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Molecular Target-Oriented Phytochemical Database and Its Application to the Network Analysis of Action Mechanisms of Herbal Medicines

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Kampo medicines, the Japanese adaptation of traditional Chinese medicines, are formed by combining several herbs containing multiple phytochemicals. The considerable ambiguity of pharmacological profiles of Kampo medicines is expected to be clarified by identifying the molecular targets of constituent phytochemicals and analyzing the combined effects of the phytochemicals on the pharmacological pathways formed by those targets. To facilitate this line of study, we constructed paired databases named PhytodamaTarget and PhytodamaTaxon DBs which treat molecular targets of phytochemicals and constituent phytochemicals of plant taxa, respectively, by utilizing information from the literature. We then used the databases to explore possible mechanisms of synergism in analgesic activity between *Glycyrrhiza globra* and *Paeonia lactiflora*

Keyword: Phytochemicals, Database, Molecular Target, Kampo Medicines, Synergism, Analgesic

1. Introduction

Kampo and traditional Chinese medicines are characterized by a complex composition with multiple pharmacologically active phytochemicals[1]. A tremendous number of these medicines have been developed by combining various specified herbs to achieve the greatest possible pharmacological effect[2]. However, most of the mechanisms of pharmacological action of Kampo medicines have yet to be fully elucidated at a molecular level[3]. To elucidate these molecular mechanisms, it is necessary to identify the molecular targets of the herbal ingredients and to clarify the synergism or antagonism of molecular actions. The purpose of this study is to construct a phytochemical database that facilitates identification of the molecular targets of phytochemicals contained in Kampo formulations and to examine its usefulness for exploring the molecular mechanisms behind the medicines actions.

2. Methods

2.1 Database generation

The data for molecular targets and the taxonomy of producers of phytochemicals were compiled from both primary and secondary sources, particularly Gideon Polya's Biochemical Targets of Plant Bioactive Compounds[4]. The chemical

information on phytochemicals was also obtained from primary and secondary sources, the latter of which included databases such as PubChem (http://pubchem.ncbi.nlm.nih.gov/) and NikkajiWeb (http://nikkajiweb.jst.go.jp/nikkaji_web/pages/top_e.html). PhytodamaTarget DB and PhytodamaTaxon DB were constructed by using Microsoft Office Access 2003.

2.2 Graphical presentation of relationship between phytochemicals and their molecular targets

CellDesigner 4.2, a tool for modeling biochemical networks with a graphical user interface developed by H. Kitano *et al.*[5], was used to present graphically the relationship between phytochemicals and their molecular targets.

2.3 Screening of molecular targets involved in analgesic activity

A list of 371 genes involved in pain was obtained from the database PainGenesdb (http://www.jbldesign.com/jmogil/enter.html).

The molecular targets of phytochemicals involved in analgesic activity were identified among the targets of either *Glycyrrhiza globra* or *Paeonia lactiflora* by checking whether they were included in the pain gene list.

3. Results and Discussion

3.1 Construction of PhytodamaTarget and PhytodamaTaxon Databases

We constructed two databases named PhytodamaTarget and PhytodamaTaxon DBs, the former covering information on molecular targets of phytochemicals together with their chemical properties, and the latter providing information on the taxonomy of the producers of phytochemicals together with herbal information.

The basic information in PhytodamaTarget DB consists of phytochemical names, phytochemical synonyms, target names, target subfamilies, target categories, modes of action, IC_{50}/EC_{50}, *Kd/Ki*, chemical structure, chemical formulae, smiles strings, molecular weights, biosynthetic pathways, links to other databases such as PubChem, KEGG (http://www.genome.jp/kegg/) and GeneCards (http://www.genecards.org/) and references (Fig.1) whereas that in PhytodamaTaxon DB consists of taxonomic names including genus, family, order, clade, Japanese, Chinese and English herb names, and phytochemical names (Fig.2).

Fig.1. Representative form of PhytodamaTarget DB.

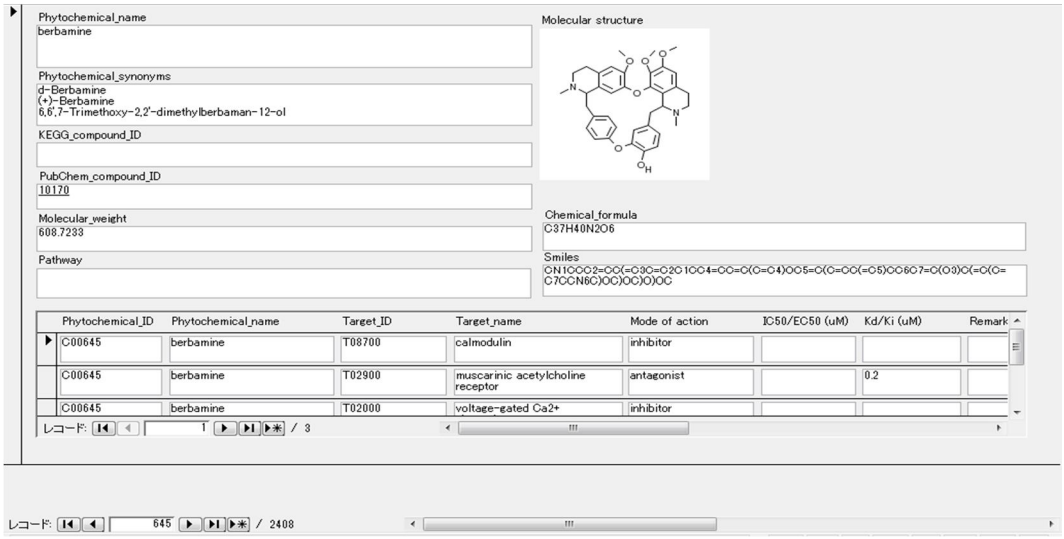
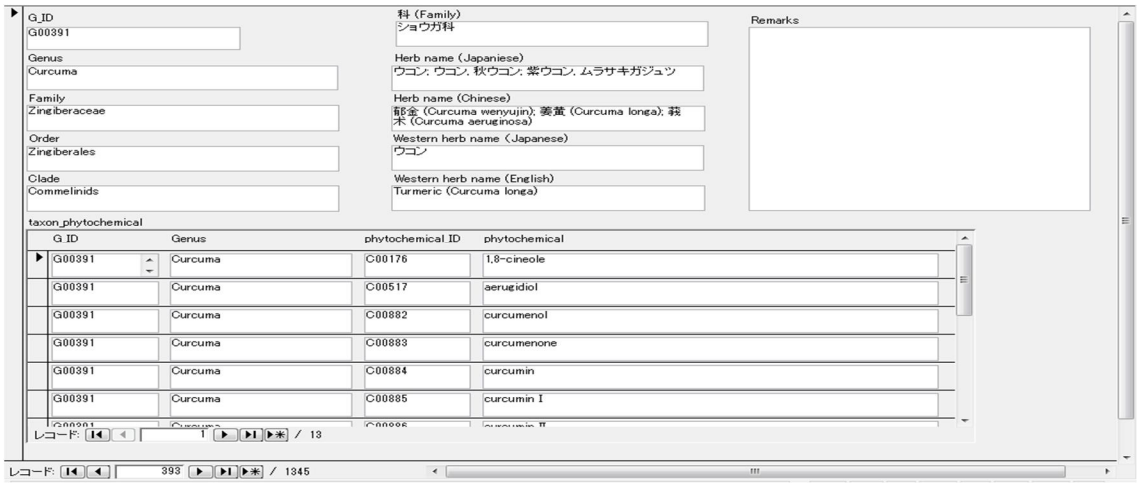


Fig. 2. Representative form of PhytodamaTaxon DB.



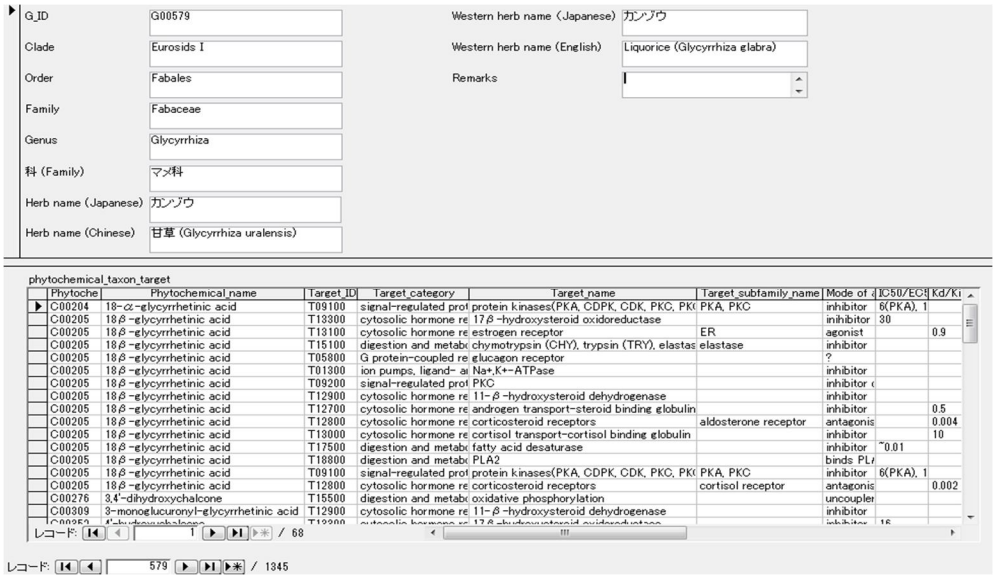
The data set used in the classification of targets: target category, target name and target subfamily designates pharmacologically distinct groups such as G protein-coupled receptors, gene product families such as receptor families, and gene product subfamilies such as receptor subtypes, respectively. The plant classification was performed according to the APG III system[6].

Registered numbers of phytochemicals, targets, plant genuses and herbs in the databases are 2408, 210, 1345 and 159, respectively.

3.2 Retrieval of Targets of Herbs

Identification of molecular targets of phytochemicals contained in herbs is helpful in elucidating the pharmacological effect of herbs. This identification is easily accomplished by combined use of PhytodamaTarget and PhytodamaTaxon DBs. Namely, data on plant genus can be obtained instantly together with that on its constituent phytochemicals and their targets by using the "subform" and "query" functions of Microsoft Access (Fig. 3).

Fig.3. Presentation of phytochemicals and their targets corresponding to a plant genus record.

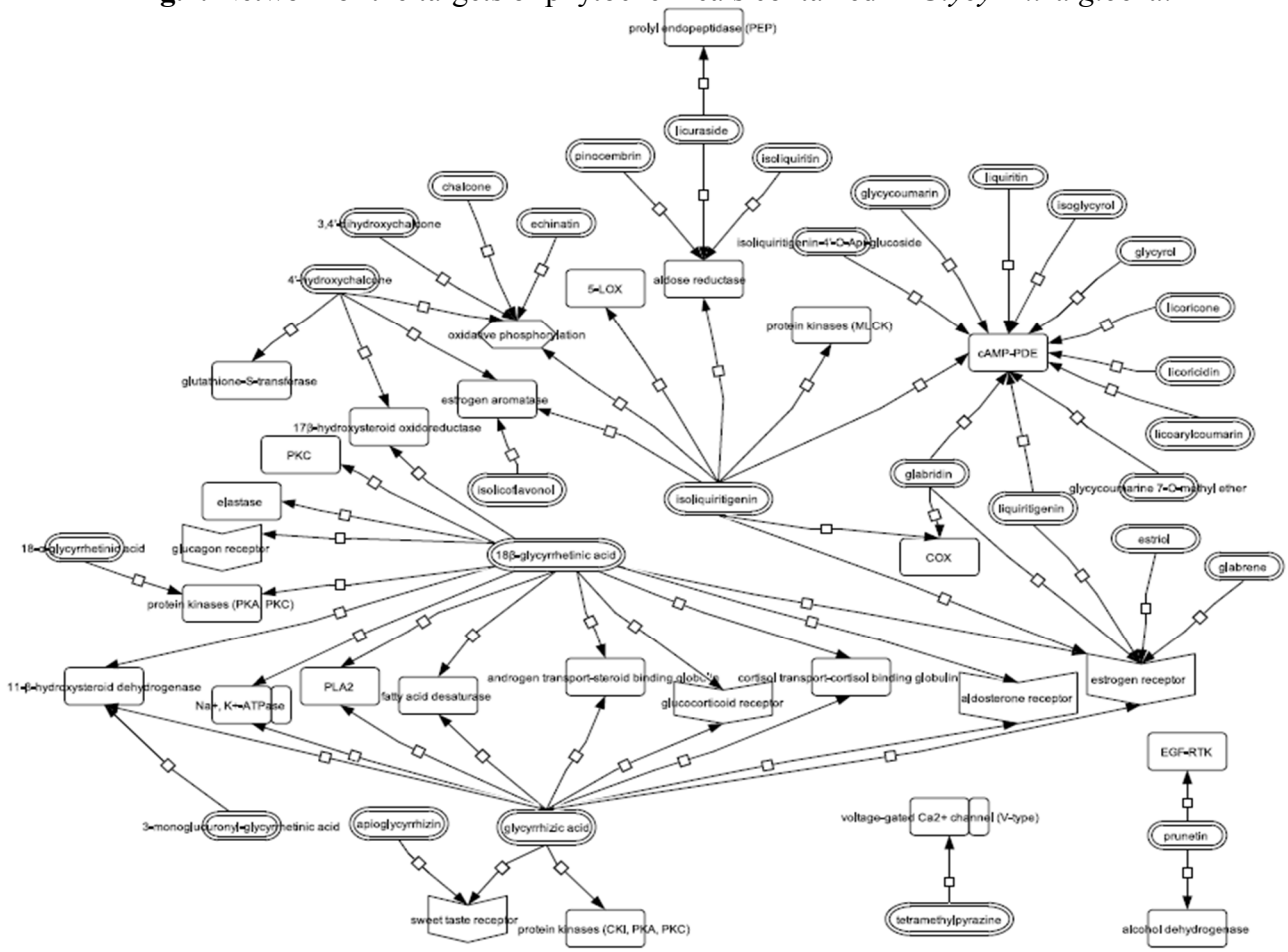


3.3 Possible mechanism of synergism in analgesic activity between herbal medicines derived from *Glycyrrhiza globra and Paeonia lactiflora*

To validate the usefulness of our databases for exploring the molecular mechanisms responsible for the pharmacological activities of Kampo medicines, we used them to analyze the synergistic analgesic activity between the herbs from *Glycyrrhiza globra* and *Paeonia lactiflora.*

Synergism in analgesic activity between the herbs from *Glycyrrhiza globra* and *Paeonia lactiflora* was first described in a Chinese book named Treatise on Cold Damage Disorders collated by Zhang Zhongjing. Presently *Shakuyakukanzoto*, a Kampo formulation composed of *Glycyrrhiza globra* and *Paeonia lactiflora*, is mostly used to relieve muscle pain[7]. The pharmacological mechanism of this analgesic activity has been investigated by several groups. Kimura *et al.* found that a combination of paeoniflorin and glycyrrhizin, major ingredients of *Paeonia lactiflora* and *Glycyrrhiza globra*, respectively, synergistically inhibited twitch tensions of indirectly stimulated diaphragm muscles of mice[8]. Satoh and Tsuruo also found that *Shakuyakukanzoto* relaxed carbachol-induced contractions of rat intestinal smooth muscles and assumed that the relaxation was caused by anti-cholinergic and phosphodiesterase inhibitory actions[9]. However, anti-cholinergic activity of the ingredients of both *Glycyrrhiza globra* and *Paeonia lactiflora* has yet to be shown directly although phosphodiesterase inhibitory activities of several compounds of *Glycyrrhiza globra* were reported.

Fig.4. Network of the targets of phytochemicals contained in *Glycyrrhiza globra.*

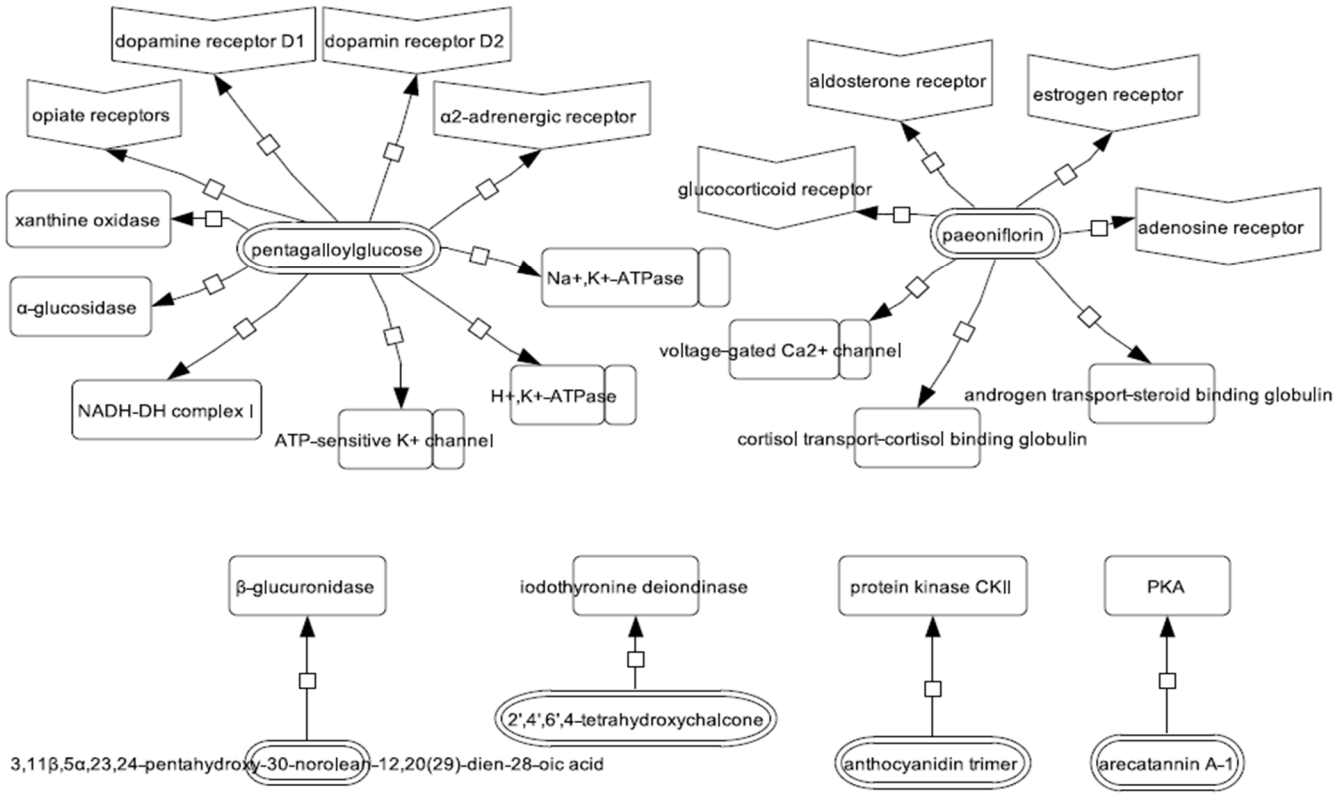


Meanwhile, other mechanisms of the analgesic activity of *Shakuyakukanzoto* and its constituents have been reported. Omiya *et al.* found that *Shakuyakukanzoto* showed antinociceptive activity in diabetic mice and assumed the activity to be caused by activation of the descending noradrenergic neurons[10]. Liu *et al.* ascribed the antinociceptive effect of paeoniflorin in mice to its ability to increase the binding and antinociceptive effect of an adenosine A1 agonist by binding with A1 receptors[11]. According to our database, *Paeonia lactiflora* contains a constituent with α2-adrenergic agonist activity, namely β-1,2,3,4, 6-penta-O-galloyl-D-glucose (PGG), together with paeoniflorin. Further studies are necessary to conclude whether PGG, paeoniflorin or same as yet unidentified ingredient is responsible for the analgesic activity of *Shakuyakukanzoto* in diabetic mice.

The molecular mechanism behind the synergistic analgesic effect of *Shakuyakukanzoto* remains largely unclear despite extensive studies. To elucidate it, it is necessary to clarify the interactions among constituents whose molecular targets are involved in the analgesia. Accordingly, the molecular targets of phytochemicals contained in *Glycyrrhiza globra* and *Paeonia lactiflora* were first retrieved by combined use of our databases. The number of hits for phytochemicals in *Glycyrrhiza globra* and *Paeonia lactiflora* was 29 and 6, respectively and the total number of hits for the molecular targets of these phytochemicals was 28 and 21, respectively (Fig.4 and Fig.5). The number of molecular targets common to both genuses was 6. Furthermore, some molecular targets were shared by multiple phytochemicals within the genus (Fig.4).

The symbols used to represent molecules and actions are as follows; ovoids, concave hexagons, and divided and simple round-cornered squares represent phytochemicals, receptors, ion channels and generic proteins, respectively. Arrows represent actions of phytochemicals against targets.

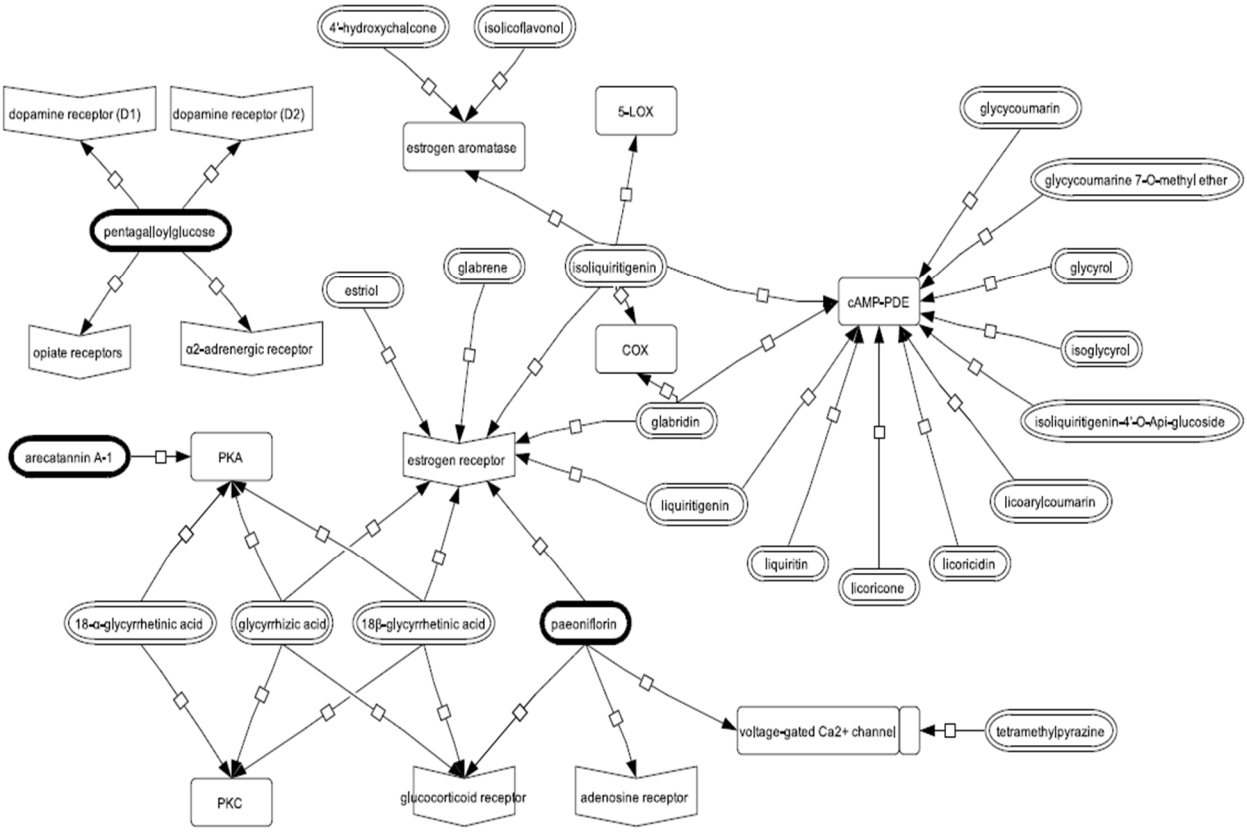
Fig.5. Network of the targets of phytochemicals contained in *Paeonia lactiflora.*



The symbols used are explained in the legend of Fig. 4.

To identify the molecular targets involved in analgesic activity among those listed, those coinciding with the gene products registered in the pain genes database[12] were screened. Eight out of 28 targets for *Glycyrrhiza globra* and 8 of 21 targets for *Paeonia lactiflora* were hit. They are cyclooxygenase, estrogen aromatase, the estrogen receptor, 5-lipooxygenase, protein kinase A, protein kinase C, cAMP phosphodiesterase and voltage-gated Ca^{2+} channels for *Glycyrrhiza globra* and the adenosine receptor, dopamine receptors, the estrogen receptor, opiate receptors, protein kinase A, voltage-gated Ca^{2+} channels and the α2-adrenergic receptor for *Paeonia lactiflora*. Although the glucocorticoid receptor, a target for both genuses, is missing in the pain genes database, it may be added as a pain gene, referring to the literature[13,14]. Among the targets identified as pain-related, cyclooxygenase, 5-lipooxygenase and estrogen aromatase are involved in the biosynthesis of algesic and analgesic substances[15,16], the adenosine, estrogen and glucocorticoid receptors are receptors for algesic or analgesic substances[13,14,17,18], the α2-adrenergic, dopamine and opiate receptors are receptors for neurotransmitters[19-21], protein kinases A, C and cAMP phosphodiesterase are involved in postreceptor signaling pathways[22] and voltage-gated Ca^{2+} channels are involved in neural transmission including regulation of the release of neurotransmitters[23]. Fig. 6 summarizes the interaction between the herbal ingredients and their molecular targets involved in analgesia.

Fig.6. Network of the pain-involved targets of phytochemicals contained in either *Glycyrrhiza globra* or *Paeonia lactiflora.*



The symbols are the same as those in Fig.4 except that ovoids with double outlines represent phytochemicals contained in *Glycyrrhiza globra* whereas ovoids with a thick outline represent those in *Paeonia lactiflora*.

The network of herbal ingredients and their pain-involved molecular targets shows that there are hubs, targets highly connected with ingredients, such as cAMP phosphodiesterase and the estrogen receptor, suggesting their possible greater roles for analgesic activity of the herbal medicine. Cyclic AMP phosphodiesterase catabolizes cAMP which is a pain-mediating second messenger.[24] Hence, the inhibitory activity of phytochemicals against cAMP phosphodiesterase would lead to pain enhancement. However, contrary to this speculation, cAMP phosphodiesterase inhibitors were shown to elevate nociceptive thresholds in the central nervous system by increasing antinociceptive natural epoxy-fatty acids[25]. The estrogen receptor was shown to be involved in lowering nociceptive thresholds by using the estrogen receptor knockout female mice although its mechanism has yet to be deciphered[26]. The phytochemicals would exert analgesic activity not by their agonistic activity but by their antagonistic activity against the estrogen receptor in the presence of endogenous estrogen[27].
Meanwhile, among targets shared by multiple components, those shared by components from different herbs, such as voltage-gated Ca^{2+} channels, the glucocorticoid receptor, the estrogen receptor and protein kinase A, are particularly noteworthy since one possible mechanism of synergism between agonists/antagonists or inhibitors is differential activities against a shared target. However, targets other than those mentioned above could be involved in the synergism, because simultaneous activities against targets closely related to analgesic activity is another possible mechanism of synergism. Though the ideas discussed above need to be examined further in wet experiments, the information provided by our database should help to elucidate the molecular mechanism of synergism in the analgesic activity of *Shakuyakukanzoto*. Furthermore, PhytodamaTarget and PhytodamaTaxon DBs should prove useful for analyzing the synergism or antagonism of pharmacological activities among herbal components of Kampo and other traditional medicines.

4. Conclusion

PhytodamaTarget and PhytodamaTaxon DBs were shown to facilitate identification of the molecular targets of phytochemicals contained in Kampo formulations and to be useful for exploring the molecular mechanisms of the synergistic pharmacological actions of these medicines.

5. References

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