Research Article

Systems Pharmacological Approach to the Effect of Bulsu-san Promoting Parturition

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Bulsu-san (BSS) has been commonly used in oriental medicine for pregnant women in East Asia. The purpose of this research was to elucidate the effect of BSS on ease of parturition using a systems-level in silico analytic approach. Research results show that BSS is highly connected to the parturition related pathways, biological processes, and organs. There were numerous interactions between most compounds of BSS and multiple target genes, and this was confirmed using herb-compound-target network, target-pathway network, and gene ontology analysis. Furthermore, the mRNA expression of relevant target genes of BSS was elevated significantly in related organ tissues, such as those of the uterus, placenta, fetus, hypothalamus, and pituitary gland. This study used a network analytical approach to demonstrate that Bulsu-san (BSS) is closely related to the parturition related pathways, biological processes, and organs. It is meaningful that this systems-level network analysis result strengthens the basis of clinical applications of BSS on ease of parturition.

1. Introduction

The name of Bulsu-san (BSS) originated from its therapeutic effects that help to promote easy labor as if being touched by merciful Buddha’s hand [1]. BSS is composed of Angelicae Sinensis Radix (Danggui, DG) and Cnidium officinale Makino (Cheongung, CG), which is one of the most commonly used herb pairs in Traditional Medicine of East Asia and the usual component ratio is 2:3 (CG:DG) or 1:1 [2]. BSS is widely used in women’s medicine in East Asia; its recognized therapeutic effects are as follows: removal of impure blood, blood making, easy parturition, acceleration of labor, elimination of dead fetus or placenta, amelioration of pain, nourishing blood, and promoting blood circulation [3].

What is more, recent experimental research on the CG-DG herb pair indicated that they affect the nourishment of blood [4], activate blood circulation, and prevent blood stasis [5]. In addition, the CG-DG herb pair showed significant inhibitory effects on the proliferation and protein synthesis of vascular smooth muscle cells [6]. It was suggested BSS could affect the activities of Akt kinase and eNOS by increasing intracellular Ca\(^2\) and reducing ROS levels [7] and regulate menstruation and provide relief from pain by enabling the management of uterine smooth muscle contractions [8]. Although BSS has therapeutic effects on various pathological symptoms in pregnant or childbearing aged women, this research focused on the molecular mechanisms and impact of BSS on easing parturition and the acceleration of labor.

In terms of parturition onset, numerous studies have described the complex hormone interactions between estrogen, progesterone, oxytocin, corticosteroid, and prostaglandin. Among these, corticotrophin releasing hormone (CRH) is regarded as a trigger that initiates the labor [9]. The placenta releases substantial amounts of CRH, which stimulates the pituitary glands of both mother and fetus to secrete adrenocorticotropic hormone [10]. This in turn induces the release of estrogen precursor, which is converted into estrogen by the placenta that induces smooth muscle contraction [10]. Additionally, dilatation of cervical connective tissue and smooth muscle is induced by the following changes: a shift from progesterone to estrogen dominance, increased response to oxytocin via the upregulation of myometrial oxytocin receptor, increased prostaglandins synthesis in uterus, increased myometrial gap junction formation, decreased nitric oxide activity, and increased influx of calcium into myocyte [11].
The hypothesis of this study was that BSS may promote the positive-feedback of hormone loops as well as a series of myometrial and cervical changes to ease parturition and safely accelerate labor. A network based in silico approach was used to identify the effect of BSS on parturition related systems and the aim of this study was to elucidate the effect of BSS on the parturition by system-level analysis. The workflow of the network pharmacological study is summarized in Figure 1.

2. Material and Methods

2.1. Identification of Active Compounds. Compounds in CG and DG were identified using a phytochemical database that is the Traditional Chinese Medicine Systems Pharmacology (TCMSP, http://ibts.hkbu.edu.hk/LSP/tcmsp.php). We applied parameters related to absorption, distribution, metabolism, and excretion (ADME), namely, human drug-likeness (DL) [12], oral bioavailability (OB) [13], and Caco-2 permeability (Caco-2) to screen the Potential active compounds in BSS [14].

2.1.1. Drug-Likeness Evaluation. DL helps filter “drug-like” compounds in oriental herbs, as DL represents a qualitative concept for valuations based on how “drug-like” a prospective compound is [15]. Accordingly, a high DL may lead to a greater possibility of therapeutic success, and compounds with a higher DL value are more likely to possess certain biological properties [16]. The calculations of DL in TCMSP database were based on Tanimoto coefficient formula [17] as follows:

\[ F(A, B) = \frac{A \times B}{A^2 + B^2 - A \times B}, \]

where \( A \) represents the molecular parameters of herbal compounds and \( B \) is the average molecular parameters of all compounds in the Drugbank database (http://www.drugbank.ca/) [18]. In the present study, we excluded compounds with a DL of \(<0.08\). Other previous researches of herbal formulas set a higher threshold in the range of 0.1 to 0.18. However, we found out that most compounds of DG have low DL. In detail, only 36 compounds of 125 in DG show higher or equal DL value than 0.08. For this reason, this study sets a lower threshold of DL than other previous researches to see the most potential targets of BSS.

2.1.2. Oral Bioavailability (OB) Prediction. OB is defined as the ratio of active compounds’ absorption into the systemic circulation, which represents the convergence of the ADME process [13]. OB values are dependent on drug dissolution in the gastrointestinal (GI) tract and hepatic and intestinal first-pass metabolism, as well as on intestinal membrane permeation, which makes it a major pharmacokinetic parameter for drug evaluations [16]. In this study, the OB threshold was set as \( \geq 15\% \).

2.1.3. Caco-2 Permeability Screening. Caco-2 permeability is used to predict the absorption of an orally administered
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drug [14]. Surface absorptivity of the small intestine is maximized with the presence of villi and microvilli, for this reason most orally administered drug absorption occurs in the small intestine [19]. Moreover, the movement of orally administered drugs across the intestinal epithelial barrier determines the rate and extent of human absorption and ultimately affects drug bioavailability [20]. In the present study, compounds with OB, DL and Caco-2 values of greater than 15%, 0.08, and >−0.4, respectively, were regarded as active compounds and subjected to further analysis.

2.1.4. Lipinski’s Rule (LR) Screening. In addition, the screening standard used was defined based on Lipinski’s rule (LR), which identifies druggable compounds as having molecular weight (MW) of ≤500 Da (MW ≤ 500), chemical composition with ≤5 hydrogen-bond donors, ≤10 hydrogen-bond acceptors, and an octanol-water partition coefficient, AlogP of ≤ 5 [21]. AlogP can be used to estimate local hydrophobicity, to produce molecular hydrophobicity maps, and to evaluate hydrophobic interactions in protein-ligand complexes [22]. Hdon and Hacc are the number of possible hydrogen-bond donors and acceptors, and the hydrogen-bonding capacity of a drug solute is recognized as a crucial determinant of permeability; moreover high hydrogen-bonding potential is often related to low permeability and absorption [23]. Eventually, in the present study, we selected active compounds satisfying the following criteria: OB ≥ 15%; DL ≥ 0.08; Caco-2 ≥ −0.4; MW ≤ 500; H-bond donors ≤ 5; H-bond acceptors ≤ 10; AlogP ≤ 5.

2.2. Target Fishing. Aside from filtering active compounds, we also sought to identify the molecular targets of these active compounds. Compound-target interaction profiles were established based on a systematic prediction of multiple drug-target interactions tool which employs random forest (RF) and support vector machine (SVM) methods and integrates chemical, genomic, and pharmacological information for drug targeting and discovery on a large scale [24]. Compound-target interactions satisfying SVM score ≥ 0.8 and RF score ≥ 0.7 were selected for further study. Additionally, filtered compound-target interaction profile mapping was performed using the UniProt database (http://www.uniprot.org/) [25].

2.3. Gene Ontology (GO) Analysis. Biological process (BP) of gene ontology (GO) analysis was employed to determine the biological properties of target genes [26]. GO annotation indicates the possibility of direct statistical analysis on gene function information. In this research, GO BP terms with P values < 0.01 were employed and the data was collected using the DAVID 6.8 Gene Functional Classification Tool (http://david.abcc.ncifcrf.gov/).

2.4. Network Construction and Analysis. In order to understand the multiscale interactions between the active compounds of BSS and targets, two types of networks were built: (1) the herb-compound-target network (H-C-T network), in which nodes represent either compounds, target genes, or herbs and edges indicate herb-compound-target connections; and (2) the target-pathway network (T-P network) to extract the pathways from KEGG database (http://www.genome.jp/kegg/), and the terms highly associated with parturition with P values < 0.05 were selected as the related pathways of targets in this work. Related targets were mapped onto relevant pathways, which resulted in the T-P network. Both networks were generated in Cytoscape 3.5.1, an open-source biological network visualization and data integration software package [27].

2.5. Target Organ Location Map. Tissue-specific patterns of mRNA expression can indicate important associations with biological events or gene functions [28]. To explore the beneficial effects of BSS during parturition, it is important that the tissue mRNA expression profiles of target genes at the organ level be known [29]. The target organ location map was built according to the Dataset: GeneAtlas U133A, gcrma (http://biogps.org). BioGPS database provides expression data acquired by direct measurements of gene expression obtained by microarrays analysis [30]. First, the mRNA expression patterns of each target gene in 176 parts of organ tissues were obtained. Second, average values were calculated for each gene. Third, frequency of above average mRNA expression tissue organs was inspected. Forth, based on the result from the third step and parturition mechanism theory, mRNA expression data of relevant organ tissues were extracted and categorized into 6 groups, namely, uterus and/or uterus corpus, fetus and/or placenta, hypothalamus and/or pituitary, smooth muscle, and whole blood.

3. Results

3.1. Identification of Active Compounds. 314 compounds of BSS were identified, including 189 molecules in CG and 125 in DG (as shown in Supplementary Material Table S1 in Supplementary Material available online at https://doi.org/10.1155/2017/7236436) and active compounds met the criteria OB ≥ 15%, Caco-2 ≥ −0.4, and DL ≥ 0.08, as well as the standards of Lipinski’s rule (LR) (as shown in Table 1). In detail, 60 active compounds were initially chosen, but 8 compounds were present in both herbs, namely, 3-butylidene-7-hydroxyphthalide, adenine, BdPh, beta-selinene, palmitic acid, senkyunolide-C, senkyunolide-D, and senkyunolide-E, and 14 had no target protein information and were thus excluded from the list of active compounds, whereas 27 compounds with lower ADME properties than above thresholds were included, which were reported to be related to oxytocin. In total, 65 active compounds were filtered.

Although ligustilide and ferulic acid have a DL of <0.08, both were included in this study. Since ligustilide (C12, DL = 0.07, OB = 53.72, Caco-2 = 1.3) was reported to be the main compound of DG in uterine contraction [31], and ferulic acid (C42, DL = 0.06, OB = 54.97, Caco-2 = 0.53) has been reported to be useful for the treatment of vascular diseases [6, 32] and blood deficiency syndrome [33] in China and to suppress inflammatory responses and tumor progression [34]. Some other compounds also have been shown experimentally to have various biological activities; for example, crysophanol (C42, DL = 0.21, OB = 18.64, Caco-2 = 0.62) can be used to treat menorrhagia and thrombocytopenia [35]. Perlyone
Table 1: Potential active compounds of BSS (compound with * was present in both herbs).

| ID | Active compounds                                                                 | OB (%) | Caco-2  | DL  | Herb |
|----|----------------------------------------------------------------------------------|--------|---------|-----|------|
| C1 | ()-alpha-Terpineol                                                                | 46.3   | 1.28    | 0.03| DG   |
| C2 | ()-Aromadendrene                                                                  | 55.74  | 1.81    | 0.1 | CG   |
| C3 | ()-Terpinen-4-ol                                                                  | 81.41  | 1.36    | 0.03| CG   |
| C4 | (+)-alpha-Funebrene                                                                | 52.87  | 1.79    | 0.1 | CG   |
| C5 | (+)-Ledol                                                                          | 16.96  | 1.43    | 0.12| DG   |
| C6 | (1R,5R,7S)-4,7-Dimethyl-7-(4-methylpent-3-eny)bicyclo[3.1.1]hept-3-ene             | 16.23  | 1.86    | 0.09| CG   |
| C7 | (1S,4aR,8aR)-1-Isopropyl-7-methyl-4-methylene-2,3,4a,5,6,8a-hexahydro-1H-naphthalene | 19.8   | 1.86    | 0.08| DG   |
| C8 | (1S,4E,8E,10R)-4,8,11,11-tetramethylbicyclo[8.1.0]undeca-4,8-diene                | 21.69  | 1.86    | 0.08| CG   |
| C9 | (3E)-3-butylidene-7-hydroxy-2-benzofuran-1-one                                     | 42.17  | 1.03    | 0.08| DG   |
| C10| (L)-alpha-Terpineol                                                                | 48.8   | 1.39    | 0.03| CG   |
| C11| (R)-Linalool                                                                       | 39.8   | 1.33    | 0.02| CG   |
| C12| ()-Ligustilide                                                                    | 53.72  | 1.3     | 0.07| CG   |
| C13| 1-Acetyl-beta-caroline                                                             | 67.12  | 1.18    | 0.13| CG   |
| C14| 1-beta-Ethylacylate-7-aldehyde-beta-carbone                                        | 28.53  | 0.45    | 0.31| CG   |
| C15| 1H-Cycloprop(e)azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-                | 82.33  | 1.37    | 0.12| CG   |
| C16| 1-Terpinol                                                                        | 49.83  | 1.24    | 0.03| CG   |
| C17| 2,6-Di(phenyl)thiophiyan-4-thione                                                  | 69.13  | 1.74    | 0.15| DG   |
| C18| 2-[(2S,5S,6S)-6,10-Dimethylspiro[4.5]dec-9-en-2-yl]propan-2-ol                    | 37.62  | 1.44    | 0.09| CG   |
| C19| 3-Butylidene-7-hydroxyphthalalde                                                    | 62.68  | 1       | 0.08| CG&DG|
| C20| 4,7-Dihydroxy-3-butylphthalide                                                     | 106.09 | 0.69    | 0.1 | CG   |
| C21| 49070 FLUKA                                                                       | 85.51  | 1.29    | 0.12| CG   |
| C22| 4-Hydroxy-3-butylphthalalde                                                         | 70.31  | 0.9     | 0.08| CG   |
| C23| 58870 FLUKA                                                                       | 49.01  | 1.82    | 0.1 | CG   |
| C24| Adenine                                                                           | 62.81  | -0.3    | 0.03| CG&DG|
| C25| ADO                                                                                | 15.98  | -1.56   | 0.18| CG   |
| C26| alpha-Cubebene                                                                    | 16.73  | 1.83    | 0.11| CG   |
| C27| alpha-Selinene                                                                     | 31.81  | 1.82    | 0.1 | CG   |
| C28| Aromadendrene oxide 2                                                              | 65.1   | 1.56    | 0.14| CG   |
| C29| BdPh                                                                               | 42.44  | 1.32    | 0.07| CG&DG|
| C30| beta-Chamigrene                                                                    | 31.99  | 1.82    | 0.08| DG   |
| C31| beta-Selinene                                                                      | 24.39  | 1.83    | 0.08| CG&DG|
| C32| beta-Cubebene                                                                      | 32.16  | 1.82    | 0.11| CG   |
| C33| Cadinene                                                                          | 17.12  | 1.88    | 0.08| DG   |
| C34| Caffeic acid                                                                       | 25.76  | 0.21    | 0.05| CG   |
| C35| Carotol                                                                           | 149.03 | 1.46    | 0.09| CG   |
| C36| Cedrene                                                                            | 51.14  | 1.82    | 0.11| CG   |
| C37| Chuanxiongol                                                                       | 22.19  | 0.94    | 0.1 | CG   |
| C38| cis-Thujopsene                                                                     | 56.43  | 1.84    | 0.12| DG   |
| C39| Coniferyl furate                                                                   | 4.54   | 0.71    | 0.39| DG   |
| C40| Cryosphanol                                                                        | 18.64  | 0.62    | 0.21| CG   |
| C41| FA                                                                                 | 68.96  | -1.5    | 0.71| CG   |
| C42| Ferulic acid (CIS)                                                                  | 54.97  | 0.53    | 0.06| DG   |
| C43| InChI=1/C15H24/c1-10-7-8-15-9-12(10)14(3,4)13(15)6-5-11(15)2/h7,11-13H,5-6,8-9H2,1-4H | 55.56  | 1.79    | 0.1 | DG   |
| C44| L-Bornyl acetate                                                                    | 65.52  | 1.29    | 0.08| CG   |
| C45| Methyl palmitate                                                                   | 18.09  | 1.37    | 0.12| CG   |
| C46| Myricanone                                                                         | 40.6   | 0.67    | 0.51| CG   |
| C47| Nicotinic acid                                                                     | 47.65  | 0.34    | 0.02| DG   |
Table 1: Continued.

| ID  | Active compounds | OB (%) | Caco-2 | DL | Herb   |
|-----|------------------|--------|--------|----|--------|
| C48 | Oleic acid       | 33.13  | 1.17   | 0.14 | CG     |
| C49 | Palmitic acid    | 19.3   | 1.09   | 0.1  | CG&DG  |
| C50 | Perlolyrine      | 65.95  | 0.88   | 0.27 | CG     |
| C51 | PLO              | 14.07  | 0.69   | 0.43 | CG     |
| C52 | Scopoletol       | 27.77  | 0.71   | 0.08 | DG     |
| C53 | Senkyunolide A   | 26.56  | 1.3    | 0.07 | CG     |
| C54 | Senkyunolide G   | 39.52  | 0.63   | 0.08 | CG     |
| C55 | Senkyunolide-C   | 46.8   | 0.87   | 0.08 | CG&DG  |
| C56 | Senkyunolide-D   | 79.13  | 0.12   | 0.1  | CG&DG  |
| C57 | Senkyunolide-E   | 34.4   | 0.55   | 0.08 | CG&DG  |
| C58 | Senkyunolide-K   | 61.75  | 0.52   | 0.08 | CG     |
| C59 | Sinapic acid     | 64.15  | 0.48   | 0.08 | CG     |
| C60 | Sphingomyelin    | 0.31   | −0.46  | 0.51 | DG     |
| C61 | Stearic acid     | 17.83  | 1.15   | 0.14 | CG     |
| C62 | Stigmasterol     | 43.83  | 1.44   | 0.76 | DG     |
| C63 | Succinic acid    | 29.62  | −0.44  | 0.01 | DG     |
| C64 | Sucrose          | 7.17   | −2.89  | 0.23 | CG     |
| C65 | Wallichilide     | 42.31  | 0.82   | 0.71 | CG     |

(C52, DL = 0.27, OB = 65.95, Caco-2 = 0.88) was confirmed to have a protective effect on injured human umbilical vein endothelial cells [36], and myricanone (C48, DL = 0.51, OB = 57.61, Caco-2 = 0.67) was found to best inhibit mouse skin tumor progression [37].

3.2. Target Fishing. The 65 active compounds interact with 185 target proteins, as shown in Table 2; in other words, on average, each compound on average interacts with 2.85 target proteins. This result confirms the polypharmacological character of oriental medicine and demonstrates the synergistic effects of multiple compounds on multiple targets [38]. Different compounds in CG and DG can directly affect common targets, for example, the target protein "calmodulin (CALM1)" interacts with crysophanol from CG and coniferyl ferulate from DG at the same time, which implies the synergic or cumulative effects of herbal medicine.

3.3. GO Analysis. 397 biological process terms with P values of <0.01 were sorted using the functional annotation chart of the DAVID 6.8 Gene Functional Classification Tool, based on 185 filtered target genes, and P values were adjusted using the Benjamini-Hochberg method. 30 enriched GO BP terms extracted by P value and gene counts are displayed in Figure 2. It is meaningful that most of the target genes are significantly related to the various BP involved in parturition. For instance, 30 extracted GO BP terms include "MAPK signaling pathways," "steroid hormone mediated signaling pathway," "response to glucocorticoid," "response to estradiol," and "positive regulation of ERK1 and ERK2 cascade." "MAPK signaling pathways" were reported to be activated in human uterine cervical ripening during parturition [39]. "Steroid hormone mediated signaling pathway" is highly related to parturition process as estrogen and progesterone play important roles in pregnancy and parturition, and estrogen induces the principal stimulatory myometrial contractility [40]. Also, estradiol takes key place in parturition process [41]. It was identified that increased ERK activation is observed at the onset of labor, and it promotes myometrial contractility and development of parturition [42, 43]. To sum up, the target genes of BSS are highly associated with the biological process (BP) of parturition.

3.4. Network Construction and Analysis. Network analysis is an efficient tool for visualizing and understanding multiple targeted drug actions and demonstrates drug actions within the context of the whole genome [44, 45]. For a better insight of therapeutic impacts, H-C-T and T-P networks were constructed and displayed in Figures 3 and 4, respectively. In the H-C-T network, nodes represent herb names, compounds, and targets. Also in the T-P network, circular nodes represent targets and triangle nodes represent pathways. Besides node size is relative to the degree and edges show interactions between nodes.

H-C-T network confirmed that there were 739 interactions between 185 targets and 65 active compounds of CG and DG: oleic acid (C48, degree = 42) with the highest number of interactions with targets, followed by succinic acid (C63, degree = 40) and stigmasterol (C62, degree = 37). It shows that single molecules target multiple receptors [46]. Also, some compounds from CG and DG were found to share common targets. Likewise, prostaglandin G/H synthase 2 (PTGS2, degree = 56) displayed the most affinitive connections with compounds, followed by gamma-amino butyric acid receptor subunit alpha-1 (GABRA1, degree = 48), prostaglandin G/H synthase 1 (PTGS1, degree = 37),...
### Table 2: Related targets of potential compounds in BSS.

| UniProt ID | Target name | Gene Name |
|------------|-------------|-----------|
| P80404     | 4-aminobutyrate aminotransferase, mitochondrial | ABAT |
| P33121     | Long-chain-fatty-acid--CoA ligase 1 | ACSL1 |
| O60488     | Long-chain-fatty-acid--CoA ligase 4 | ACSL4 |
| P00813     | Adenosine deaminase | ADA |
| P07327     | Alcohol dehydrogenase 1A | ADH1A |
| P00325     | Alcohol dehydrogenase 1B | ADH1B |
| P00326     | Alcohol dehydrogenase 1C | ADH1C |
| P29274     | Adenosine A2a receptor | ADORA2A |
| P35348     | Alpha-1A adrenergic receptor | ADRA1A |
| P35368     | Alpha-1B adrenergic receptor | ADRA1B |
| P25100     | Alpha-1D adrenergic receptor | ADRA1D |
| P08913     | Alpha-2A adrenergic receptor | ADRA2A |
| P18089     | Alpha-2B adrenergic receptor | ADRA2B |
| P18825     | Alpha-2C adrenergic receptor | ADRA2C |
| P08588     | Beta-1 adrenergic receptor | ADRB1 |
| P07550     | Beta-2 adrenergic receptor | ADRB2 |
| Q5S84      | Adenylosuccinate synthetase | ADSS |
| P21549     | Serine--pyruvate aminotransferase | AGXT |
| O43865     | Putative adenosylhomocysteinase 2 | AHCY1 |
| P15121     | Aldose reductase | AKR1B1 |
| P13716     | Delta-aminolevulinic acid dehydratase | ALAD |
| P51649     | Succinate semialdehyde dehydrogenase, mitochondrial | ALDH5A1 |
| P04745     | Alpha-amylase 1 | AMY1A |
| P04746     | Pancreatic alpha-amylase | AMY2A |
| P04114     | Apolipoprotein B-100 | APOB |
| P10275     | Androgen receptor | AR |
| P06576     | ATP synthase subunit beta, mitochondrial | ATP5B |
| P06276     | Cholinesterase | BCHE |
| P10415     | Apoptosis regulator Bcl-2 | BCL2 |
| Q06187     | Tyrosine-protein kinase BTK | BTK |
| P00915     | Carbonic anhydrase 1 | CA1 |
| P62158     | Calmodulin | CALM1 |
| P42574     | Caspase-3 | CASP3 |
| P04040     | Catalase | CAT |
| P06307     | Cholecystokinin | CCK |
| P20248     | Cyclin-A2 | CCNA2 |
| P30305     | M-phase inducer phosphatase 2 | CDC25B |
| P24941     | Cell division protein kinase 2 | CDK2 |
| P11597     | Cholesterol ester transfer protein | CETP |
| P28329     | Choline O-acetyltransferase | CHAT |
| O14757     | Serine/threonine-protein kinase Chkl | CHEK1 |
| P36222     | Chitinase-3-like protein 1 | CHI3L1 |
| P11229     | Muscarinic acetylcholine receptor M1 | CHRM1 |
| P08172     | Muscarinic acetylcholine receptor M2 | CHRM2 |
| P20309     | Muscarinic acetylcholine receptor M3 | CHRM3 |
| Q15822     | Neuronal acetylcholine receptor subunit alpha-2 | CHRNA2 |
| P36544     | Neuronal acetylcholine receptor protein, alpha-7 chain | CHRNA7 |
| Q99966     | Cbp/p300-interacting transactivator 1 | CITED1 |
| P02452     | Collagen alpha-1(I) chain | COL1A1 |
| Q02388     | Collagen alpha-1(VII) chain | COL7A1 |
| P17538     | Chymotrypsinogen B | CTRB1 |
| P07339     | Cathepsin D | CTSD |
| P04798     | Cytochrome P450 1A1 | CYPIA1 |
Table 2: Continued.

| UniProt ID | Target name | Gene Name |
|------------|-------------|-----------|
| P05177     | Cytochrome P450 1A2 | CYP1A2 |
| Q9ULA0     | Aspartyl aminopeptidase | DNPEP |
| P27487     | Dipeptidyl peptidase IV | DPP4 |
| P21728     | Dopamine D1 receptor | DRD1 |
| P14416     | D(2) dopamine receptor | DRD2 |
| P25101     | Endothelin-1 | EDNRA |
| Q07075     | Glutamyl aminopeptidase | ENPEP |
| Q6UWV6     | Ectonucleotide pyrophosphatase/phosphodiesterase family member 7 | ENPP7 |
| P04626     | Receptor tyrosine-protein kinase erbB-2 | ERBB2 |
| P03372     | Estrogen receptor | ESR1 |
| Q92731     | Estrogen receptor beta | ESR2 |
| P00742     | Coagulation factor Xa | F10 |
| P00734     | Thrombin | F2 |
| P08709     | Coagulation factor VII | F7 |
| P07148     | Fatty acid-binding protein, liver | FABP1 |
| P01100     | Proto-oncogene c-Fos | FOS |
| P15408     | Fos-related antigen 2 | FOSL2 |
| P35575     | Glucose-6-phosphatase | G6PC |
| P14867     | Gamma-aminobutyric acid receptor subunit alpha-1 | GABRA1 |
| P47869     | Gamma-aminobutyric acid receptor alpha-2 subunit | GABRA2 |
| P34903     | Gamma-aminobutyric acid receptor alpha-3 subunit | GABRA3 |
| P48169     | Gamma-aminobutyric acid receptor subunit alpha-4 | GABRA4 |
| P31644     | Gamma-aminobutyric acid receptor alpha-5 subunit | GABRA5 |
| Q16445     | Gamma-aminobutyric acid receptor subunit alpha-6 | GABRA6 |
| P17677     | Neurromodulin | GAP43 |
| P47871     | Glucagon | GCGR |
| P14136     | Glial fibrillary acidic protein | GFAP |
| Q2TU84     | Growth-inhibiting protein 18 | GIG18 |
| P23415     | Glycine receptor alpha-1 chain | GLRA1 |
| P00367     | Glutamate dehydrogenase 1, mitochondrial | GLUD1 |
| P17174     | Aspartate aminotransferase, cytoplasmic | GOT1 |
| P00505     | Aspartate aminotransferase, mitochondrial | GOT2 |
| P42262     | Glutamate receptor 2 | GRIA2 |
| P49841     | Glycogen synthase kinase-3 beta | GSK3B |
| Q15486     | Putative beta-glucuronidase-like protein SMA3 | GUSBP1 |
| P19367     | Hexokinase-1 | HK1 |
| P04035     | 3-hydroxy-3-methylglutaryl-coenzyme A reductase | HMGCR |
| P00738     | Haptoglobin | HP |
| O14756     | Oxidoreductase | HSD17B6 |
| P08238     | Heat shock protein HSP 90 | HSP90AB1 |
| P28223     | 5-hydroxytryptamine 2A receptor | HTR2A |
| P01344     | Insulin-like growth factor II | IGF2 |
| P01857     | Ig gamma-1 chain C region | IGHG1 |
| P22301     | Interleukin-10 | IL10 |
| P05231     | Interleukin-6 | IL6 |
| P01308     | Insulin | INS |
| Q12809     | Potassium voltage-gated channel subfamily H member 2 | KCNH2 |
| Q12791     | Calcium-activated potassium channel subunit alpha 1 | KCNMA1 |
| P35968     | Vascular endothelial growth factor receptor 2 | KDR |
| P09848     | Lactase-phlorizin hydrolase | LCT |
| Q32P28     | Prolyl 3-hydroxylase 1 | LEPRE1 |
| Q8IVL6     | Prolyl 3-hydroxylase 3 | LEPREL2 |
| P06858     | Lipoprotein lipase | LPL |
| UniProt ID | Target name | Gene Name |
|-----------|-------------|-----------|
| P09960    | Leukotriene A-4 hydrolase | LTA4H     |
| P21397    | Amine oxidase [flavin-containing] A | MAOA      |
| P27338    | Amine oxidase [flavin-containing] B | MAOB      |
| Q16539    | Mitogen-activated protein kinase 14 | MAPK14    |
| Q00266    | S-adenosylmethionine synthetase isomorph type-1 | MAT1A     |
| P31153    | S-adenosylmethionine synthetase isomorph type-2 | MAT2A     |
| P23368    | NAD-dependent malic enzyme, mitochondrial | ME2       |
| Q16798    | NADP-dependent malic enzyme, mitochondrial | ME3       |
| Q35YC2    | 2-acetylgluceral O-acetyltransferase 2 | MOGAT2    |
| P05164    | Myeloperoxidase | MPO       |
| Q15788    | Nuclear receptor coactivator 1 | NCOA1     |
| Q15596    | Nuclear receptor coactivator 2 | NCOA2     |
| P29475    | Nitric-oxide synthase, brain | NOS1      |
| P35228    | Nitric oxide synthase, inducible | NOS2      |
| P29474    | Nitric oxide synthase, endothelial | NOS3      |
| P16083    | NRH dehydrogenase [quinone] 2 | NQO2      |
| Q14994    | Nuclear receptor subfamily 1 group I member 3 | NRII3     |
| P04150    | Glucocorticoid receptor | NR3C1     |
| P08235    | Mineralocorticoid receptor | NR3C2     |
| Q16620    | BDNF/NT-3 growth factors receptor | NTRK2     |
| P04181    | Ornithine aminotransferase, mitochondrial | OAT       |
| P00480    | Ornithine carbamoyltransferase, mitochondrial | OTC       |
| Q9BYC2    | Succinyl-CoA:3-ketoacid-coenzyme A transferase 2, mitochondrial | OXCT2     |
| Q15460    | Prolyl 4-hydroxylase subunit alpha-2 | P4HA2     |
| P49585    | Choline-phosphate cytidylyltransferase A | PCYT1A    |
| Q14432    | CGMP-inhibited 3',5'-cyclic phosphodiesterase A | PDE3A     |
| O00330    | Pyruvate dehydrogenase protein X component, mitochondrial | PDHX      |
| P52945    | Pancreas/duodenum homeobox protein 1 | PDX1      |
| P06401    | Progestosterone receptor | PGR       |
| P48736    | Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform | PIK3CG    |
| P11309    | Proto-oncogene serine/threonine-protein kinase Pim-1 | PIM1      |
| P3925    | cAMP-dependent protein kinase inhibitor alpha | PKIA      |
| P14618    | Pyruvate kinase isozymes M1/M2 | PKM2      |
| P04054    | Phospholipase A2 | PLA2G1B    |
| P00749    | Urokinase-type plasminogen activator | PLAU      |
| P00747    | Plasminogen | PLG       |
| P00491    | Purine nucleoside phosphorylase | PNP       |
| P27169    | Serum paraoxonase/arylesterase 1 | PON1      |
| Q07869    | Peroxisome proliferator-activated receptor alpha | PPARA     |
| Q03181    | Peroxisome proliferator-activated receptor delta | PPARD     |
| P37231    | Peroxisome proliferator activated receptor gamma | PPARG     |
| Q9UBK2    | Peroxisome proliferator-activated receptor gamma coactivator 1-alpha | PPARGCG1A |
| P17612    | mRNA of PKA Catalytic Subunit C-alpha | PRKACA    |
| P05771    | Protein kinase C beta type | PRKCB     |
| P35030    | Trypsin-3 | PRSS3     |
| P60484    | Phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN | PTEN      |
| P43115    | Prostaglandin E2 receptor EP3 subtype | PTGER3    |
| P23219    | Prostaglandin G/H synthase 1 | PTGSI     |
| P35354    | Prostaglandin G/H synthase 2 | PTGS2     |
| P18031    | mRNA of Protein-tyrosine phosphatase, non-receptor type 1 | PTPN1     |
| P10082    | Peptide YY | PYY       |
| P63000    | Ras-related C3 botulinum toxin substrate 1 | RAC1      |
| P50120    | Retinol-binding protein 2 | RBP2      |
| P08100    | Rhodopsin | RHO       |
Table 2: Continued.

| UniProt ID | Target name                                                                 | Gene Name |
|------------|------------------------------------------------------------------------------|-----------|
| P19793     | Retinoic acid receptor RXR-alpha                                               | RXRA      |
| O00767     | Acyl-CoA desaturase                                                            | SCD       |
| Q14524     | Sodium channel protein type 5 subunit alpha                                   | SCN5A     |
| P31040     | Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial      | SDHA      |
| P16109     | P-selectin                                                                    | SLP       |
| P05121     | Plasminogen activator inhibitor 1                                              | SERPINE1  |
| P14410     | Sucrase-isomaltase, intestinal                                                 | SI        |
| O76082     | Solute carrier family 22 member 5                                              | SLC22A5   |
| Q9UBX3     | Mitochondrial dicarboxylate carrier                                            | SLC25A10  |
| P1168      | Solute carrier family 2, facilitated glucose transporter member 2              | SLC2A2    |
| P23975     | Sodium-dependent noradrenaline transporter                                     | SLC6A2    |
| Q01959     | Sodium-dependent dopamine transporter                                          | SLC6A3    |
| P31645     | Sodium-dependent serotonin transporter                                         | SLC6A4    |
| P35610     | Sterol O-acyltransferase 1                                                    | SOAT1     |
| P00441     | Superoxide dismutase [Cu-Zn]                                                   | SOD1      |
| P08047     | Transcription factor Sp1                                                        | SPI       |
| P12931     | Proto-oncogene tyrosine-protein kinase SRC                                     | SRC       |
| P36956     | Sterol regulatory element-binding protein 1                                   | SREBF1    |
| Q12772     | Sterol regulatory element-binding protein 2                                   | SREBF2    |
| Q99973     | Succinyl-CoA ligase [ADP-forming] beta-chain, mitochondrial                  | SUCLA2    |
| P0137      | Telomerase protein component 1                                                 | TEP1      |
| Q16881     | Tumor necrosis factor                                                          | TNF       |
| P55916     | Thioredoxin reductase, cytoplasmic                                             | TXNRD1    |
| Q55916     | Mitochondrial uncoupling protein 2                                             | UCP2      |
| Q55916     | Mitochondrial uncoupling protein 3                                             | UCP3      |

and muscarinic acetylcholine receptor M1 (CHRM1, degree = 37). Except for C60 (PLA2G1B, degree = 1), the rest of the 64 active compounds are connected with more than one target; likewise, 73 (39.5%) target genes out of 185 interacted with more than one compound. This result demonstrates the multicomponents and multtarget properties of herbal compounds and there was a report that compounds with multiple targets could have greater therapeutic efficacy [47].

In addition, the top 40 pathways were extracted based on gene counts and P value (<0.05), and P value was adjusted by Benjamini-Hochberg method. T-P network using relevant targets of herbal compounds is demonstrated in Figure 4. There were 485 interactions between the top 40 pathways and 135 of 185 target genes. "Metabolic pathways" (degree = 49) and "neuroactive ligand-receptor interaction pathway" (degree = 32) had the highest and the second highest numbers of connections with the targets, followed by "calcium signaling" (degree = 21), "cAMP signaling pathway" (degree = 17), and "cGMP PKG signaling pathway" (degree = 15). These are compelling results that parturition processes are the complex hormone interactions and it is well known that calcium signals within the myometrium are pivotal for uterine contractions [48]. In the same manner, some target genes demonstrated higher degree centrality with top 40 pathways, namely, PI3-kinase subunit gamma (PIK3CG, degree = 23), cAMP-dependent protein kinase catalytic subunit alpha (PRKACA, degree = 20), protein kinase C beta type (PRKCB, degree = 18), and calmodulin (CALM1, degree = 11). We can confirm the same result in the previous researches. For instance, PI3-kinase subunit gamma plays the key role in regulating cAMP, calcium cycling, and beta-adrenergic signaling [49]. Moreover, during the labor, calmodulin-calcium complex activates myosin light-chain kinase, which causes the generation of ATPase activity; eventually, uterine contraction is promoted [50].

H-C-T network explains the multitarget, multicomponents properties and accumulates effect of herbal medicines and T-P network shows that target genes of BSS are highly related to the pathway associated with parturition process.

3.5. Target Organ Location Map. It is important to confirm the tissue mRNA expression profiles of the target genes at the organ level to identify the effects of BSS on parturition. Since there was no mRNA expression information in BioGPS of muscarinic acetylcholine receptor M1 (CHRM1), putative beta-glucuronidase-like protein SMA3 (GUSBP1), and retinol-binding protein 2 (RBP2), excluding these 3 targets from 185 filtered targets, totally 182 genes mRNA expression profiles were analyzed in this study. There were 519 interactions between target genes and organ locations. The networks of target genes tissue mRNA expression profiles and compounds of BSS are shown in Figure 5.
As a result, 159 of 182 target genes displayed beyond average mRNA expression in relevant organ tissues, such as uterus and/or uterus corpus, fetus and/or placenta, hypothalamus and/or pituitary, smooth muscle, and whole blood. The rest of 23 genes of 182 targets did not display above average mRNA expression in above organ tissues, for example, gamma-aminobutyric acid receptor subunit alpha-6 (GABRA6) and coagulation factor X (F10).

Nevertheless, most genes of 159 demonstrated high expression patterns in several organs of parturition related tissues at the same time. In detail, 60 genes showed most significant mRNA expression in the uterus and/or uterus corpus group, 130 for placenta and/or fetus, 86 for hypothalamus and/or pituitary, 82 for smooth muscle, 80 for pituitary, and 81 for whole blood. Besides, 30 of 159 genes showed expression in all of 6 groups. For instance, muscarinic acetylcholine receptor M2 (CHRM2), neuronal acetylcholine receptor subunit alpha-2 (CHRNA2), gamma-aminobutyric acid receptor subunit alpha-3 (GABRA3), NO synthase, inducible (NOS2), cGMP-inhibited 3′,5′-cyclic phosphodiesterase A (PDE3A), and sodium-dependent dopamine transporter (SLC6A3) recorded beyond average mRNA expression in all six groups. Furthermore, 79% of targets were expressed in two or more organ tissues, which suggests that those organs and target genes of BSS are closely correlated.

4. Discussion

In this study, network pharmacology method with DL, OB, Caco-2, and LR evaluation, multiple drug-target prediction,
network analysis, and relevant organ location mapping was used to explain the targets of BSS in relation to the parturition process. There is no denying that network based analysis is powerful approach for identifying the actions of multitargeting herbal medicines at the systems level and our study shows target genes of BSS are strongly connected to parturition related pathways, biological processes, and organs. It was confirmed that 98% of the active compounds of BSS were interacted with more than two targets and 39.5% of the targets related to more than one compound. The synergetic multitarget properties of BSS were visualized, but further discussion about differentiated drug action based on degree centrality and simultaneous targeting effect of more than one compound is required [51]. Also, detailed potential pathways of BSS should be explored deeply in the future.

Similar findings were identified in a few RCT researches in China that using BSS in induction of labor can reduce the delivery time, the amount of bleeding, and the residual rate of placenta [52, 53]. In addition, BSS targets six genes of GABA receptor and NOS, which was reported to be related oxytocin neurons at the time of parturition in rats [54]. Also, BSS targets NOS and NO (nitric oxide) which are involved in the regulation of uterine contractility during pregnancy and is a key factor for the onset of labor [55], and iNOS (inducible nitric oxide synthase) can be upregulated accordantly by similar inflammatory mediators during ripening [11].

In fact, rather than DG, *Angelicae Gigantis Radix* (Danggwi, AGR) grows naturally in Korea; for that reason, the combination of AGR and CG is commonly used as BSS in Korea. Instead, DG is named as Chinese Danggwi for accurate classification in Korea. Several studies have shown AGR is differs from DG in terms of its main active constituents and genetic form. AGR is mainly composed of...
Figure 4: T-P network: in target-pathway (T-P) network, circular nodes represent compounds and triangles indicate pathways. Node size is relative to the degree and edges demonstrate interactions between nodes.

water soluble polysaccharide but coumarin, which is liposoluble including nodakenin (1), peucedanone (2), marmesin (3), decursinol (4), 7-hydroxy-6-(2R-hydroxy-3-methylbut-3-enyl) coumarin (5), demethylsuberosin (6), decursin (7), decursinol angelate (8), and isoimperatorin (9) [56]. Of these, decursin and its isomer decursinol angelate have been reported to be the active compounds in AGR [57]. It was identified in the experimental studies that AGR and DG act via different mechanisms in the cardiovascular, central nervous system, and anticancer activity but both have similar pharmacological effects [57]. Since the compositions of DG and AGR differ, further study on BSS with AGR is required. Currently, BSS is commonly prescribed to treat cerebra vascular and cardiovascular diseases in China [33], but, in Korea, BSS is widely applied in obstetrics.

The similarity between cervical ripening during parturition and inflammatory reaction has been pointed out in earlier studies; this has been attributed to the induction of leukocyte migration into tissue, thus promoting cervical remodeling and parturition by estrogen [58]. Further study is needed in terms of the effect of BSS on inflammatory reactions and parturition.

Furthermore, the CG-DG herb pair has other names, such as, Gunggui-tang (weight ratios of 2:3 or 1:1), Ogeum-san (1:1), Iphyo-san (1:1), and Sinmyo Bulsu-san (1:2), those are prepared at different weight ratios [3]. Accordingly, weight ratio should be determined based on considerations of targeted symptoms for relevant clinical applications.

5. Conclusion

This study results show that Bulsu-san (BSS) is highly connected to the parturition related pathways, biological processes, and organs. Most compounds in BSS work together with multiple target genes in a synergetic way, and this was confirmed using herb-compound-target network and target-pathway network analysis. The mRNA expression of relevant target genes of BSS was elevated significantly in parturition.
related organ tissues, such as those of the uterus, placenta, fetus, hypothalamus, and pituitary gland. This study employed the network analytical methods to show the multicomponent, multitarget properties of BSS. The results not only support clinical applications of BSS on easing childbirth but also suggest the related target genes and pathways of BSS on promoting parturition according to a systems-level in silico analytic approach. However, detailed mechanisms and other functions of BSS should be discussed further.

**Conflicts of Interest**

The authors declare that there are no conflicts of interest.

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