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Original Research Article

Active Compounds from *Polyscias scutellaria* Stimulate Breast Milk Production: *In Silico* Study on Serotonergic 5-HT2A Receptors and Prolactin Receptors

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ABSTRACT

Mother's milk plays a significant role in infant nutrition. It is well known that exclusive breastfeeding can reduce the risk of various pathogens infection in infants. Colostrum in mother's milk contains various bioactive factors, especially immunoglobulin A (IgA). However, hormones such as serotonin and prolactin can affect the secretion of a mother's milk. Serotonin is a neurotransmitter that plays a role in stimulating prolactin secretion by the pituitary gland. Shield Aralia (*Polyscias scutellaria*), *daunmangkokan* in Java, has various active compounds, including afzelin, kaempferol, quercetin, quercitrin, and rutin. This study aimed to analyze the potential of the active compounds of *P. scutellaria* leaves in enhancing breast milk production through *in silico* analyses of two main proteins, serotonin 5-hydroxytryptamine-2A receptors (5-HT2AR) and prolactin receptors (PRLR). Results of molecular docking studies revealed that active compounds present in *P. scutellaria* docked at the same site as Risperidone, a known drug that influences serotonin reuptake and used in this study as the positive control agent. These results showed that the active compounds in the *P. scutellaria* leaves could be used as an alternative medicine to increase breast milk production.

Keywords: *P. scutellaria*, 5-hydroxytryptamine-2A receptors, Prolactin receptors, *In silico*.

Introduction

Exclusive breastfeeding for the first six months is deemed essential for infant nutrition until 1-2 years.[1,2] Breast milk provides all the nutritional components required by infants, particularly during the first six months of a newborn. Human milk contains proteins, fats, starch, minerals, vitamins, and water.[2,3] Breast milk has no *β*-lactoglobulin, a known cause of allergies in infants.[4] It also contains numerous bioactive factors relating to the immune system, defense against various infections, and other ingredients that help digest and absorb food.[3,4] Colostrum is one of the components present in breast milk fluids just after delivery. Colostrum contains high immunoglobulin A (IgA), lactoferrin, leukocytes, and various growth factors. Lactoferrin has been reported to be one of the most effective antibacterial, anti-viral, and anti-fungal proteins.[5,6]

However, low milk production remains an issue for infants who may not complete their exclusive breastfeeding nutritional phase. Low milk production is caused by lifestyle, especially in terms of a high-fat diet, the environment that affects the mother's emotional condition[7], nutrition shortages,[8] and stress.[9] Several antipsychotic drugs, for example, Risperidone,[10] are used to increase milk production. However, consumption of antipsychotic medications has limitations due to safety reasons and side effects for both mother and infants.[11,12] Against this backdrop, the utilization of galactagogue herbal plants could replace antipsychotics drugs in boosting breast milk production in lactating mothers.[13]

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The Indonesian society has traditionally used fenugreek (*Trigonella foenum-graecum*)[14] and shield aralia leaves (*Polyscias scutellaria*) or *daun mangkokan* to help increase milk production among new mothers. Fenugreek is commonly used worldwide and has been scientifically reported to increase breast milk production. However, there is a paucity of scientific research related to the use of *P. scutellaria* leaves to increase breast milk production.

This study aimed to analyze active compounds in *P. scutellaria* leaf through *in silico* analyses and their role in increasing breast milk production. *In Silico* study is a computational method for identifying, designing, and discovering drugs (drug design and discovery). *In silico* techniques can be used to predict the potential(s) of a compound as a possible drug candidate by using published bank data.[15]

Materials and Methods

Preparation of proteins and compounds structures

The compounds used as ligands in this study were selected from *P. scutellaria* active compounds[16] namely afzelin, kaempferol, quercetin, quercitrin, and rutin. The ligands' 3D chemical structures were obtained in .sdf format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Accession numbers for ligands were CID 5316673 for afzelin, CID 5280863 for kaempferol, CID 5280343 for quercetin, CID 5280459 for quercitrin, CID 5280805 rutin, and CID 5073 for risperidone as control. All ligands were then converted into Protein Data Bank (PDB) using PyMoL software. The 3D structure of the target protein was obtained from PDB (https://www.rcsb.org/) with accession ID 6A93 for protein 5-Hydroxytryptamine 2A receptors (5-HT2AR) and ID 3D48 for prolactin receptor (PRLR). Removal of water molecules from ligands and target proteins was done by the use of the PyMoL software.

Molecular docking analysis

Molecular docking between ligands and target proteins was analyzed using PyRx 0.8 software.[17,18] Visualization, interaction of ligands and target protein binding took place using PyMoL software and BIOVIA

Discovery Studio 2016. Binding affinity values were analyzed to determine the strength of the interaction between ligands and target protein before being compared with control drugs.[19]

Protein-ligand interaction and network

The BioGRID Database was used to analyzed protein-protein interactions (https://biogrid.org/)[20], and STRING database for protein-protein interaction network specific pathway (https://string-db.org/).[21] Subsequently, protein and ligand interaction networks were examined using the STITCH database (http://stitch.embl.de/).[22]

Pathway analysis

Pathway analysis for 5-HT2AR and PRLR was examined using Kyoto Encyclopedia Gene and Genome (KEGG) databases (http://www.genome.jp/kegg/).[23] Using the STRING database (https://string-db.org/), the role of 5-HT2AR and PRLR on lactogenesis in various molecular pathways over breast milk production was identified. Meanwhile, the interaction of active compounds in various proteins involved in breast milk production was analyzed using the STITCH database (http://stitch.embl.de/).[22]

Results and Discussion

Molecular docking analysis

Molecular docking results on 5-HT2AR showed a binding affinity value of -11.8 kcal/mol for Risperidone, the standard control drug. However, the active compounds in *P. scutellaria* leaf extract, kaempferol, and quercetin have lower binding affinity values (-8.9 kcal/mol) than Risperidone. Gibb's free energy measures the amount of free energy derived from interactions between ligands and proteins. The relationship between ligand and protein is significant and more stable when Gibb's free energy value is negative.[24,25] Similarly, docking on PRLR showed that the binding affinity value of Risperidone was -7.6 kcal/mol, while kaempferol, quercetin, and rutin, derived from *P. scutellaria*, have a binding affinity value of -6.7 kcal/mol. These results showed that active compounds found in *P. scutellaria* leaves showed good docking poses for the target proteins 5-HT2AR and PRLR.

Visualization of molecular docking and ligands interactions (Figure 2 and 3) showed interactions between ligand-proteins. For example, docking of 5-HT2AR showed that all the ligands docked to identical amino acid residues, namely Leu229, Phe234, Ser159, Asp155, Val366, Leu228, and Phe339 (Table 2). Moreover, docking of PRLR showed that all ligands bind to identical amino acid residues, namely Tyr94, Tyr99, Tyr190, Ile100, and Lys11 (Table 3).

Risperidone is an antipsychotic drug widely used to treat schizophrenia. Risperidone has a high binding affinity for 5-HT2AR.[26,27] The 5-HT2AR binding increases serotonin production as a dopamine antagonist. Decreasing dopamine levels will increase the secretion of lactocyte prolactin[28]. Further examination evaluated the position of the *P. scutellaria* active compounds when binding with 5-HT2AR. This evaluation showed that the active compounds shared a similar site with Risperidone.

Furthermore, the orientation of docking analysis on the PRLR active site demonstrated that *P. scutellaria* active compounds bound to PRLR in a similar position as Risperidone. Comparatively, the binding affinity value of Risperidone and *P. scutellaria* active compound revealed that though Risperidone exhibited better binding affinity to PRLR than *P. scutellaria* active compounds, this value was not significant. The data suggested that active compounds found in *P. scutellaria* can increase breast milk production by binding to 5-HT2AR and PRLR.

Molecular docking results showed that active compounds from aqueous leaf extract of *P. scutellaria* could bind to the target protein 5-HT2AR. In *P. scutellaria* leaf extract, Kaemferol and quercetin exhibited the lowest binding affinity for the target proteins compared to the other compounds evaluated except Risperidone. A previous study reported that Asp155 and Leu229 are critical residues in the binding pocket of 5-HT2AR.[29] The four factors that strongly influenced the binding of the protein target and ligands are hydrophobic, hydrogen-bond, electronic, and π-π stacking. Further, the hydrophobic residues play significant roles in initiating the active conformation between ligands and protein targets.

Interestingly, our result demonstrated that Leu228 and Val366 are found in all ligands. These two amino acid residues take place in the hydrophobic pocket. Lin *et al.* reported that hydrophobic interaction occurs at ring C of 5-HT2AR with Leu28 and Val366 and plays a role in helping induction on the active conformation of antagonist.[30] Meanwhile, other studies reported that the other amino acid residue, namely, Phe339, was involved in allosteric communication and 5HT2AR activation, producing the second messenger as an intracellular signaling pathway.[31]

Our present work demonstrated that kaempferol, quercetin, and rutin have a lower binding affinity than afzelin and quercitrin, even though Risperidone still has the lowest binding affinity value in PRLR interaction. Interestingly, the bioactive compounds of *P. scutellaria* have the same binding site as Risperidone at Tyr94, Tyr99, Tyr190, Ile100, and Lys11 (Table 3). A previous study had demonstrated that tyrosine phosphorylation by prolactin is required for maximum STAT5 activation.[32] Further, Tyr473, 479, and 580 are seen as essential amino acid residues necessary for activating STAT5 and STAT5-dependent gene transcription. Meanwhile, our study showed that all ligands bond at tyrosine residues, although to different tyrosine sites. Therefore, tyrosine phosphorylation in JAK2 will further activate STAT, including STAT5, to produce a lactogenic response.[33] However, further evaluation is still required on the potential role of Tyr90, Tyr94, and Tyr190 to bind with PRLR. Based on the docking results, we hypothesize that the bioactive compounds from *P. scutellaria* may be involved in PRLR activation, making it possible to respond to induce JAK/STAT signaling pathway and induce a lactogenic response.

Protein interaction and pathway analysis

Breast milk production involves various complex physiological processes, emotional factors, and the interaction of multiple hormones. The primary hormone involved in lactation is prolactin.[28] Serotonin mediates prolactin secretion indirectly through 5-hydroxytryptamine 2A receptors (5-HT2AR).[34] Lactogenesis pathways were investigated to reveal proteins involved in breast milk production using the STRING database. This analysis provided the protein network map in the path and the false discovery rate for each pathway. The proteins participate in two pathways related to breast milk production, lactation, and receptor signaling pathway via JAK-STAT (Figure 3-5). The lactation pathway is closely associated with the signaling pathway via JAK-STAT for mammary gland development and differentiation process.[35]

Under normal conditions, the blood level of prolactin is low. This low blood level is attributed to an inhibitory mechanism by neurons, tuberoinfundibular dopamine (TIDA) (Figure 4). Inhibition of TIDA activity, primarily dopamine D2 receptors (D2R), will remove inhibition in lactotroph cells and the subsequent prolactin release.[36,37] Liang (2000) reported that 5-HT2AR showed an inhibitory effect on TIDA activity, which increased prolactin production in female mice.[36,38] The current study results reported that five compounds chosen from *P. scutellaria* were predicted to increase breast milk production by binding to 5-HT2AR and PRLR. According to analysis from the STITCH database, Risperidone has been reported to bind to D2R, 5-HT2AR, and PRLR.

Table 1: Binding affinity value from Shield aralia active compounds with 5-Hydroxytryptamine 2A receptors (5-HT2AR) and Prolactin receptor (PRLR)

Ligands	Binding Affinity (kcal/mol)	
	5-HT2AR	PRLR
Risperidone (control)	-11.8	-7.6
Afzelin	-7.7	-6.3
Kaemferol	-8.9	-6.7
Quercetin	-8.9	-6.7
Quercitrin	-7.1	-6.2
Rutin	-8.6	-6.7

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Table 2: Amino acid residues from molecular docking of 5-HT2AR

5-HT2AR	
Interactions	**Residues**
Risperidone	
Van der walls	Cys227, Leu362, Asn343, Ile152, Leu229, Ile163, Phe332, Phe234, Thr134, Tyr139, Asn363
Halogen	Ser159
CHB	Thr160, Tyr370
Carbon HB	Ser131, Asp155, Ser242
Pi-Anion	-
Pi-sigma	Val366
Pi-Pi T-shaped	Trp336, Phe340
Pi-Alkyl	Trp151, Leu228, Phe339
Afzelin	
Van der walls	Ser159, Ile152, Val366, Asn363, Ser242, Gly238, Ile210, Phe234, Ser239, Trp336
Halogen	-
CHB	Asp155, Leu229, Asn343, Val235
Carbon HB	Gly288
Pi-Anion	Asp155
Pi-sigma	-
Pi-Pi T-shaped	Phe339
Pi-Alkyl	Val156, Leu228, Leu362, Phe340
Kaempferol	
Van der walls	Trp336, Val366, Trp151, Leu228, Asn343, Phe234, Gly238, Ser242, Thr160, Ser159
Halogen	-
CHB	Cys227, Val235, Ser239
Carbon HB	Val156
Pi-Anion	Asp155
Pi-sigma	Leu229
Pi-Pi T-shaped	Phe339, Phe340
PI-Alkyl	Ile152
Quercetin	
Van der walls	Trp336, Thr160, Ser242, Gly238, Phe234, Leu229, Ile152, Leu228, Trp151, Tyr370
Halogen	-
CHB	Val235, Ser239
Carbon HB	Val156, Ser159
Pi-Anion	Asp155
Pi-sigma	Val156
Pi-Pi T-shaped	Phe339, Phe340
Pi-Alkyl	Val366
Quercitrin	
Van der walls	Trp336, Thr160, Ser242, Ser239, Phe234, Cys227, Leu362, Phe339, Ile152
Halogen	-
CHB	Tyr370, Leu229, Asn343, Val235, Gly238
Carbon HB	-
Pi-Anion	Asp155
Pi-sigma	Leu229
Pi-Pi T-shaped	Trp151, Phe340
Pi-Alkyl	Val366, Val156, Leu228
Rutin	
Van der walls	Ser226, Cys227, Val235, Trp151, Val156, Tyr370, Ser159, Thr160, Ser242, Gly238, Ile210, Trp367, Phe234, Ser239, The134, Tyr139
Halogen	-
CHB	Ser131, Asn343, Ile152
Carbon HB	Asp155
Pi-Anion	-
Pi-sigma	-
Pi-Pi T-shaped	Phe339
Pi-Alkyl	Val366, Leu228, Leu229, Leu362, Phe340

CHB: Conventional Hydrogen Bond; HB: Hydrogen Bond

Table 3: Amino acid residues from molecular docking of PRLR

PRLR	
Interactions	**Residues**
Risperidone	
Van der walls	Thr74, Asp96, Val95, Cys12
Halogen	Gln102
CHB	Tyr94
Carbon HB	-
Pi-Anion	-
Pi-sigma	-
Pi-Pi T-shaped	Tyr99, Tyr190
Pi-Alkyl	Ile100, Arg13, Lys11
Afzelin	
Van der walls	Phe10, Lys11, Ile100, Val95
Halogen	-
CHB	Ile9, Tyr190
Carbon HB	-
Pi-Anion	-
Pi-sigma	-
Pi-Pi T-shaped	Tyr99, Tyr190
Pi-Alkyl	Ile100, Arg12, Lys11
Kaempferol	
Van der walls	Tyr99, Arg13, Phe10, Lys11, Leu 93, Val95, Tyr94
Halogen	-
CHB	Tyr190, Gln102
Pi-sigma	Ile100
Pi-Pi T-shaped	-
Pi-Alkyl	-
Quercetin	
Van der walls	Tyr99, Val95, Leu93, Lys11, Arg13, Gln 102
Halogen	-
CHB	Tyr94, Cys12
Carbon HB	-
Pi-Anion	-
Pi-sigma	Ile100
Pi-Pi T-stacked	Tyr190
Pi-Alkyl	-
Quercitrin	
Van der walls	Arg13, Lys11, Val95, Tyr99
Halogen	-
CHB	Tyr190, Ile9
Carbon HB	-
Pi-Anion	-
Pi-sigma	-
Pi-Pi T-sulfur	Cys12
Pi-Alkyl	Arg13, Ile100
Rutin	
Van der walls	Leu93, Val95, Thr74, Tyr94, Lys11, Gln102
Halogen	-
CHB	Tyr190, Asp96
Carbon HB	-
Pi-Anion	-
Pi-sigma	-
Pi-Pi T-shaped	Tyr99
Pi-Alkyl	Arg12, Ile100

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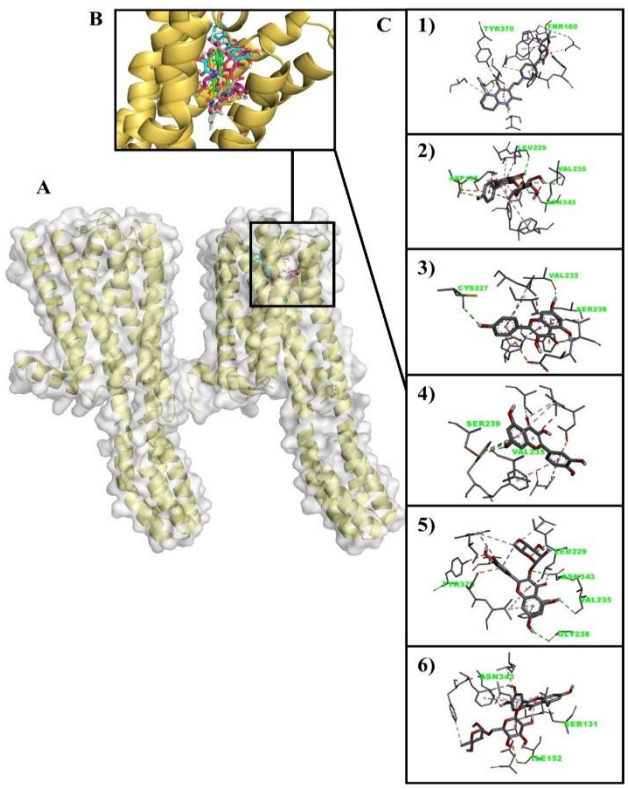


Figure 1: Molecular docking visualization of *P. scutellaria* active compounds and control drug over 5-HT2AR protein.
(A) Three-dimensional structure with black rectangle showed the ligands attached in active site of 5-HT2AR. (B) *P. scutellaria* active compounds at the same site with risperidone as control drug. (C) Amino acid residues with hydrogen-bond interaction between 5-HT2AR and all ligands. (1) risperidone (control); (2) afzelin; (3) kaempferol; (4) quercetin; (5) quercitrin; and (6) rutin.

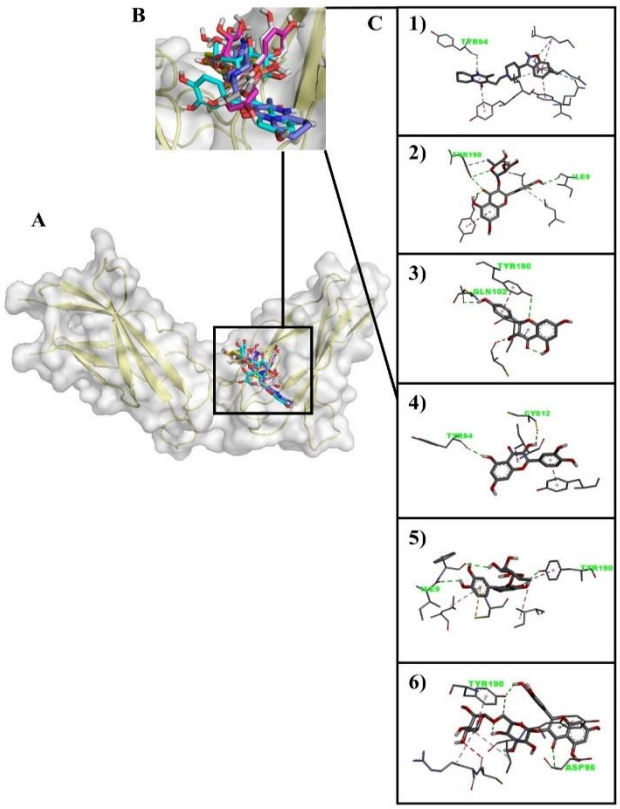
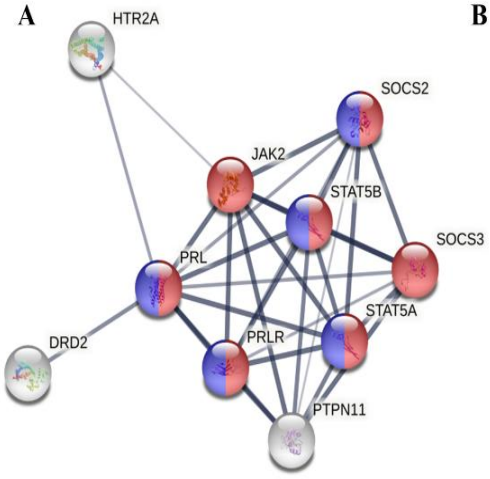


Figure 2: Molecular docking visualization of *P.scutellaria* active compounds and control drug over PRLR protein.
(A) Three-dimensional structure with black rectangle showed the ligands attached in active site of PRLR. (B) *P. scutellaria* active compounds at the same site with risperidone as control drug. (C) Amino acid residues with hydrogen-bond interaction between PRLR and all ligands. (1) risperidone (control); (2) afzelin; (3) kaempferol; (4) quercetin; (5) quercitrin; and (6) rutin.

A



B

Pathway	False Discovery	Genes
Lactation	2.66×10^{-09}	PRL, PRLR, SOCS2, STAT5A, STAT5B
Receptor signaling pathway via JAK-STAT	2.01×10^{-11}	PRL, PRLR, JAK2, STAT5A, STAT5B, SOCS2, SOCS3

Figure 3: Protein-protein interaction. (A) The network of protein interaction by STRING database involved lactation pathways (violet) and growth hormone receptor signaling pathway via JAK-STAT (red). (B) Involved genes in lactation and growth hormone receptor signaling pathway via JAK-STAT following with false discovery rates. The thickness for each line indicated the amount of evidence that has been found.

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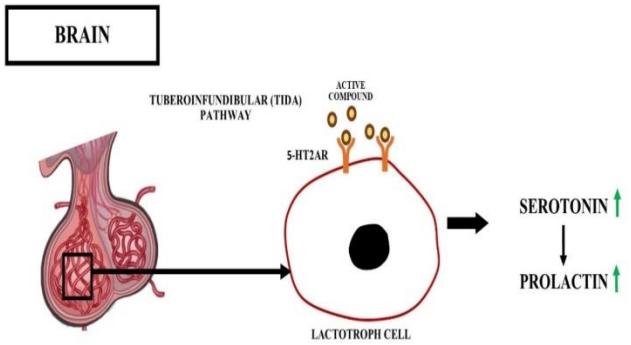

Figure 4: The proposed mechanism of active compounds binds with 5-HT2AR in tuberoinfundibular (TIDA) pathway as an agonist to increase prolactin secretion by lactotroph cell in the anterior pituitary.

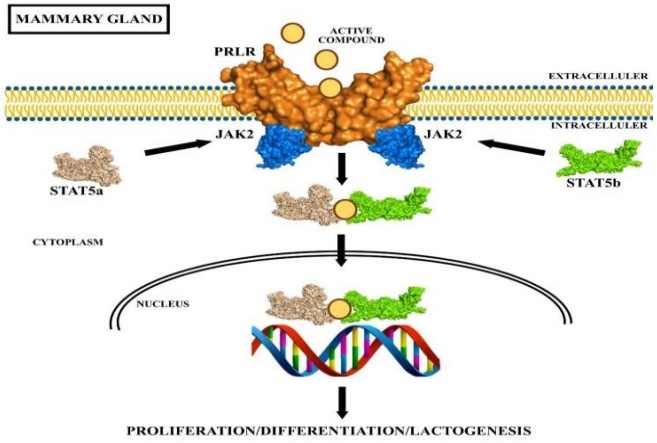

Figure 5: The proposed mechanism of active compounds found in *P. scutellaria* involved in mammary gland development along the lactation stage in breastfeeding mothers. PRLR simultaneously activated the JAK-STAT signaling pathway plays a role in adequate breast milk production. PRLR, prolactin receptor; JAK, Janus kinase; STAT, signal transducer and activator of transcription.

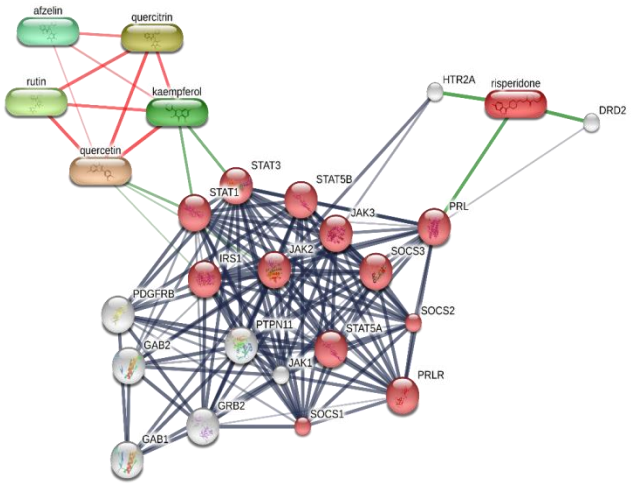

Figure 6: Protein and ligand interaction in JAK-STAT cascade involved in growth hormone signaling pathway via (red circle).
P. scutellaria active compound as the ligands (afzelin, kaempferol, quercetin, quercitrin, and rutin) and risperidone as drug control. Red line indicated a relation between ligands. The green line indicated the interaction between the ligands into the target protein. The grey line indicated the interaction between proteins. The thickness for each line indicated the amount of evidence that has been found for certain interactions.

At the same time, kaempferol and quercetin found in *P. scutellaria* bind to STAT3 and STAT1 within the JAK-STAT cascade involved in the growth hormone signaling pathway (Figure 6). Furthermore, the results also predicted the involvement of prolactin and prolactin receptors in the signaling pathway. To the best of our knowledge, our study is the report of the binding of the active compounds of *P. scutellaria* to either 5-HT2AR or PRLR targets.

Human milk has a dynamic/variable composition that depends on lactation time, changing needs of growing infants, and the foods consumed by the mother.[39] Dietary phytochemicals have been shown to alter the flavonoids content in human milk 12 h after ingestion.[40,41] Interestingly, numerous studies reported that some phytochemicals, such as kaempferol and quercetin, are found in the mother's milk.[40,42] These phytochemicals are known to prevent oxidative stress and intestinal diseases in infants.[43] The utilization of natural products believed to increase milk production has been traditionally used in some societies.[44] In this case, based on our molecular docking and pathway analysis, the bioactive compounds of *P. scutellaria* were identified as promising candidates as plant galactagogues through 5-HT2AR and PRLR signaling pathways. The dietary intake of phytochemicals, such as *P. scutellaria*, may protect infants from oxidative damages and other allied diseases by primarily increasing the mother's milk production during lactation.

Conclusion

The present study suggested that the bioactive compounds from *P. scutellaria* might have potential as a natural candidate involved in 5-HT2AR and PRLR complex. Kaempferol and quercetin were forecasted to elevate prolactin secretion by lactotroph cells via the 5-HT2AR signaling pathway. Meanwhile, kaempferol, quercetin, and rutin were predicted to enhance lactogenesis via JAK/STAT signaling pathway. These bioactive compounds may be expected to regulate proliferation, differentiation, and lactogenesis during breastfeeding. Besides, *P. scutellaria* may become a viable alternative for synthetic drugs to overcome low milk production problems during breastfeeding. Further *in vitro* and *in vivo* experiments should be performed to validate the use of bioactive compounds of *P. scutellaria* as a breast milk booster.

Conflict of Interest

The authors declare no conflict of interest.

Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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