated by *in vitro* or *in vivo* assays and, ideally, clinical trials (Viceconti *et al.*, 2021). Second, longitudinal studies investigating the long-term effects of ISP on patients with IBS could provide valuable insights into remission rates, quality of life measures, and the potential for preventing flare-ups of IBS symptoms (Gracie *et al.*, 2018). Additionally, future research should aim to elucidate the specific mechanisms underlying the effects of ISP on identified targets. A deeper understanding of these pathways may lead to more targeted approaches and the development of adjunct therapies.

CONCLUSIONS

In summary, this study presents a comprehensive investigation into ISP derived from *E. japonicum*, highlighting its potential as a therapeutic agent for IBS. Through HPLC quantitative analysis, target prediction,

Table 3. Detailed information on the target proteins.

Protein	^a Binding affinity (kcal/mol)	Interacted amino acid
EGFR	−10.6	ARG, ASP, LYS, ASN, HIS, ASN, THR, PRO, ALA, GLU, LEU, VAL, CYS
TNF- α	−11.8	LYS, ILE, PRO, TYR, GLN, ILE, LEU, TRP, ALA, GLU

^a Free energy change that occurs when a ligand binds to a receptor, expressed in kilocalories per mole (kcal/mol).

network pharmacology, ADME, and molecular docking analyses, ISP demonstrated significant interactions with key inflammatory mediators, particularly TNF- α . Despite its pharmacokinetic and physicochemical limitations, including poor membrane permeability and low oral bioavailability, ISP's ability to target extracellular or membrane-bound proteins, combined with its anti-inflammatory properties, positions it as a promising candidate for gut-restricted therapies. As research progresses, ISP shows potential not only as a functional food

AUTHOR CONTRIBUTIONS

N. P. Uy performed the experiments, analyzed the data, and wrote the manuscript. C.-D. Lee performed the data curation. T. Son designed the study and edited the manuscript. H. Kim organized the research results. K. Sakai reviewed and edited the manuscript. S. Lee was supervision, provided the resources, and reviewed the manuscript. All authors assisted in editing of the manuscript and approved the final version.

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REFERENCES

- Abera, B., Y. Melaku, K. Shenkute, A. Dekebo, N. Abdissa, M. Endale, T. Negassa, H. Woldemariam and M. Hunsen 2024 *In vitro* antibacterial, antioxidant, *in silico* molecular docking and ADEMT analysis of chemical constituents from the roots of *Acokanthera schimperi* and *Rhus glutinosa*. *Appl. Biol. Chem.*, **67**: 77
- Berman, H.M. 2000 The protein data bank. *Nucleic Acids Res.*, **28**: 235–242
- Chagas, M. D. S. S., M. D. Behrens, C. J. Moragas-Tellis, G. X. M. Penedo, A. R. Silva and C. F. Gonçalves-De-Albuquerque 2022 Flavonols and flavones as potential anti-inflammatory, antioxidant, and antibacterial compounds. *Oxid. Med. Cell Longev.*, **2022**: 1–21
- Cho, C. H., S. H. Chae, S. H. Kim and K. H. Kim 2024 Phenolic compounds isolated from *Juncus decipiens* and their effects on osteoblast differentiation in the mouse mesenchymal stem cell line C3H10T1/2. *Nat. Prod. Sci.*, **30**: 135–142
- Czarnomska, Z., M. Markowski, E. K. Nawrocka, W. Koźmiński, A. Bazyłko and W. J. Szypuła 2024 *Gentiana capitata* Buch.-Ham. ex D. Don cell suspension culture as a new source of isosaponarin and 3,7,8-trimethoxy-9-oxo-9H-xanthen-1-yl 6-O- β -D-ribofuranosyl- β -D-allopyranoside and their effect on PC-12 cell viability. *Int. J. Mol. Sci.*, **25**: 8576
- Daina, A., O. Michielin and V. Zoete 2017 SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. *Sci. Rep.*, **7**: 42717
- Daina, A., O. Michielin and V. Zoete 2019 SwissTargetPrediction: updated data and new features for efficient prediction of protein targets of small molecules. *Nucleic Acids Res.*, **47**: W357–W364
- Eawasakul, K. and K. Bunluepuech 2024 Exploring synergistic inhibition of inflammatory and antioxidant potential: integrated *in silico* and *in vitro* analyses of *Garcinia mangostana*, *Curcuma comosa*, and *Acanthus ebracteatus*. *Adv. Pharmacol. Pharm. Sci.*, **2024**: 8584015
- Eberhardt, J., D. Santos-Martins, A. F. Tillack and S. Forli 2021 AutoDock Vina 1.2.0: new docking methods, expanded force field, and python bindings. *J. Chem. Inf. Model.*, **61**: 3891–3898
- Faris, J. H., E. Adaligil, N. Popovych, S. Ono, M. Takahashi, H. Nguyen, E. Plise, J. Taechalertraisarn, H. W. Lee, M. F. T. Koehler, C. N. Cunningham and R. S. Lokey 2024 Membrane permeability in a large macrocyclic peptide driven by a saddle-shaped conformation. *J. Am. Chem. Soc.*, **146**: 4582–4591
- Gracie, D.J., J. P. Hamlin and A. C. Ford 2018 Longitudinal impact of IBS-type symptoms on disease activity, healthcare utilization, psychological health, and quality of life in inflammatory bowel disease. *Am. J. Gastroenterol.*, **113**: 702–712
- Han, Y., X. Zhang, Y. Kang, Y. Gao, X. Li, R. Qi, R. Cai and Y. Qi 2021 Sophoraflavanone M, a prenylated flavonoid from *Sophora flavescens* Ait., suppresses pro-inflammatory mediators through both NF- κ B and JNK/AP-1 signaling pathways in LPS-primed macrophages. *Eur. J. Pharmacol.*, **907**: 174246
- Hashimoto, T., J. Long and K. Kanazawa 2023 Dietary isosaponarin is intestinally metabolized to isovitexin, most of which are excreted in feces without being absorbed. *Asian J. Food Res. Nutr.*, **2**: 104–116
- Hu, Q. R., H. Hong, Z. H. Zhang, H. Feng, T. Luo, J. Li, Z. Y. Deng and F. Chen 2023 Methods on improvements of the poor oral bioavailability of ginsenosides. *J. Ginseng Res.*, **47**: 694–705
- Jang, D. I., A. H. Lee, H. Y. Shin, H. R. Song, J. H. Park, T. B. Kang, S. R. Lee and S. H. Yang 2021 The role of tumor necrosis factor alpha (TNF- α) in autoimmune disease and current TNF- α inhibitors in therapeutics. *Int. J. Mol. Sci.*, **22**: 2719
- Kang, J. H., S. Choi, J. E. Jang, P. Ramalingam, Y. T. Ko, S. Y. Kim and S. H. Oh 2017 *Wasabia japonica* is a potential functional food to prevent colitis via inhibiting the NF- κ B signaling pathway. *Food Funct.*, **8**: 2865–2874
- Kim, S., J. Chen, T. Cheng, A. Gindulyte, J. He, S. He, Q. Li, B. A. Shoemaker, P. A. Thiessen, B. Yu, L. Zaslavsky, J. Zhang and E. E. Bolton 2024 PubChem 2025 update. *Nucleic Acids Res.*, **53**: D1516–D1525
- Kumar, S., P. Singh and A. Kumar 2021 Targeted therapy of irritable bowel syndrome with anti-inflammatory cytokines. *Clin. J. Gastroenterol.*, **15**: 1–10
- Lucak, S., L. Chang, A. Halpert and L. A. Harris 2017 Current and emergent pharmacologic treatments for irritable bowel syndrome with diarrhea: evidence-based treatment in practice. *Therap. Adv. Gastroenterol.*, **10**: 253–275
- Morris, G. M., R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson 2009 AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.*, **30**: 2785–2791
- Muro, P., L. Zhang, S. Li, Z. Zhao, T. Jin, F. Mao and Z. Mao 2024 The emerging role of oxidative stress in inflammatory bowel disease. *Front. Endocrinol.*, **15**: 1390351
- Nasim, N., I. S. Sandeep and S. Mohanty 2022 Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus*, **65**: 399–411
- Ng, Q. X., A. Y. S. Soh, W. Loke, D. Y. Lim and W. S. Yeo 2018 The role of inflammation in irritable bowel syndrome (IBS). *J. Inflamm. Res.*, **11**: 345–349
- Nickel, J., B. O. Gohlke, J. Erehman, P. Banerjee, W. W. Rong, A. Goede, M. Dunkel and R. Preissner 2014 SuperPred: update on drug classification and target prediction. *Nucleic Acids Res.*, **42**: W26–W31
- Nitulescu, G. M., M. Van De Venter, G. Nitulescu, A. Ungurianu, P. Zuzenas, Q. Peng, O. T. Olaru, D. Grădinaru, A. Tsatsakis, D. Tsoukalas, D. A. Spandidos and D. Margina 2018 The Akt pathway in oncology therapy and beyond (Review). *Int. J. Oncol.*, **53**: 2319–2331
- Oliveros, J. C. 2015 Venny. An interactive tool for comparing lists with Venn's diagrams. [online] Available at: <https://bioinfo.gp.cnb.csic.es/tools/venny/index.html>
- Parameswaran N. and S. Patil 2010 Tumor necrosis factor- α signaling in macrophages. *Crit. Rev. Eukaryot. Gene Expr.*, **20**: 87–103
- Park, B. K., E. Chun, J. J. Choi, Y. Shin, Y. T. Kho, S. H. Oh, S. Y. Kim, T. H. Lee, T. W. Kim, E. Shin, S. G. Do and M. Jin 2017 Administration of *Wasabia koreana* ameliorates irritable bowel

- syndrome-like symptoms in a zymosan-induced mouse model. *J. Med. Food.*, **20**: 474–484
- Park, J. H., S. B. Kim, E. P. Lee, S. Y. Lee, E. J. Kim, J. M. Lee, J. H. Park, K. T. Cho, H. M. Jeong, S. S. Choi, H. K. Park and Y. H. You 2021 Study on the photosynthetic characteristics of *Eutrema japonica* (Siebold) Koidz. under the pulsed LEDs for simulated sunflecks. *J. Ecol. Environ.*, **45**: 6
- Saha, L. 2014 Irritable bowel syndrome: pathogenesis, diagnosis, treatment, and evidence-based medicine. *World J. Gastroenterol.*, **20**: 6759–6773
- Schrödinger, L. and W. DeLano 2020 PyMOL. [online] Available at: <http://www.pymol.org/pymol>
- Shaikh, S. D., N. Sun, A. Canakis, W. Y. Park and H. C. Weber 2023 Irritable bowel syndrome and the gut microbiome: a comprehensive review. *J. Clin. Med.*, **12**: 2558
- Shannon, P., A. Markiel, O. Ozier, N. S. Baliga, J. T. Wang, D. Ramage, N. Amin, B. Schwikowski and T. Ideker 2003 Cytoscape: a software environment for integrated models of bio-molecular interaction networks. *Genome Res.*, **13**: 2498–2504
- Simrén, M., H. Törnblom, O. S. Palsson and W. E. Whitehead 2017 Management of the multiple symptoms of irritable bowel syndrome. *Lancet. Gastroenterol. Hepatol.*, **2**: 112–122
- Stelzer, G., N. Rosen, I. Plaschkes, S. Zimmerman, M. Twik, S. Fishilevich, T. I. Stein, R. Nudel, I. Lieder, Y. Mazor, S. Kaplan, D. Dahary, D. Warshawsky, Y. Guan-Golan, A. Kohn, N. Rappaport, M. Safran and D. Lancet 2016 The GeneCards suite: from gene data mining to disease genome sequence analyses. *Curr. Protoc. Bioinformatics*, **54**: 1.30.1–1.30.33
- Sultana, T. and G. P. Savage 2008 Wasabi – Japanese horseradish. *Bangladesh J. Sci. Ind. Res.*, **43**: 433–448
- Szewczyk, K. D. S., W. Skowrońska, A. Kruk, A. Makuch-Kocka, A. Bogucka-Kocka, M. Miazga-Karska, A. Grzywa-Celińska and S. Granica 2023 Chemical composition of extracts from leaves, stems and roots of wasabi (*Eutrema japonicum*) and their anti-cancer, anti-inflammatory and anti-microbial activities. *Sci. Rep.*, **13**: 9142
- Szklarczyk, D., R. Kirsch, M. Koutrouli, K. Nastou, F. Mehryary, R. Hachilif, A. L. Gable, T. Fang, N. T. Doncheva, S. Pyysalo, P. Bork, L. J. Jensen and C. von Mering 2023 The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Res.*, **51**: D638–D646
- Viceconti, M., F. Pappalardo, B. Rodriguez, M. Horner, J. Bischoff and F. Musuamba Tshinanu 2021 *In silico* trials: Verification, validation and uncertainty quantification of predictive models used in the regulatory evaluation of biomedical products. *Methods*, **185**: 120–127
- Vijayan, R.S.K., J. Kihlberg, J. B. Cross and V. Poongavanam 2022 Enhancing preclinical drug discovery with artificial intelligence. *Drug Discov. Today*, **27**: 967–984
- Wang, H., Y. Chen, L. Wang, Q. Liu, S. Yang and C. Wang 2023 Advancing herbal medicine: enhancing product quality and safety through robust quality control practices. *Front. Pharmacol.*, **14**: 1265178
- Weaver, K.R., G. D. Melkus and W. A. Henderson 2017 Irritable bowel syndrome. *Am. J. Nurs.*, **117**: 48–55
- Wee, P. and Z. Wang 2017 Epidermal growth factor receptor cell proliferation signaling pathways. *Cancers*, **9**: 52
- Yamane, K., T. Yamada-Kato, N. Haga, K. Ishida, S. Murayama, K. Kobayashi and I. Okunishi 2023 Allyl isothiocyanate and 6-(methylsulfinyl) hexyl isothiocyanate contents vary among wild and cultivated wasabi (*Eutrema japonicum*). *Breed. Sci.*, **73**: 237–245
- Zhang, S., J. P. Thompson, J. Xia, A. T. Bogetti, F. York, A. G. Skillman, L. T. Chong and D. N. LeBard 2022 Mechanistic insights into passive membrane permeability of drug-like molecules from a weighted ensemble of trajectories. *J. Chem. Inf. Model.*, **62**: 1891–1904
- Zia, K., S. Ashraf, A. Jabeen, M. Saeed, M. Nur-E-Alam, S. Ahmed, A. J. Al-Rehaily and Z. Ul-Haq 2020 Identification of potential TNF- α inhibitors: from *in silico* to *in vitro* studies. *Sci. Rep.*, **10**: 20974

