Original Article

Exploring Natural Compounds of Sungihwajungtang for TRPV1 Antagonistic and Anti-inflammatory Effect using *in silico* Method

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ABSTRACT

Background: Temporomandibular joint disorder (TMD) is a neuromusculoskeletal disorder that mainly affects the temporomandibular joint. Due to the multifactorial disease etiology inflammation and chronic pain are considered as main targets for drug design and development. Surgery and pharmacologic interventions are used to treat TMD. However, these therapies have their advantages and disadvantages and require the discovery of safer and non-invasive therapies. Natural medicinal extracts remained a last resort for treating different types of diseases and possess a wide range of pharmaco-biological activities. The current study investigated the phytochemicals of the Korean oriental concoction known as Sungihwajungtang against the transient receptor potential cation channel subfamily V member 1 (TRPV1) and inflammatory targets implicated in TMD. **Materials and Methods:** 1,191 phytochemicals from 11 medicinal plants used in the preparation of this concoction were evaluated through a virtual screening followed by *in silico* gene expression and pharmacokinetic prediction. **Results:** Four promising phytochemicals such as Cnidimoside A, Isorhamnetin-3-mono-β-D-glucoside, Kaempferol-7-O-α-D-glucopyranoside, and Isolaricinosinol-3-α-O-β-D-glucopyranoside formed a strong hydrogen bond with the adequate binding and ligRMSD values. Moreover, these ligands interact with the inflammatory genes by reducing the mRNA expression of *CXC10*, *SDC4*, *CCR6*, and *IL1R1* while preventing the degeneration of the articular disc by decreasing *WNT7A*, *MAPK8*, *MAP4K4* expression. The toxic effect of these ligands is cautionary as they require high doses to incite nephron- and hepatotoxicity and affect the urinary bladder, kidney, and vascular system. **Conclusion:** The ADMET properties of these compounds are adequate but require pharmacokinetic adjustments to be further used for TMD therapy.

Keywords: Temporomandibular joint disorder, Sungihwajungtang, Medicinal Plants, Virtual Screening, *in silico* Gene Expression, Pharmacokinetics.

Submission Date: 24-01-2022;
Revision Date: 17-03-2022;
Accepted Date: 29-04-2022.

DOI: 10.5530/ijper.56.3.137

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INTRODUCTION

Temporomandibular joint disorder (TMD) is a neuro- and musculoskeletal condition characterized as chronic craniofacial pain, headache, joint clicking during mastication followed by degeneration of articular disc and inflammation.[1-2] The disease etiology of TMD seems to be elusive, however, it is believed to be multifactorial. Several factors such as bruxism, psychological conditions i.e., anxiety, stress, craniofacial injury, neoplasia, degenerative joint disorders, and infection.[3] The prevalence data show that 15% of adults are affected by TMD, however, only 5% opt for treatment.[4-5] This disease is more prevalent in women than men and the incidence rate of TMD falls between 20-40 years of age.[4] The symptoms of this disease are comprised of mild to chronic pain, and limited movement of the jaw and joint grating. In most of the cases, TMD spontaneously resolves using pharmacologic interventions such as anxiolytics, muscle relaxants, anti-inflammatory agents, and anti-depressants. Adjunctive therapies have shown little to no benefit but also exacerbate the diseased

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condition due to muscle fatigue and contractures and decreased production of synovial fluid.[4] Invasive and laser surgery have shown promise in alleviating TMD but there is a chance of microtrauma or injury that might lead to recrudescence.[6] Whereas alternative herbal therapies have shown not only to dampen the inflammatory response but also to promote tissue rehabilitation and pain alleviation.[7]

Sungihwajungtang, a Korean herbal concoction is widely explored in Korean Oriental medicine. As it is used to treat severe pain, headaches, and revive Qi deficiency.[8] The phytochemicals mixture used for making this concoction was explored to predict its pain alleviating and inflammatory activity. In this study, we have identified major phytochemicals in Sungihwajungtang concoction involved in interacting with TRPV1 protein and also downregulating the expression of inflammatory genes instigating joint degeneration and chronic pain.

MATERIALS AND METHODS

Collection of ligands information and screening for drug likeness

The Korean herbal concoction selected for *in silico* analysis for the discovery of a suitable drug candidate for the temporomandibular joint disease is Sungihwajungtang. This concoction is used in Korean oriental medicine to relieve and manage severe pain. Sungihwajungtang herbal concoction is made from different plants such as: *Astragalix radix*, *Panax ginseng*, *Astractylodis rhizome alba*, *Angelica gigantis radix*, *Paeoniae radix alba*, *Citrus unshius pericarpum*, *Cimicifugae rhizoma*, *Bupleuri radix*, *Viticis fructus*, *Asiasari radix*, and *Cnidii rhizoma*. The identified phytochemical information about these plants was procured from the TCMSP database,[9] and EnsolbioBank. The ligand structures of these phytochemicals were downloaded from TCMSP and PubChem Database and converted into mol2 file format. Openbabel program was used to extract the canonical SMILES information of these ligands which was then submitted to the Molsoft database (https://www.molsoft.com/mprop/) for druglikeness prediction.

Retrieval of protein structure and active site residues identification

The structure of transient receptor potential cation channel subfamily V member-1 has been utilized in the current study. TRPV1 is involved in instigating chronic pain and inflammation in TMD. The structure of this protein was retrieved from the RCSB protein databank (PDB ID: 7LQZ) which was further processed and structurally minimized with Modrefiner.[10] The structure of TRPV1 was already prebound with resiniferatoxin (RTX) which is a known TRPV1 antagonist.[11] The amino acid residues (6EU: THR552, ASN553, ILE575, and LEU671) to which RTX bounded to were used as reference active site residues for site specific molecular docking.

Virtual screening of ligands for TRPV1 antagonist

The ligand candidates obtained from druglikeness prediction were used for virtual screening investigation. The screening process was aided by the virtual drug screening tool iGEMDOCK v2.1. This program was used for molecular docking analysis to obtain ligands showing probable interaction with TRPV1 protein. Site specific docking was performed by specifying 6EU (Active Site Pocket) of TRPV1 in the binding site settings and a drug screening algorithm was selected in which a number of resolved solutions was kept at 3, Generations 70 and population size: 200 respectively.[12] The ligands were then directed at the specified active site using these settings.

Drug induced gene expression prediction

The canonical SMILES of ligands were analyzed with the DIGEP-Pred database[13] to predict their effect on vital gene targets of Inflammation and other important pathways implicated in TMD.

Acute rat toxicity, adverse effects and organ damage profiling

The obtained ligands from the virtual screening analysis were used to predict toxic attributes of these compounds. The canonical SMILES of these compounds were deposited in the GUSAR database[14] to predict the acute rat toxicity. For adverse and organ damaging effects, Adver-Pred,[15] and ROSC pred database[16] was used.

Pharmacokinetics

The pharmacokinetic attributes of these ligands were gathered by using the pKCSM database. The methodology used to predict this activity is reported elsewhere.[12]

RESULTS

Ligands druglikeness results

A total of 1,196 compounds from 11 plants are utilized in making Sungihwajungtang was analyzed for druglikeness analysis. For selecting drug likeable compounds, DL>0.4 criteria were applied, and duplicate compounds found in different plants, or the same plants were removed to avert redundancy. Of 1,196 ligands, 50 ligands qualified

the DL criteria, and their DL values range from 0.40 to 1.14. These ligands are given in Table 1.

Virtual interaction results

The ligands obtained from the druglikeness analysis (Table 1) were further investigated for probable interaction with the TRPV1 protein using the virtual drug screening program IGEMDOCK v2.1. The results obtained from the virtual screening analysis were classified. The classification criteria were comprised of hydrogen bonding of ligands with the protein target active site residues, low binding energy >100 Kj/mol and ligRMSD 1-7 Å. Out of 50 ligands, 4 ligands such as Cnidimoside A, Isorhamnetin-3-mono-β-D-glucoside, Kaempferol-7-O-α-D-glucopyranoside and Isolaricinosinol-3- α-O-β-D-glucopyranoside showed chemical affinity to the TRPV1 active site residues (Supplementary Figure 1-4). These ligands established the hydrogen bonds in addition to hydrophobic bonds i.e., (Pi-Alkyl, Pi-Sigma, Pi-Sulfur, Vander Waal's and Carbon Hydrogen Bond) as summarized in Table 2. The binding energy of these ligands was between -100.01-114.04 Kj/mol. However, the ligRMSD results of Cnidimoside A and Isorhamnetin-3-mono-β-D-glucoside were 4.34-4.84 Å and Kaempferol-7-O-α-D-glucopyranoside and Isolaricinosinol-3- α-O-β-D-glucopyranoside as 5.11 and 6.66 Å respectively.

Gene Expression Results

After a virtual screening investigation, the canonical SMILES of these four ligands were generated. The SMILES were used to predict the effect of these ligands on the major genes in the pathogenesis of TMD. The data obtained from the DIGEP-Pred database showed that these ligands have four common genes in the target such as *MAPK8*, *SDC4*, *CXC10* and *OLFML1* (Table 3). These genes activity is reported to be upregulated in this disease. These ligands can inhibit the expression of these genes up to 70-90%. Moreover, Cnidimoside A affects the expression of *MFAP4*, Isorhamnetin-3-mono-β-D-glucoside on MAP4K4, and *CCR6*, Kaempferol-7-O-α-D-glucopyranoside on *MAP4K4*, *WNT7A* and *IL1R1* respectively.

Toxicity Investigation Results

These four ligands were investigated to gather information about the lethal dose, possible side effects, and the common organ affected by the administration of these agents (Table 4). GUSAR prediction evaluated the toxic dose of Cnidimoside A is situated at > 600,000 mg/kg for intraperitoneal administration, 1014,000 for intravenous administration, 1616,000 mg/kg for oral and 2671,000 mg/kg for subcutaneous administration. Cnidimoside A is non-toxic when administered via intravenous and subcutaneous routes, however, has a cautionary profile when administered through intraperitoneal and oral routes. Isorhamnetin-3-mono-β-D-glucoside has the lethal dose of 459,700 mg/kg when administered intraperitoneally, 2073,000 mg/kg via intravenous route, 3012,000 mg/kg orally and 2924,000 mg/kg subcutaneously. Kaempferol-7-O-α-D-glucopyranoside incites toxic response at 587,000 mg/kg intraperitoneally, 1598,000 mg/kg orally and 3343,000 mg/kg subcutaneously. Both Isorhamnetin-3-mono-β-D-glucoside and Kaempferol-7-O-α-D-glucopyranoside have a cautionary profile when administered intraperitoneally and orally. Isolaricinosinol-3- α-O-β-D-glucopyranoside has a lethal dose predicted at 516,400 mg/kg intraperitoneally, 79,450 mg/kg for the intravenous route, 2301,000 mg/kg orally and 364,000 subcutaneously and has a cautionary profile for all administration routes. These organs affected by these ligands are: Vascular System, Kidney, Small Intestine, Urinary Bladder, and Induces Nephro- and Hepatotoxicity.

Pharmacokinetic attributes of screened ligands

The ADMET properties of these ligands were predicted to evaluate the mechanics inside the human body (Table 5). pKCSM prediction indicated that the water solubility of these ligands is -2.482 to 3.09 log mol/L, Caco-2 permeability is 0.33 log Papp in 10^{-6} cm/s for Cnidiomoside A, -0.67 log Papp in 10^{-6} cm/s for Isorhamnetin-3-mono-β-D-glucoside, 0.168 log Papp in 10^{-6} cm/s for Kaempferol-7-O-α-D-glucopyranoside, and -0.23 log Papp in 10^{-6} cm/s for Isolaricinosinol-3-α-O-β-D-glucopyranoside. The intestinal absorption is the highest for Kaempferol-7-O-α-D-glucopyranoside (57.822%), while for other ligands, it was between 43.5-45.5% and the skin permeability of these ligands is the same which is -2.735 log Kp. All these ligands are P-glycoprotein substrates and are non-inhibitors of P-glycoprotein I and II except for Isolaricinosinol-3-α-O-β-D-glucopyranoside. The value of distribution at a steady rate of these ligands was around -0.097 to 1.079 log L/Kg while the fraction unbound was between 0.094-0.331. The BBB permeability of these ligands were -1.377 log BB for Cnidimoside A, -1.942 log BB for Isorhamnetin-3-mono-β-D-glucoside, -1.48 log BB for Kaempferol-7-O-α-D-glucopyranoside, and -1.46 log BB for Isolaricinosinol-3-α-O-β-D-glucopyranoside. The CNS permeability of these ligands was recorded between -3.815 and -4.884 (log PS). However, these ligands did not affect CYP proteins and renal OCT2. The total clearance value was found to

Table 1: Druglike-able Compounds Selected from Sungihwajungtang Concotion.

No	Chemical Name	Druglikeness	Source
1	Astraisoflavnin	0.5	TCMSP
2	5-O-Methylvisammioside	0.56	TCMSP
3	Isomucronulatol-7,2'-di-O-glucosiole	0.85	TCMSP
4	7,2'-dihydroxy-3',4'-dimethoxyisoflavone-7-O-β-D-glucoside	0.41	TCMSP
5	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol		TCMSP
6	Hirsutrin	0.68	TCMSP
7	Rhamnocitrin-3-O-glucoside	0.63	TCMSP
8	FA	1.09	TCMSP
9	Rutin	0.91	TCMSP
10	Alexandrin	0.50	TCMSP
11	12-O-Nicotinoylisolineolone	1.52	TCMSP
12	Deoxyharringtonine	0.81	TCMSP
13	Pandamine	0.56	TCMSP
14	7-alpha-L-Rhamnosyl-6-methoxylutcolin	0.93	TCMSP
15	Ginsenoside Rg5	0.42	TCMSP
16	Notoginsenoside R2	0.46	TCMSP
17	20(S)-Ginsenoside-Rh1	0.46	TCMSP
18	(3S,5R,6S,8R,9R,10R,12R,13R,14R,17S)-17-[(2R)-2-hydroxy-6-methylhept-5-en-2-yl]-4,4,8,10,14-pentamethyl-2,3,5,6,7,9,11,12,13,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthrene-3,6,12-triol	0.55	TCMSP
19	6'-Malonylginsenoside Rd1	0.68	TCMSP
20	Ginsenoside-Rg3	0.49	TCMSP
21	20(s)-Protopanaxadiol	0.68	TCMSP
22	Gypnoside V	0.68	TCMSP
23	Ginsenoside-Rh3	0.40	TCMSP
24	Folinic acid	0.76	TCMSP
25	Kaempferol-3-arabofuranoside	0.59	TCMSP
26	Campesteryl ferulate	1.14	TCMSP
27	(3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R,5S)-5-propan-2-yloctan-2-yl]-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	0.85	TCMSP
28	Levistolid A	0.82	TCMSP
29	Pulchinenoside A	0.55	TCMSP
30	Hederagenin	0.47	TCMSP
31	Naringin	1.05	TCMSP
32	(2S)-7-[(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-methylol-3-[(2S,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyl-tetrahydropyran-2-yl]oxy-tetrahydropyran-2-yl]oxy-5-hydroxy-2-(3-hydroxy-5-methoxy-phenyl)chroman-4-one	0.96	TCMSP
33	Hesperidin	0.94	TCMSP
34	Isorhamnetin-3-mono-beta-D-glucoside	0.59	TCMSP
35	Kaempferitrin	0.73	TCMSP
36	Oroxindin	0.70	TCMSP
37	Hyperin	0.68	TCMSP
38	Pulsatillic acid	0.85	TCMSP
39	Baicalin	0.58	TCMSP
40	3',6'-O,O-diacetylsaikosaponin b2	0.69	TCMSP
41	kaempferol-7-O-α-L-rhamnoside	0.76	TCMSP
42	Narcissoside	0.79	TCMSP
43	(+)-Isolariciresinol-3α-O-β-D-glucopyranoside	0.77	TCMSP
44	Norkhelloside	0.66	TCMSP
45	Cnidimoside A	1.03	EnsolBio Bank
46	Pregnolone	0.56	EnsolBio Bank
47	5,7-di-O-beta-D-glucopyranosyl 2(R)-naringenin	1.05	Ramalingam *et al.*, 2015
48	1-O-feruloyl-Beta-D-glucopyranoside	0.50	Ramalingam *et al.*, 2015
49	Higenamine	0.78	Ramalingam *et al.*, 2015
50	Campesterol	0.59	Ramalingam *et al.*, 2015

Table 2: List of Qualified Ligands Establishing an Interaction with TRPV1 Protein.						
No.	**Qualified Ligand**	**Protein**	**Active Residues of Protein**	**Established Interaction**	**Binding Energy**	**LigRMSD**
1	Cnidimoside A	The transient receptor potential cation channel subfamily V member 1 (7LQZ)	THR552, ASN553, ILE575, LEU671	Hydrogen Bond: TYR513, THR552, LEU555, TYR556, ARG559, ALA568, GLU572 Hydrophobic Bond: LEU517, PHE545, ALA548, MET549, ILE571, ILE575	-114.04 KJ/mol	4.34 Å
2	Isorhamnetin-3-mono-beta-D-glucoside			Hydrogen Bond: TYR513, ASN553, ALA548, ILE575 Hydrophobic Bond: LEU579, CYS580	-106.91 KJ/mol	4.84 Å
3	Kaempferol-7-O-α-L-rhamnoside			Hydrogen Bond: TYR513, ASN553, ARG559, GLU572, ILE575, CYS580 Hydrophobic Bond: LEU517, ILE571	-113.01 KJ/mol	5.11 Å
4	Isolaricinesinol-3 ά-o-β-D-glucopyranoside			Hydrogen Bond: THR552, ASN553, ALA568, GLU572 Hydrophobic Bond: MET549, PHE545, ILE575	-100.01 KJ/mol	6.66 Å

Table 3: List of Major TMD Genes Targeted by Ligands.				
No	**Ligand Name**	**Target Gene Name**	**Gene Abbreviation**	**Percentage % mRNA inhibition**
1	Cnidimoside A	Mitogen-Activated Protein Kinase 8	*MAPK8*	89.2%
		Syndecan 4	*SDC4*	86.3%
		C-X-C Motif Chemokine Ligand 10	*CXC10*	78.4%
		Olfactomedin Like 1	*OLFML1*	72.6%
		Microfibril Associated Protein 4	*MFAP4*	62.0%
2	Isorhamnetin-3-mono-beta-D-glucoside	Olfactomedin Like 1	*OLFML1*	98.0%
		Mitogen-Activated Protein Kinase 8	*MAPK8*	88.3%
		Syndecan 4	*SDC4*	85.2%
		C-X-C Motif Chemokine Ligand 10	*CXC10*	81.9%
		Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4)	*MAP4K4*	64.3%
		C-C Motif Chemokine Receptor 6	*CCR6*	53.9%
3	Kaempferol-7-O-α-L-rhamnoside	Mitogen-Activated Protein Kinase 8	*MAPK8*	88.6%
		C-X-C Motif Chemokine Ligand 10	*CXC10*	87.1%
		Syndecan 4	*SDC4*	85.3%
		Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4)	*MAP4K4*	70.8%
		Wnt Family Member 7A	*WNT7A*	70.4%
		Interleukin 1 Receptor Type 1	*IL1R1*	69.5%
4	Isolarcinesinol-3-alpha-O-Beta-D-glucopyransoide	Mitogen-Activated Protein Kinase 8	*MAPK8*	88.1%
		Syndecan 4	*SDC4*	79.0%
		C-X-C Motif Chemokine Ligand 10	*CXC10*	71.1%
		Olfactomedin Like 1	*OLFML1*	70.3%

Table 4: Toxic Influence of Ligands and Adverse Effects on Rat's Organs.

No	Ligand Name	Rat IP LD50 (mg/kg) and Classification	Rat IV LD50 (mg/kg) and Classification	Rat Oral LD50 (mg/kg) and Classification	Rat SC LD50 (mg/kg) and Classification	Organ Affected	Adverse Effect
1	Cnidimoside A	601,400 (Class 5)	1014,000 (Non-Toxic)	1616,000 (Class 4)	2671,000 (Non-Toxic)	vascular system, Kidney	Nephrotoxicity
2	Isorhamnetin-3-mono-beta-D-glucoside	459,700 (Class 4)	2073,000 (Non-Toxic)	3012,000 (Class 5)	2924,000 (Non-Toxic)	kidney vascular system, Small Intestine	Nephrotoxicity
3	Kaempferol-7-O-α-L-rhamnoside	587,100 (Class 5)	1598,000 (Non-Toxic)	3227,000 (Class 5)	3343,000 (Non-Toxic)	vascular system urinary bladder, Kidney	Nephrotoxicity, Hepatotoxicity
4	Isolaricinesinol-3 ά-o-β-D-glucopyranoside	516,400 (Class 5)	79,450 (Class 4)	2301,000 (Class 5)	364,000 (Class 4)	Kidney	Nephrotoxicity

Table 5: Results of pKCSM Predicted Pharmacokinetic Properties of Ligands.

ADMET Property	Compounds			
	Cnidimoside A	Isorhamanetin-3-mono-beta-D-glucoside	Kaempferol-7-O-α-L-rhamnoside	Isolarcinesinol-3-alpha-O-Beta-D-glucopyransoide
ABSORPTION (Predicted Value)				
Water solubility (log mol/L)	-2.482	-3.09	-2.987	-2.956
Caco2 permeability (log Papp in 10-6 cm/s)	0.33	-0.671	0.168	-0.23
Intestinal absorption (human) (% Absorbed)	45.039	45.502	57.822	43.529
Skin Permeability (log Kp)	-2.735	-2.735	-2.735	-2.735
P-glycoprotein substrate	Yes	Yes	Yes	Yes
P-glycoprotein I inhibitor	No	No	No	Yes
P-glycoprotein II inhibitor	No	No	No	Yes
DISTRIBUTION (Predicted Value)				
VDss (human) (log L/kg)	0.522	-0.097	1.079	0.44
Fraction unbound (human) (Fu)	0.331	0.094	0.097	0.261
BBB permeability (log BB)	-1.377	-1.942	-1.48	-1.466
CNS permeability (log PS)	-3.876	-4.884	-3.815	-4.261
METABOLISM (Predicted Value)				
CYP2D6 substrate	No	No	No	No
CYP3A4 substrate	No	No	No	No
CYP1A2 inhibitior	No	No	No	No
CYP2C19 inhibitior	No	No	No	No
CYP2C9 inhibitior	No	No	No	No
CYP2D6 inhibitior	No	No	No	No
CYP3A4 inhibitior	No	No	No	No
EXCRETION (Predicted Value)				
Renal OCT2 substrate	No	No	No	No
Total Clearance (log ml/min/kg)	0.264	0.528	0.421	0.472
TOXICITY (Predicted Value)				
AMES toxicity	No	No	No	No
hERG I inhibitor	No	No	No	No
hERG II inhibitor	No	Yes	No	Yes
Hepatotoxicity	No	No	No	No
Skin Sensitisation	No	No	No	No
T.Pyriformis toxicity (log ug/L)	0.285	0.285	0.287	0.285
Minnow toxicity (log mM)s	3.839	6.179	3.511	6.219

be ~0.264-0.528 log ml/min/kg. The toxicity of these compounds are relatively low except for Isorhamnetin-3-mono-β-D-glucoside and Isolaricinosinol-3- α-O-β-D-glucopyranoside as they interact with the hERG II protein and has high Minnow toxicity (~6.0 log mM).

DISCUSSION

The temporomandibular joint disorder is a multifactorial disease whose molecular mechanism is still under exploration. However, exacerbated inflammation and chronic pain in the ginglymo-arthrodial joint is one of the discerning manifestations of TMD.[3] Here in this research, we attempted to analyze the Korean oriental herbal concoction "Sungihwajungtang" by predicting its effect on pain and inflammation. The concoction of Sungihwajungtang is comprised of 11 different plants harboring more than > 1000 compounds. In Korean oriental medicine, this concoction is exploited to treat severe pain and revive Qi. Due to their pain-relieving properties, we attempted to investigate those active compounds implicated in alleviating the TRPV1 channels. Moreover, these medicinal plants have been reported to have anti-inflammatory properties which were explored by checking their effect on the inflammatory and other related genes.

A total of 1190 compounds competed for TRPV1 protein's active site in the virtual screening process. Four candidates Cnidimoside A, Isorhamnetin-3-mono-β-D-glucoside, Kaempferol-7-O-α-D-glucopyranoside and Isolaricinosinol-3-α-O-β-D-glucopyranoside showed probable interaction TRPV1 by forming hydrogen bonds with the reported active site residues followed by multiple hydrophobic bonds with suitable binding energy and ligRMSD values. It is established in the recent literature that a ligand bonded to the active site residues via a hydrogen bond brings functional changes in the protein structure resulting in the inhibition of protein function.[17-18] Therefore, these compounds could be involved in alleviating pain response in different types of disease which might have a promising role in alleviating chronic pain in TMD.

These four compounds reduce the expression of *CXC10*, *CXCR6* and *IL1R1* and *SDC4* levels. As these proteins instigate the inflammation and modulate the inflammatory signaling pathways leading to TMD arthritis.[19-20] Whereas *MAPK8*, *MAP4K4* and *WNT7A* contribute to the dedifferentiation of chondrocytes and prevent their regulation in response to an inflammatory insult to the cell.[21-22] On the other hand, the level of *OLFML1* and *MFAP4* is upregulated in TMD,[23] however, their role in TMD is still not understood.

The toxicity of these compounds is moderate except for Cnidimoside A and Isolaricinosinol-3- α-O-β-D-glucopyranoside which are relatively safe. All these compounds either induce nephrotoxicity or hepatotoxicity and the organ affected by these agents are the vascular systems, urinary bladder, and kidney. The pharmacokinetics of these compounds show that they have relatively low intestinal absorption and BBB and CNS penetrance which can be resolved by introducing a bioavailability agent or using nanotherapeutic carriers,[24-25] to increase their absorption and penetration. These compounds have a low toxic effect except for Isorhamnetin-3-mono-β-D-glucoside and Isolaricinosinol-3- α-O-β-D-glucopyranoside as they inhibit hERG II protein which has a role in regulating cardiac action potential.

CONCLUSION

Our research results identified the major compounds in Sungihwajungtang that are involved in alleviating pain by binding to the reported active site of the TRPV1. Moreover, they also have an anti-inflammatory effect as they inhibit the expression of inflammatory gene markers. These compounds have adequate toxic and ADMET attributes which require pharmacokinetic attention to improve their effectivity, penetrance, and permeability. These compounds in this *in silico* study discovered four drug candidates that could be efficacious in the treatment of chronic pain and inflammation in TMD. But these results require further *invitro* validation to be promulgated for clinical trials.

ACKNOWLEDGEMENT

This work was supported by the National Research Foundation of Korea (NRF) funded by the Korean Government (2020R1I1A306969913).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TMD: Temporomandibular Joint Disorder; **TRPV1:** Transient Receptor Potential Cation Channel Subfamily V Member 1; **ADMET:** Absorption, Distribution, Metabolism, Excretion and Toxicity; **BBB:** Blood Brain Barrier; **CNS:** Central Nervous System; **hERG:** Human

Ether-a-go-go-related gene; **OCT2:** Organic Cation Transporter.

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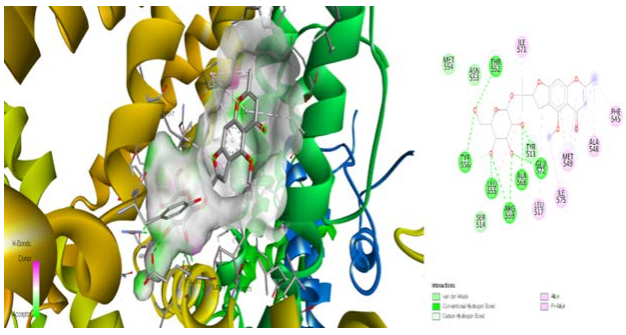
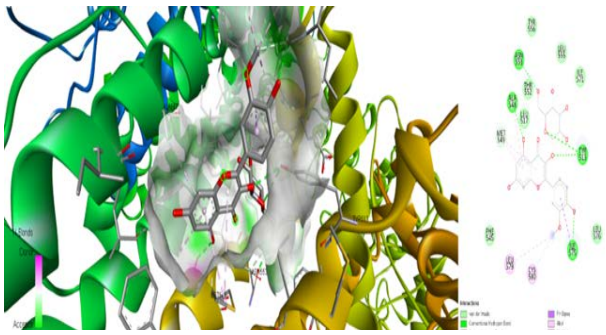
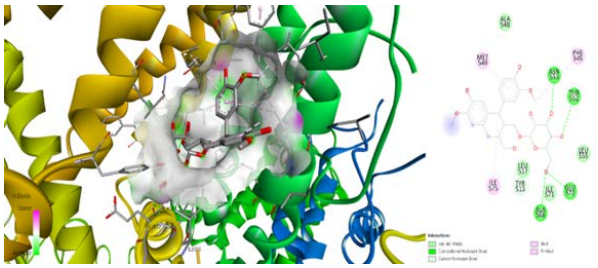
SUPPLEMENTARY FIGURES



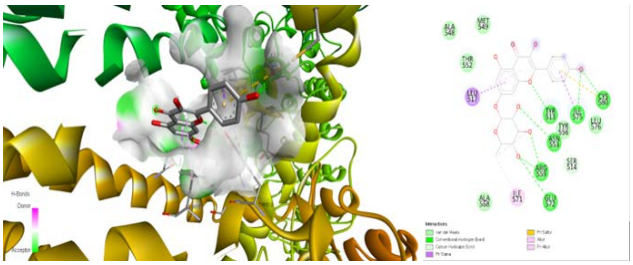
Supplementary Figure 1: Molecular Bonding Between Cnidimoside A with TRPV1 Protein



Supplementary Figure 3. Molecular Bonding Between Isorhamnetin-3-mono-β-D-glucoside with TRPV1 Protein.

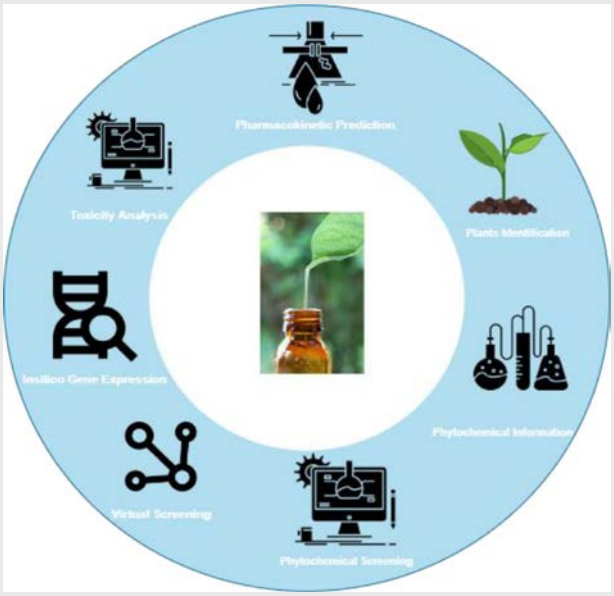


Supplementary Figure 2. Molecular Bonding Between Isolaricinosinol-3- α-O-β-D-glucopyranoside with TRPV1 Protein



Supplementary Figure 4. Molecular Bonding Between Kaempferol-7-O-α-D-glucopyranoside with TRPV1 Protein.

PICTORIAL ABSTRACT



SUMMARY

- Sungihwajungtang is Korean medicinal plants concoction which has been explored by *in silico* method to discover potential phytochemicals for TMD therapy.
- 1,191 identified compounds were obtained from 11 different medicinal plants used for the preparation of Sungihwajungtang concoction.
- Five potential phytochemicals showed probable interaction with TRPV1 protein implicated in pain signaling.
- These phytochemicals inhibited major molecular targets of inflammation by preventing the expression of inflammatory proteins.
- The pharmacokinetics and ADMET properties of these compounds are moderately toxic and should be utilized wisely for *in vitro* and clinical trials.

About Authors

Mr. Fahad Hassan Shah, working as a Research Associate and Postgraduate student in the area of Molecular Pharmacology at Kongju National University. He is currently working on the molecular mechanism behind Joint Related Disorders, Cancer, and Neuropsychiatric disorders and developing treatments using Natural and Synthetic Agents.

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Prof. Song Ja Kim is currently working as a Professor at the Department of Biological Sciences, Kongju National University, South Korea. She is focused on the molecular process behind different Joint Related Disorders, and Cancer and introduced several drug targets involved in these diseases. These drug targets are further exploited in their laboratory for drug design and development purposes. Prof. Kim has published more than 200 articles in both International and National Journals, written 3 books and presented several researches in International and National Conferences.

Cite this article: Shah FH, Eom YS, Kim SJ. Exploring Natural Compounds of Sungihwajungtang for TRPV1 Antagonistic and Anti-inflammatory Effect using *in silico* Method. Indian J of Pharmaceutical Education and Research. 2022;56(3):846-54.