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# Molecular docking analysis of *Carica papaya* leaf's bioactive components as prolactin production modulator

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**ABSTRACT**

Prolactin plays an important role in lactation, particularly in mammary epithelial cell differentiation, milk synthesis, and milk secretion. A sufficient level of prolactin during the lactation phase can support the success of breastfeeding and the baby's growth. Some breastfeeding mothers consume a variety of herbs to enhance their breast milk production, including *Carica papaya* leaves. This study aimed to identify and analyze the potential of active compounds in *C. papaya* leaves for the production of prolactin hormones. Molecular docking was conducted with Swiss target prediction, PASS SERVER, SEA, STITCH, STRING, PyRx, PyMol, and BioVia Discovery Studio 2019. The results showed that the active compounds of *C. papaya*, beta-carotene, have the lowest binding affinity to estrogen (17.4 kcal/mol) and leptin (−6.9 kcal/mol) than other active compounds. Kaempferol (−7.7 kcal/mol) and quercetin (−7.7 kcal/mol) have the lowest binding affinity through aromatase than other active compounds. The *C. papaya* active compounds were predicted to interfere with the prolactin hormone production through estrogen, leptin, and aromatase. All compounds interact either directly or indirectly with these proteins. Therefore, *C. papaya* leaves have the potential to be used as one of the natural resources acting out as galactagogue.

## 1. INTRODUCTION

Breastmilk is a complex biological nutrient that supports babies' growth and development. The success of lactation requires the expansion and differentiation of extensive breast tissues during pregnancy, followed by a sufficient amount of breast milk production post-labor. These two processes require mechanism coordination in nutrition transport, breastmilk production, and mammary gland secretion and are supported by several molecular events regulated by reproduction hormones [1].

The prolactin hormone plays a key role in the mammary glands' growth and development, particularly in mammogenesis, lactogenesis, and galactopoesis [2]. In lactogenesis, prolactin stimulates the absorption of some amino acids, milk and protein synthesis (such as α-lactalbumin and casein), glucose absorption, and lactose and milk fat synthesis [2-4]. Prolactin, together with progesterone and other metabolic hormones like insulin, is needed in the differentiation process of mammary epithelial cells that can synthesize and secrete the specific components of breastmilk [5]. High prolactin level during breast feeding can influence metabolism, to supply glucose and fat for milk production [6].

One of the prolactin's functions during breastfeeding is to provide nutrition in the breast milk's composition [7-10]. Prolactin can enhance arginase's activity and the mammary tissue's polyamine transport rate and stimulate decarboxylase activities. That mechanism can improve polyamine synthesis, which is necessary for milk production. The polyamine stabilizes the membrane structure, improves transcriptional and translational activities, and regulates enzymes [1].

Prolactin also activates the STAT5 pathway, which induces β-casein gene expression and the morphogenesis and differentiation of alveolar cells in the mammary gland [7-9]. Thus, prolactin is the primary

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hormone that controls the function and growth process of the mammary gland [9]. The prolactin gene in the mammary gland is expressed in the mid to late gestation period as the mammary gland develops, then continuously expressed throughout the lactation period, and finally decreases in the early weaning phase [11].

Various factors influenced the success of the lactation process. Internal factors, such as genetic variations, affect breast milk quantity and quality, while external factors consist of nutritional status, partner support, stress levels, and the baby's attachment to the mother [5]. The frequently emerging issues causing the failure in immediate breastfeeding after labor and the lack of breastmilk production are caused by nutritional and non-nutritional factors, including hormonal issues, parity, pregnancy, age, and psychological factors [12-14].

Forinash *et al*. [15] recommend non-pharmacological therapy using herbal galactagogues (plants that increase breast milk production) to boost breast milk production. The most commonly used herbs are fenugreek (*Trigonella foenum-graecum*), milk thistle (*Silybum marianum*), Shatavari (*Asparagus racemosus*), alfalfa (*Medicago sativa*), blessed thistle (*Cnicus benedictus*), goat's rue (*Galega officinalis*), fennel (*Foeniculum vulgare*), and brewer's yeast (*Saccharomyces cereviceae*).

Using galactagogue in diet during lactation can be an alternative [16] to increase breast milk production [17]. Moreover, it can also affect breast milk's nutritional composition [13-16]. In various cultures, the knowledge of herbal galactagogues is passed down from generation to generation. Papaya (*Carica papaya*) leaves have been used by Indonesian society to increase breast milk production. However, the scientific empirical evidence and the mechanism of action have yet to be elucidated. Papaya leaves contain alkaloids (carpain and pseudocarpain), enzymes (papain, chymopapain, cystatin), tocopherols, ascorbic acid, tannins, nicotinic acid, saponins, peonidin, chlorogenic acid, coumarin compounds, phenolic compounds (caffeic acid, p-coumaric acid), and protocatechuic acid as the major phytochemicals and flavonoids [18-24]. The extract of juvenile papaya leaves contains alkaloids, phenols, flavonoids, and several amino acids [25]. According to Miean and Mohamed [26], the flavonoid level in juvenile papaya leaves was 126 mg/100 g, with quercetin and kaempferol as the main compounds.

Flavonoids are widely found in vegetables, fruits, tea, and chocolate; these components act as nutraceuticals [27]. The previous studies reported that galactagogue herbs, such as fenugreek and malunggay (*Moringa oelifera*), are known to contain flavonoids as the main phytochemical [28,29]. Based on the liquid chromatography mass spectroscopy (LCMS)/MS analysis, it is known that papaya leaves also contain flavonoids (unpublished data). An *in vivo* study has shown that papaya leaves can increase plasma prolactin levels in lactating rats [30]. This study aims to explain the molecular mechanism of active compounds from papaya leaves in regulating or influencing the production of the prolactin hormone using a docking technique as a tool for virtual screening and optimizing the combination of active compounds (ligands) with estrogen, leptin, and aromatase as the target proteins.

Estrogen is a hormone produced by the ovaries, soluble in the membrane, and interacts with the intracellular receptors that stimulate the development of the epithelium in the mammary glands. This hormone is needed to produce lactation-competent glands [31]. Leptin is a peptide that regulates food intake, body mass, and reproductive function through a negative feedback mechanism between the adipose tissue and the hypothalamus [32]. Meanwhile, aromatase is an estrogen synthetase [33] that converts androgen into estrogen [34].

## 2. MATERIALS AND METHODS

### 2.1. Bioactive Compounds Selection

*C. papaya* leaf extract was analyzed using the LCMS method. Then, data were compared with the list of bioactive compounds from the KnapSack Kanaya database (http://www.knapsackfamily.com/KNApSAcK/) and *Dr. Duke's Phytochemical and Ethnobotanical* (https://phytochem.nal.usda.gov/phytochem/search) to explore the various aspects of metabolite relationship as well as other detailed information on the metabolites [35]. Furthermore, the list of the bioactive compounds found in the database and LCMS results were compared for further bioactive selection [Supplementary Data].

### 2.2. Sample Preparation

*C. papaya* leaves were dried in the air, extracted with hexane and ethanol, and stored in the freezer at −20°C (this process produces 15.6% ethanol extract). To obtain *C. papaya* leaf juice extract, fresh *C. papaya* leaves were mixed with a juice extractor (Panasonic, Kobe), then filtered and frozen to get lyophilized leaf juice extract and stored in the freezer at −20°C (this process produces 14% ethanol extract).

### 2.3. LCMS Analysis

0.5 g of sample was put into a 10 mL measuring flask, then added methanol for 30 min and homogenized. Then, filter with a filter membrane and inject into the ultra-performance liquid chromatography (LC) system. LC settings were carried out on column C18 with column temperature 40°C and autosampler temperature 15°C, injection volume was 10 mL, with the flow rate at 0.6 mL/min, and mobile Phase A 0.1% formic acid in acetonitrile, mobile Phase B 0.1% formic acid in aquabides [36].

### 2.4. Target Protein Prediction

The target proteins were obtained from SEA Search Server (https://sea.bkslab.org/) and STITCH (http://stitch.embl.de/) database. An analysis using Swiss Target Prediction and SEA Search Server requires each selected compound to predict its target protein separately with a SMILE structure as the keyword. The TC value or Tanimoto Similarity (SEA) of >50% (0.5) was determined as the basis for selecting the target protein for each bioactive compound. Meanwhile, the list of bioactive compounds can be used as the input in STITCH. The additional settings, such as *Rattus norvegicus* in the type of organism and the minimum required interaction score, were set to 0.700.

### 2.5. Pharmacology Network Analysis

The compiled target proteins from two databases were used as the input of the STRING database (https://string-db.org/) for analysis by the multiple protein menu. *Homo sapiens* was selected as the type of organism. The network edges were used as confidence, and the minimum required interaction score was set to 0.700. After updating the network based on the additional settings, the visualization results were downloaded as TSV (tab-separated values). The downloaded file (.tsv) was then imported into Cytoscape v.3.8.2 software for network analysis and biological process prediction involving the target proteins using BINGO on the GOlororize app menu.

### 2.6. Docking Analysis

The 3D structures of the selected target proteins were obtained from the RSCB PDB database (https://www.rcsb.org/), namely, estrogen

receptor 1 (ESR1) (PDB ID: 1ERE), LEP (GDP ID: 3V6O), and CYP19A1 (GDP ID: 5JKW). Meanwhile, the 3D structure of each papaya leaf's active compound (quercetin, kaempferol, linoleic acid, caffeic acid, and beta carotene) and control (estradiol and testosterone) was obtained from the PubChem database (https://www.pubchem.ncbi.nlm.nih.gov). Furthermore, the protein was prepared by removing the water molecules using Discovery Studio 2019 Software, while the energy of the ligand was minimized using the PyRx v.0.9.8 software. The docking was conducted using Autodock Vina integrated into PyRx v.0.9.8 [37]. The docking resulted in binding affinity from interacting with the compounds and proteins [38]. Furthermore, the interaction was visualized using the BioVia Discovery Studio 2019.

Besides BINGO, a PPI network analysis was also conducted. The variable consists of Stress, Degree, Betweenness Centrality, and Closeness Centrality. The degree centrality variable is the number of interactions of one node with other nodes, where a high value indicates that the protein has many interactions with other nodes and could be the main protein or regulatory influencer. The betweenness centrality variable compared the shortest distances used by nodes in the entire pathway and could be interpreted as having a dominant function. Meanwhile, closeness centrality was the shortest relative distance to access nodes in the pathway, where a high value could be interpreted as easy to reach and easy to become the center of other protein regulators.

## 3. RESULTS

### 3.1. Bioactive Compounds Selection

154 *C. papaya* leaf bioactive compounds were obtained from the KnapSack Kanaya, and 31 *C. papaya* leaf bioactive compounds were obtained from Dr. Dukes's Phytochemical and Ethnobotanical databases. Furthermore, these data were compared with the results of LCMS analysis. Five compounds were selected for further analysis [Table 1]. Table 1 shows the results of the analysis based on a comparison of the database and LCMS results for the bioactive components of *C. papaya* leaves. These compounds are classified based on the SMILES code in Pubchem to facilitate the pharmacology network analysis.

### 3.2. Pharmacology Network Analysis

The obtained target proteins from two databases (SEA Server and STITCH) were 177 proteins. STITCH results showed the potential of binding interaction, which is the basis of molecular dynamics [Figure 1]. The results of BINGO analysis from GOlorize showed the value of betweenness centrality [Figure 2] [39]. Each target protein that plays a role in biological processes related to breast milk is shown in Table 2. The targets with the most potential were marked in bold.

Figure 1 showed that the active compound of *C. papaya* leaves could modulate prolactin production through the Jak2 signaling pathway which is mediated by ESR1 and Cyp19a1. The compounds that play the roles are quercetin, kaempferol, and caffeic acid. The pathways that mediated by Lep are linoleic acid and carotene.

Figure 2 shows a cellular process illustrating protein interaction that can modulate prolactin production, a gene that has the strongest interaction is ESR1 and the most potential target proteins in the biological process related to breast milk are shown in Table 3.

### 3.3. Docking Analysis

The protein-compound interaction (PCI) was performed with the arrangement of the *Homo sapiens* organism to adjust as the organism of origin from the protein used as the target protein. The selected compounds as ligands were five bioactive compounds from *C. papaya* leaves that have the potential to interact with estrogen, Leptin, and Aromatase based on PCI prediction [Figure 3] and the results of network interaction analysis using Cytoscape. The control ligands, estradiol for ESR1 and testosterone for Aromatase, are also predicted for the PCI [Figure 1] and were obtained from the previous studies [40,41]. Figure 3 indicates that in the human body, quercetin has a strong interaction with Cyp19a1 to modulate prolactin production through the STAT5A signaling pathway and is mediated by ESR1. Meanwhile, linoleic acid modulate prolactin production through the STAT5B signaling pathway and mediated by Lep.

**Table 1:** Bioactive component.

| Bioactive component | Pubchem ID | Smiles structure |
|---|---|---|
| Caffeic acid | 689043 | C1=CC(=C(C=C1C=CC(=O)O)O)O |
| Beta carotene | 5280489 | CC1=C(C(CCC1)(C)C)<br>C=CC(=CC=CC(=CC=CC=C(C)<br>C=CC=C(C)C=CC2=C(CCCC2(C)C)C)C)C |
| Linoleic acid | 5280450 | CCCCCC=CCC=CCCCCCCCC(=O)O |
| Kaempferol | 5280863 | C1=CC(=CC=C1C2=C(C(=O)<br>C3=C(C=C(C=C3O2)O)O)O)O |
| Quercetin | 5280343 | C1=CC(=C(C=C1C2=C(C(=O)<br>C3=C(C=C(C=C3O2)O)O)O)O)O |

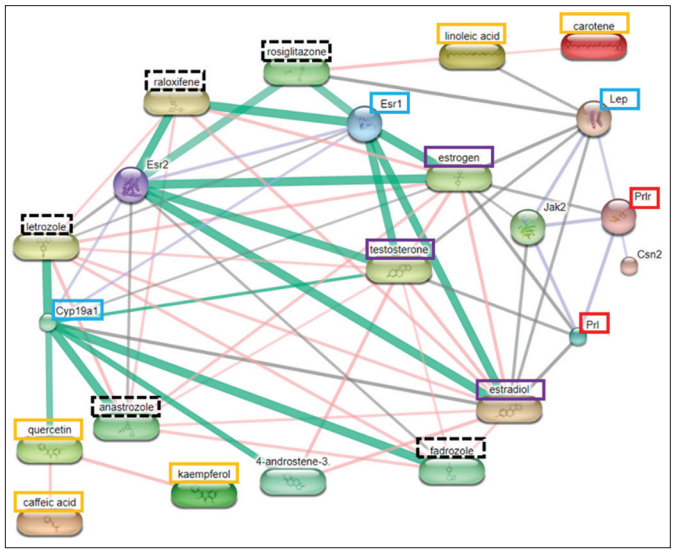


**Figure 1:** Protein-compound interaction analysis result using STITCH (*Rattus norvegicus*). The orange box shows the most potent papaya compound; the purple square indicates the essential endogenous compound; the red box shows the protein acting directly on the prolactin biological process; the blue box shows the most potential target protein; the black dotted box indicates the control compound in the form of a drug.

### 3.4. ESR1 (ESR α)

The molecular docking results of the ESR1 protein with bioactive compounds from *C. papaya* leaves showed that two flavonoid compounds had the lowest binding affinity compared to other bioactive compounds. However, the binding affinity value for the quercetin-ESR1 complex and the kaempferol-ESR1 complex was higher than the control, which was −7.6 kcal/mol compared to estradiol [Table 4]. In addition to these two compounds, fatty acid compounds have a binding affinity lower than

**Table 2:** The biological process from the network analysis

| GO-ID | Description | *P*-value | Corr *P*-value | Cluster freq | Total freq | Genes |
|---|---|---|---|---|---|---|
| 7259 | JAK-STAT cascade | 2.02E-04 | 1.11E-03 | 3/72 4.1% | 23/14291 0.1% | **LEP** PRL JAK2 |
| 7631 | Feeding behavior | 5.49E-03 | 1.54E-02 | 3/72 4.1% | 71/14291 0.4% | APP **LEP** PRLH |
| 22612 | Gland Morphogenesis | 2.95E-03 | 9.80E-03 | 3/72 4.1% | 57/14291 0.3% | MMP2 **ESR1** IGF1R |
| 30879 | Mammary gland development | 5.69E-05 | 3.98E-04 | 5/72 6.9% | 82/14291 0.5% | PRL MET XDH **ESR1** IGF1R |
| 32355 | Response to estradiol stimulus | 3.21E-10 | 9.44E-09 | 10/72 13.8% | 117/14291 0.8% | MMP2 MMP3 CYP1A2 RARA PRL PIK3R1 PTGS2 MMP9 **ESR1** PTK2 |
| 42698 | Ovulation Cycle | 6.03E-05 | 4.17E-04 | 5/72 6.9% | 83/14291 0.5% | MMP2 **LEP** PRL **ESR1** EGFR |
| 42981 | Regulation of apoptosis | 4.11E-13 | 2.96E-11 | 23/72 31.9% | 723/14291 5.0% | APP GSK3B MMP2 ALOX15 ALOX12 BRAF PIK3R1 PTGS2 **ESR1** EGFR PTK2 ESR2 NR4A1 RXRA AXL KDR AKT1 RARB NOX4 PPARG JAK2 PPARD SNCA |
| 43627 | Response to estrogen stimulus | 2.71E-09 | 6.74E-08 | 11/72 15.2% | 191/14291 1.3% | MMP2 MMP3 CYP1A2 RARA PPARG PRL PIK3R1 PTGS2 MMP9 **ESR1** PTK2 |
| 46425 | Regulation of JAK-STAT cascade | 1.34E-02 | 3.01E-02 | 2/72 2.7% | 35/14291 0.2% | PRL JAK2 |
| 46545 | Development of primary female sexual characteristics | 3.78E-04 | 1.85E-03 | 4/72 5.5% | 68/14291 0.4% | RBP4 MMP2 **LEP** **ESR1** |
| 48732 | Gland Development | 1.34E-07 | 2.22E-06 | 9/72 12.5% | 165/14291 1.1% | RXRA MMP2 RARA PRL MET **CYP19A1** XDH **ESR1** IGF1R |
| 60745 | Mammary gland branching in pregnancy | 2.00E-02 | 3.87E-02 | 1/72 1.3% | 4/14291 0.0% | **ESR1** |

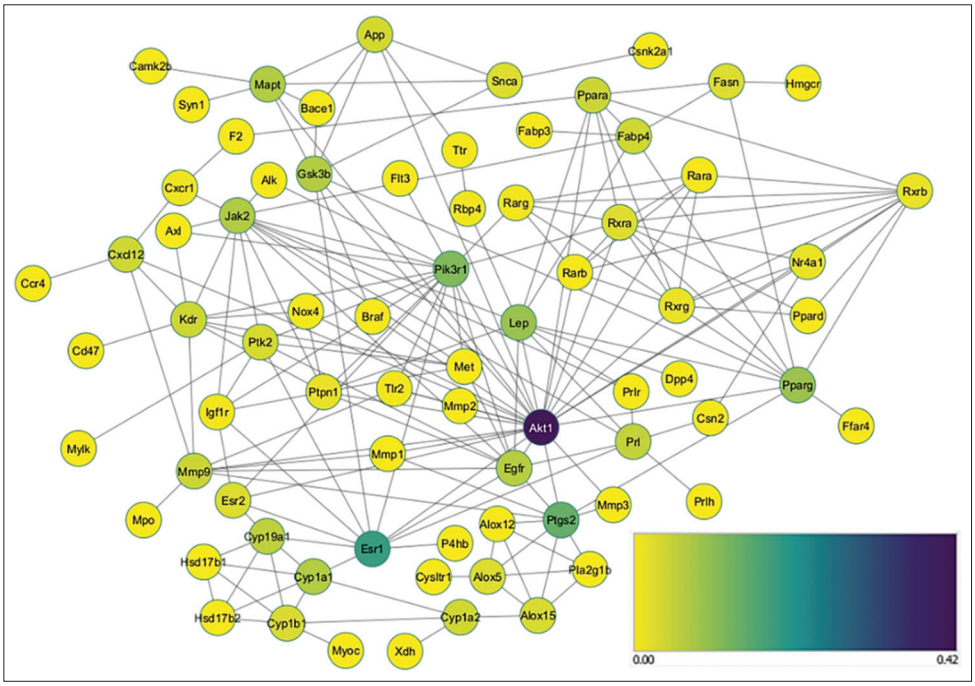


**Figure 2:** Protein-protein interaction result of BINGO analysis from GOlorize.

−7 kcal/mol. These results indicate that the bond formed between the quercetin, kaempferol, and linoleic acid complex to ESR1 is relatively stable, although they do not have a stronger bond than control.

Figure 4 showed the 3D visualization of the ESR1 protein complex with control ligands and the three most potent compounds. The figure also showed a surface hydrophobicity map. Surface hydrophobicity is displayed based on the color of the protein surface, with brown indicating

**Table 3:** Selected target protein after network analysis using Cytoscape.

| Gene | Protein | Betweenness Centrality | Closeness Centrality | Degree | Stress |
|---|---|---|---|---|---|
| Esr1 | Estrogen Receptor 1 | 0.185737484 | 0.450617284 | 11 | 3582 |
| Lep | Leptin | 0.091525895 | 0.424418605 | 11 | 1638 |
| Jak2 | Tyrosine-protein kinase JAK2 | 0.069734321 | 0.39673913 | 14 | 1214 |
| Egfr | Epidermal growth factor receptor | 0.064367392 | 0.45625 | 11 | 1304 |
| Cyp19a1 | Aromatase | 0.05599585 | 0.330316742 | 6 | 1518 |
| Prl | Prolactin | 0.051988266 | 0.382198953 | 7 | 832 |
| Esr2 | Estrogen receptor beta | 0.027615266 | 0.412429379 | 5 | 730 |

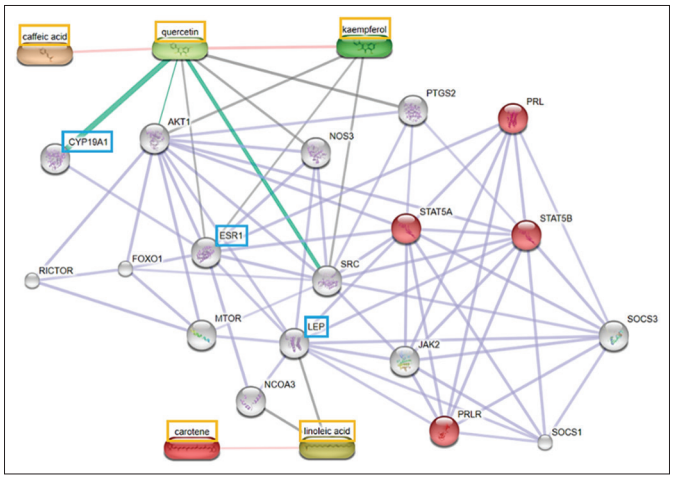


**Figure 3:** Protein compound interaction analysis using STITCH (*Homo sapiens*). The orange box shows the compound with the most potential; the red circle indicates the protein acting on the biological lactation process; the blue box shows the most potential target protein.

hydrophobic, blue indicating hydrophilic, and white indicating the intermediate between those properties. The more hydrophobic the interaction, the more stable the complex conformation will be.

### 3.5. Leptin (Lep)

The molecular docking of leptin with the selected compounds showed that three compounds from the flavonoid group had the lowest binding affinity values compared to others. In addition, the binding affinity values for the carotene-Lep (−6.9 kcal/mol) and quercetin-Lep (−6.7 kcal/mol) were lower compared to the control (−6.6 kcal/mol). At the same time, kaempferol-Lep complexes had equal binding affinity values with the control [Table 5]. However, the compound has a binding affinity value >−7 kcal/mol. This shows that the bond formed between carotene, quercetin, and kaempferol with Leptin is less stable even though it has a similar or stronger affinity than control. These results indicate that the network pathway is less precise in predicting the interaction of papaya compounds with leptin, especially for linoleic acid.

Figure 5 shows the 3D visualization of the leptin protein complex with control ligands and the three most potent compounds. The figure

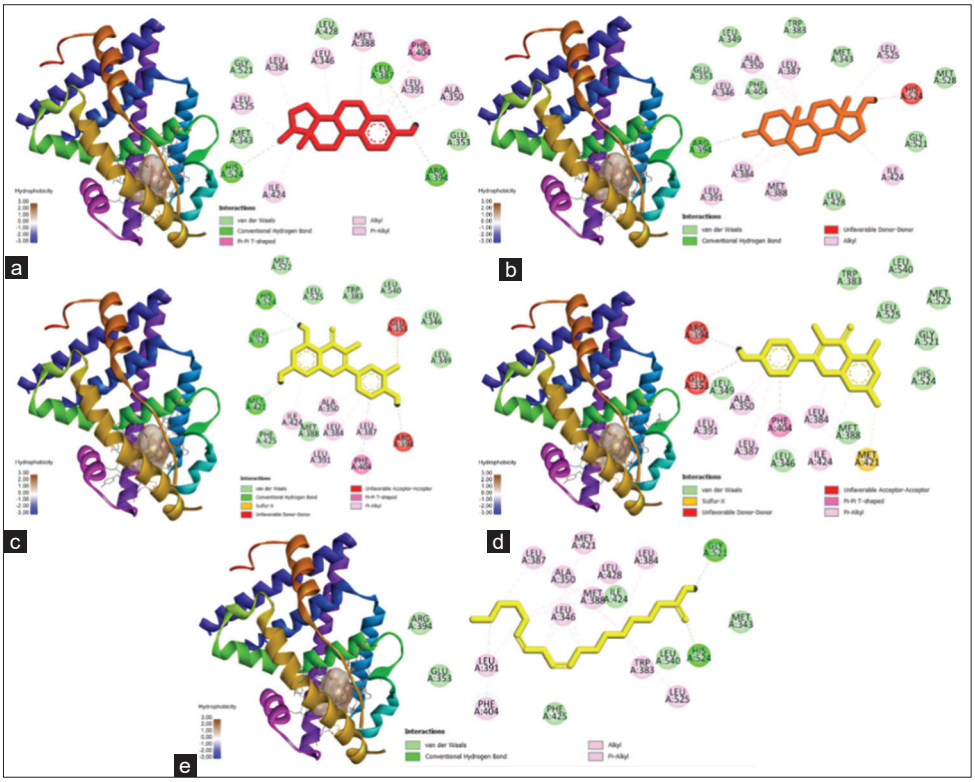


**Figure 4:** Docking results visualization of (a) ESR1-estradiol protein, (b) ESR1-testosterone protein, (c) ESR1-quercetin protein, and (d) ESR1-linoleic acid protein. The left image shows a 3D visualization, and the right shows the type of bonding produced between the ligand-proteins, (e) Protein ESR1-linoleic acid.

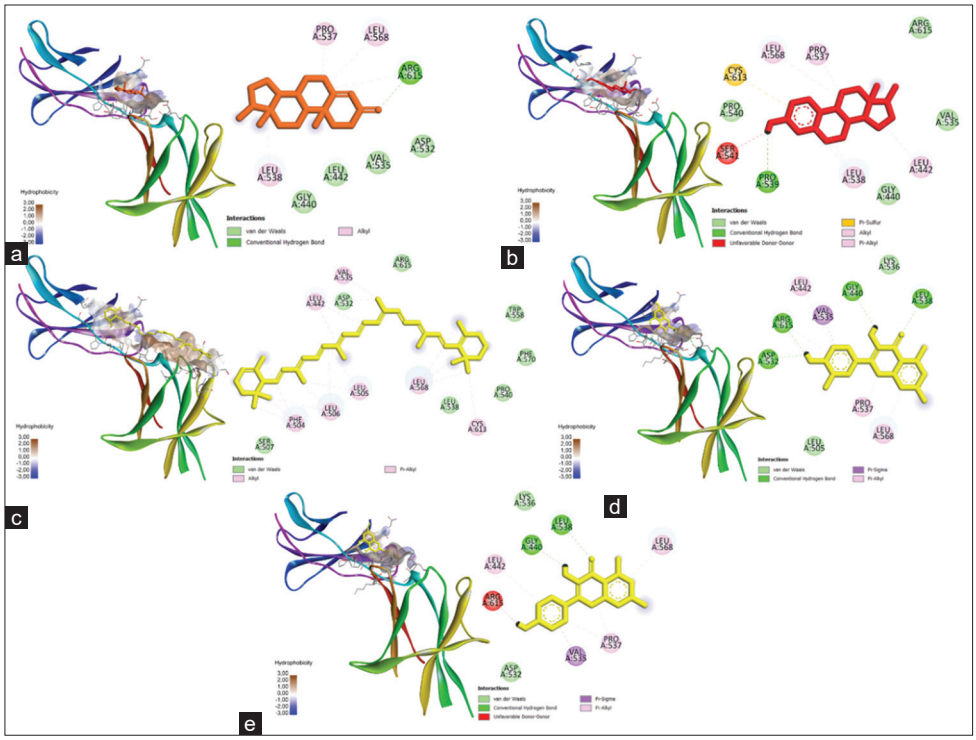

**Figure 5:** Docking results visualization of (a) Lep-testosterone protein, (b) Lep-estradiol protein, (c) Lep-carotene, (d) Lep-quercetin, and (e) Lep-kaempferol protein. The left image shows a 3D visualization, while the right one shows the type of bonding produced between the ligand-proteins.

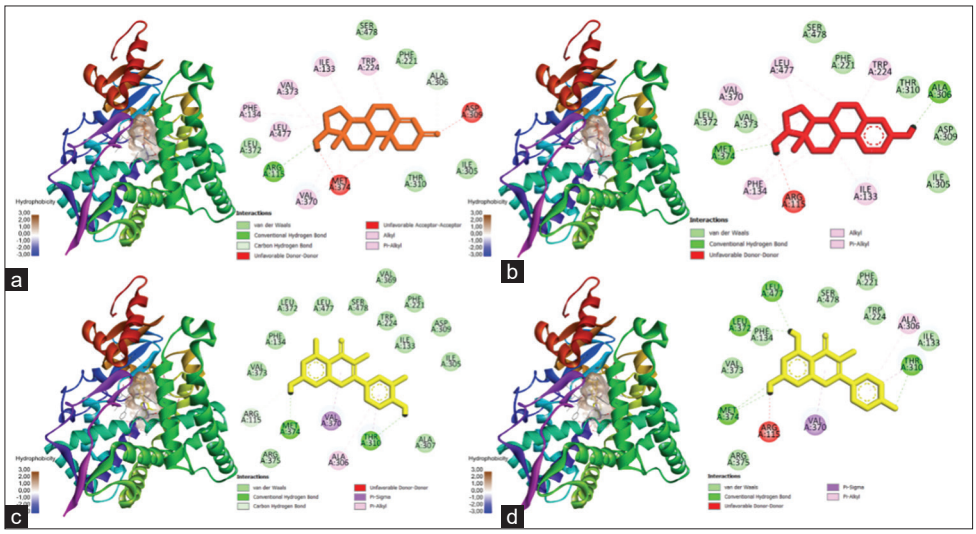

**Figure 6:** Docking results visualization of (a) CYP19A1-testosterone protein, (b) CYP19A1-estradiol protein, (c) CYP19A1-quercetin protein, and (d) CYP19A1-kaempferol protein. The left image shows a 3D visualization, and the right one shows the type of bonding produced between the ligand-proteins.

**Table 4:** The binding affinity between ESR1 protein and bioactive compounds of *Carica papaya* leaves.

| Molecule | ID Ligand | Compound | Binding affinity (kcal/mol) |
|---|---|---|---|
| Control | 5757 | Estradiol | −10.7 |
| | 6013 | Testosterone | −9.6 |
| Sample | 5280343 | Quercetin | −7.6 |
| | 5280863 | Kaempferol | −7.6 |
| | 5280450 | Linoleic acid | −7.1 |
| | 689043 | Caffeic acid | −6.6 |
| | 6419725 | Carotene | 17.4 |

**Table 5:** The binding affinity between Leptin protein and the bioactive compounds of *Carica papaya* leaves.

| Molecule | Ligand ID | Compound | Binding affinity (kcal/mol) |
|---|---|---|---|
| Control | 6013 | Testosterone | −6.7 |
| | 5757 | Estradiol | −6.6 |
| Sample | 6419725 | Carotene | −6.9 |
| | 5280343 | Quercetin | −6.7 |
| | 5280863 | Kaempferol | −6.6 |
| | 5280450 | Linoleic acid | −5.5 |
| | 689043 | Caffeic acid | −4.3 |

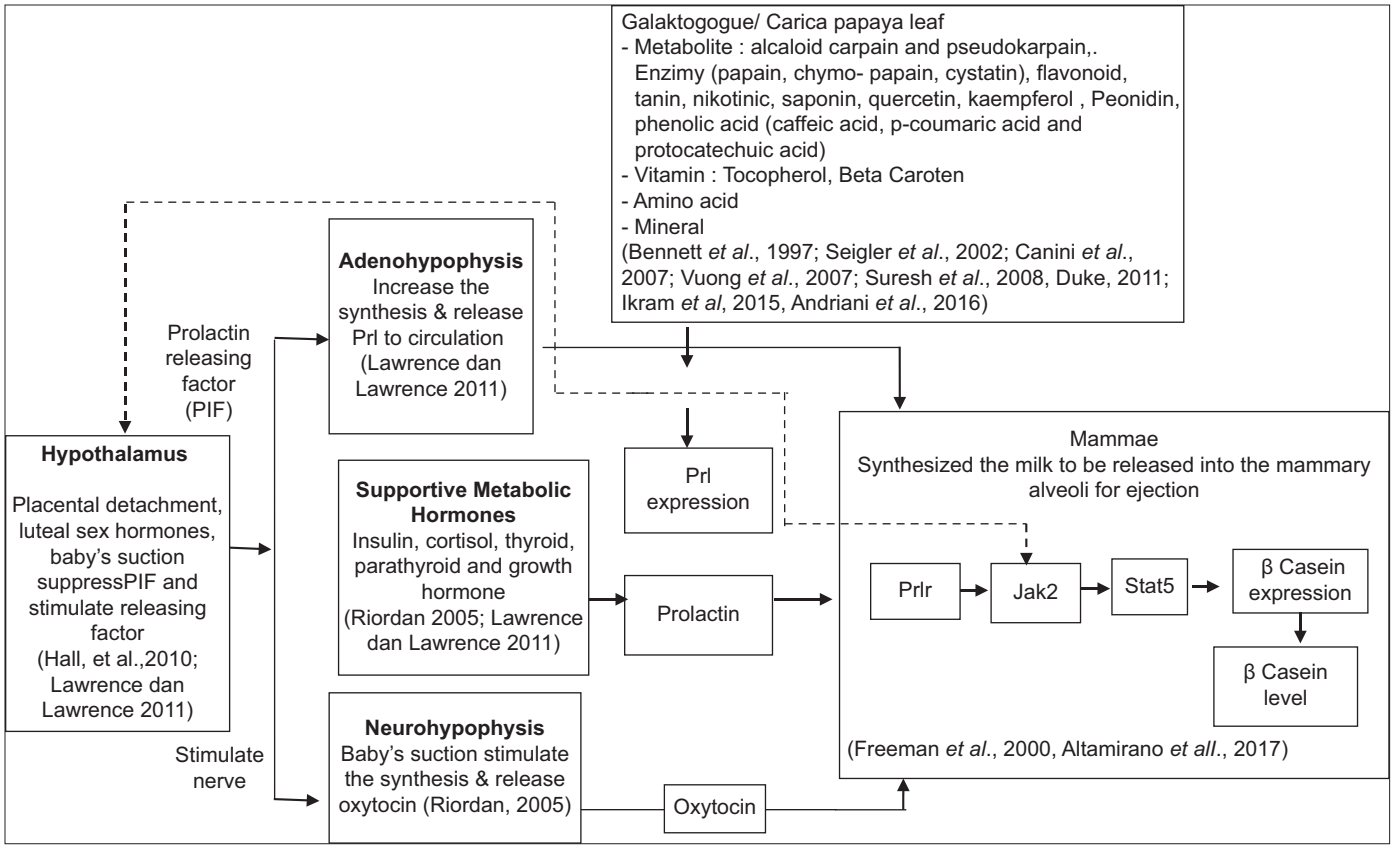


**Figure 7:** Scheme of Prolactin pathway stimulated by phytochemical of *Carica papaya* leaf extract.

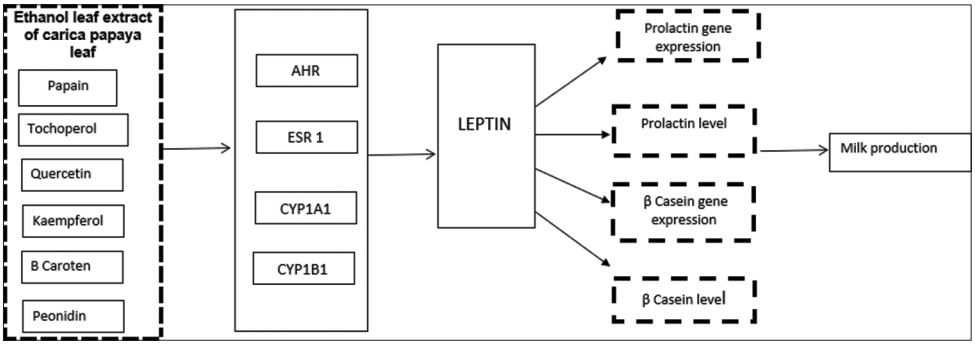


**Figure 8:** Milk production pathway stimulated by phytochemical of *Carica papaya* leaf extract.

also shows a surface hydrophobicity map. The more hydrophobic interaction, the more stable the conformation complex will be.

### 3.6. Aromatase (Cyp19a1)

The molecular docking of aromatase protein with bioactive compounds from *C. papaya* leaves showed that two compounds from the flavonoid group had the lowest binding affinity compared to other bioactive compounds. However, the binding affinity of the quercetin-CYP19A1 complex and the kaempferol-CYP19A1 complex was higher than the control [Table 6]. In addition, the two compounds also have a lower than −7 kcal/mol binding affinity value. This result indicates that the bond formed between the complex quercetin and kaempferol to aromatase is relatively stable. However, it has no potential to have a stronger bond than the control. This result also proves that the network pathway prediction is very accurate.

**Table 6:** The binding affinity between CYP19A1 protein and bioactive compounds of *Carica papaya* leaves.

| Molecule | Ligand ID | Compound | Binding affinity (kcal/mol) |
|---|---|---|---|
| Control | 6013 | Testosterone | −10.1 |
| | 5757 | Estradiol | −8.3 |
| Sample | 5280343 | Quercetin | −7.7 |
| | 5280863 | Kaempferol | −7.7 |
| | 689043 | Caffeic acid | −6.1 |
| | 5280450 | Linoleic acid | −5.6 |
| | 6419725 | Carotene | 122.4 |

Figure 6 shows the results of 3D visualization of the CYP19A1 protein complex with control ligands and the two most potent compounds.

## 4. DISCUSSION

PCI analysis using STITCH [Figure 1] showed that the most potent target proteins associated with prolactin production are Estrogen, Leptin, and Aromatase (CYP19A1), which is in agreement with previous studies related to prolactin metabolism and lactation [42-44]. The compound that has the most potential effect in this process is quercetin. Estrogen protein (ESR1) is involved in the prolactin metabolism (PRL) pathway, where both estrogen and JAK2 are required for the activation of STAT5A and STAT5B, which will induce ESR activation, especially ESR1 [45]. The scheme of the prolactin pathway is shown in Figure 7.

The leptin protein is known to have a high expression in the mammary gland during the lactation period, and it is found to be in insufficient quantity in the virgin mammary gland [42]. Meanwhile, aromatase protein was found to have the highest expression at the 148–149$^{th}$ days of gestation due to the aromatization process of androgens as the initial process of the estrogen formation in the mammary gland as a preparation for further pathways involving PRL, STAT5A, and STAT5B [46].

The result of the network analysis showed that the interaction between the active compounds of *C. papaya* leaves and ESR1 is involved in biological processes, including the branching of the mammary glands during pregnancy, differentiation, and morphogenesis of the mammary glands, estradiol response, and producing phenotypic trait [31,47]. There are two intracellular receptors for estrogen, alpha (α) and beta (β), encoded by the ESR1 and ESR2 genes, respectively. Furthermore, the interaction between the active compounds of *C. papaya* leaves and Leptin is involved in the JAK-STAT activation pathway. In contrast, the interaction with aromatase is involved in the mammary glands' development [Table 2]. Hence, the target protein in this study is estrogen, leptin, and aromatase [Table 3].

One of the important things in determining the strength of interaction between the active compound and the target protein is a binding affinity value that showed the association of the compound and target receptor, so it can be calculated how the effectiveness of the interaction between these molecules [38]. Docking analysis showed that the bioactive compounds of *C. papaya* with the potential to have the most stable bond with ESR1 are quercetin, kaempferol, and linoleic acid which the binding affinity values were −7,6; −7,6, and −7,1, respectively [Table 4]. Bai *et al*. [48] showed that quercetin strongly interacts with ESR1 and kaempferol [49]. A study by Morselli *et al*. [50] proved that fatty acids act as potential mediators in the ESR signaling activation pathway and are commonly used as anti-cancer compounds [50,51]. Another study related to prolactin metabolism has shown that PRL can stimulate the expression of the ESR gene in the corpus luteum and decidua [45].

The docking analysis between the active compound of *C. papaya* leaf and leptin showed that beta carotene, quercetin, and kaempferol have the potential for interaction, which is noted by the binding affinity values were −6,9; −6,7; and −6,6, respectively [Table 5]. Leptin is encoded by the LEP gene, consisting of 167 amino acids, and plays an important role in maintaining energy homeostasis [52]. A study conducted by Getrude *et al*. [53] showed that a diet with a high beta-carotene content could affect leptin levels. Canas *et al*. [3] reported that carotenoid combination supplementation could increase beta carotene levels in the plasma, associated with BMI decrease, thereby preventing obesity.

Likewise, quercetin, which can suppress microglia-mediated inflammatory responses through HO-1 induction, can prevent obesity-induced hypothalamic inflammation [49]. Another study stated that quercetin administration could inhibit the leptin gene's secretion and expression in cancer cells [54].

Meanwhile, kaempferol is an alternative therapy to prevent obesity and insulin resistance in rats with a high-fat diet [48]. Muni Swamy *et al.* [55] showed that kaempferol compounds isolated from *M. oleifera* could affect leptin gene expression by inducing lipolysis and suppressing adipogenesis.

Based on the docking analysis, the components of *C. papaya* leaf that can form stable bonds with aromatase are quercetin and kaempferol, with the binding affinity value of −7,7 of both compounds. Aromatase 450, encoded by the CYP19 gene, catalyzes the formation of multiple estrogens in several tissues under the control of specific promoters regulated by different signaling pathways [56]. Sanderson *et al*. [57] showed that a natural flavonoid, quercetin, can enhance aromatase activity up to 4 times, associated with the intracellular cAMP increase. Quercetin can inhibit the production of VEGF and change the redox status to affect the angiogenic process [58]. Due to its ability to influence the expression of the CYP19A1 gene, quercetin can be used as a safe chemotherapy agent for breast cancer [59].

Based on the docking analysis, the bioactive components of *C. papaya* leaves that have the most potential to increase prolactin production are flavonoid compounds, specifically quercetin, and kaempferol. These compounds play a role in the mechanism of estrogen inhibition by influencing the expression of the ESR1 and CYP19A1 genes. Flavonoids are compounds that play a role in various biological reactions [27] and have antioxidant, anti-inflammatory, anti-mutagenicity, and anti-carcinogenic properties, coupled with their capacity to modulate the function of intracellular enzymes [27,60]. Thus, many studies showed the role of flavonoids as an alternative therapy for breast cancer [61,62]. However, the studies that specifically relate to the role of flavonoids in breast milk, especially how they play a role in prolactin production, are still limited. Further testing is needed both *in vivo* and *in vitro*.

This study found that a potential pathway for papaya leaf extract to prolactin production is through ESR1. Estrogen and prolactin stimulate alveoli differentiation into specialized structures for synthesizing and secreting milk during lactation. The lack of ESR1 in the alveoli will inhibit ductal elongation, disrupting lobulo alveolar development and resulting in insufficient milk production [31]. The milk production pathway is shown in Figure 8.

The potential pathway of papaya leaf extract through signals captured by ESR1 through Leptin is expressed into the PRL gene, then expressed into the prolactin and captured by PRLR. Leptin is a potent stimulator of PRL release, and its actions are mediated through ERK1/2 stimulation. As a highly potent PRL stimulant, leptin induces an initial increase in PRL release during the first 6 h, followed by an increase in two lower levels over 1–4 and 4–16 h [63].

Cytoscape visualization results show that the five active compounds from papaya leaves can interact with the ESR1, ESR2, ERBB, and EGFR genes to activate various cellular and molecular signaling pathways, activating leptin transcription. This presence can further modulate pituitary hormone secretion, including prolactin [64].

Leptin is also a critical compound that can modulate various molecular signals, such as the JAK/STAT, PI3K, and MAPK pathways, so it can trigger the transcription of several genes or exert different hormonal effects in the other parts of the cell [65]. Münzberg and Morrison [52] stated that leptin levels and circulation constantly fluctuate depending on the nutrients and the quality of those nutrients.

## 5. CONCLUSION

The abundant phytochemical components in *C. papaya* leaves are alkaloids and flavonoids. Alkaloids are widely used as nutraceuticals to manage oxidative stress and prevent inflammation, while flavonoids are known to affect metabolism through hydroxylation, methylation, and acylation reactions. We found that the flavonoid compounds from *C. papaya* leaves, namely, quercetin, and kaempferol, have the potential as multi-activators, especially for the Estrogen protein, Leptin, and Aromatase, which are involved in the prolactin pathway and the lactation process. However, the compounds from the fatty acid group, namely, linoleic acid, are predicted to have a reasonably stable interaction in binding to estrogen. Thus, it shows that *C. papaya* leaf has the potential to be used as a galactagogue herb to increase breast milk production through increasing prolactin levels.

## 6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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## 8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

## 9. ETHICAL APPROVALS

The study protocol was approved by the Research Ethics Commission of Universitas Padjadjaran Bandung (No. 1340/UN6.KEP/EC/2019).

## 10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

## 11. PUBLISHER'S NOTE

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