THE POTENTIAL EFFECT OF APORPHINE ALKALOIDS FROM NELUMBO NUCIFERA GAERTN. AS ANTI-BREAST CANCER BASED ON NETWORK PHARMACOLOGY AND MOLECULAR DOCKING

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ABSTRACT

Objective: To demonstrate the efficacy and benefits of aporphine alkaloids from Nelumbo nucifera Gaertn, as anti-breast cancer agents.

Methods: In this study, a combination of network pharmacology and molecular docking was used to investigate the pharmacological actions and underlying mechanisms of action of nuciferine, noruciferine, and roemerine against breast cancer.

Results: Fifty-five potential targets of compounds against breast cancer were identified. The Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB), and Protein Kinase C Epsilon (PRKCE) were identified as the top five targets of compounds against breast cancer. Molecular docking demonstrated that these compounds could bind spontaneously to the screened top 4 targeted proteins.

Conclusion: The present study demonstrates that these compounds have pharmacological effects against breast cancer via a multi-target and multi-pathway manner.

Keywords: Nuciferine, Nor nuciferine, Roemerine, Breast cancer, Network pharmacology, Molecular docking

INTRODUCTION

Breast cancer is a prevalent form of malignancy among women worldwide and has emerged as a primary cause of mortality [1]. Breast cancer constitutes 24.2% of the total 8.6 million newly diagnosed cases among females and accounts for 15.0% of all 4.2 million cancer-related mortalities [2]. Over the past few decades, there has been a noticeable increase in the incidence of breast cancer in both developed and developing nations [3]. This trend is particularly evident in younger age groups [4]. Breast cancer has emerged as a significant health concern in Indonesia, surpassing other forms of cancer, with a prevalence of 16.6% [5]. This poses a serious threat to the well-being and survival of women in the country [6].

Currently, contemporary medical practice predominantly employs surgical interventions, radiotherapy, chemotherapy, and hormone therapy as primary modalities for addressing breast cancer in patients [7]. Notably, the treatment objectives for breast cancer patients include preserving quality of life and extending life expectancy [8]. However, it is pertinent to mention that breast surgery is not typically considered a conventional treatment modality in most cases [9]. While chemotherapy has demonstrated efficacy in short-term treatment, prolonged treatment is often associated with unfavorable reactions and drug resistance, which can have a detrimental impact on patients’ quality of life and physical and mental well-being [10]. Therefore, scholars are enthusiastic about discovering viable alternative treatments [11].

Recently, there has been a growing focus on utilizing traditional medicine as a means of treating cancer [12]. This is primarily due to its noteworthy clinical effectiveness, minimal adverse effects, and ability to enhance overall quality of life [13]. Traditional Indonesian Medicine known as an anticancer agent is Seroja or Nelumbo nucifera Gaertn. Seroja, a member of the Nymphaeaceae family, is an aquatic plant that possesses medicinal properties in nearly all its constituent parts [14]. Many studies have reported the anticancer activity of various parts of Seroja in anti-pancreatic cancer, anti-liver cancer, anti-cervix cancer, anti-colon cancer, and anti-breast cancer [15, 16]. This activity was demonstrated by the secondary metabolites of Seroja. The main compound found in all parts of Seroja is an aporphine alkaloid [17]. Pharmacological studies have shown that aporphine alkaloids possess diverse activities, such as metabolic regulation, antioxidation, anti-proliferation, anti-vascularization, apoptosis induction, and cell cycle arrest inhibition [15, 18]. The cellular targets of this phenomenon remain unknown.

The present investigation utilized network pharmacology analysis to anticipate the potential therapeutic targets and signaling pathways of aporphine alkaloids for the treatment of breast cancer. Hopkins established network pharmacology in 2007, which has been demonstrated as a potent approach for investigating the intricate and comprehensive activation mechanisms of natural products [19]. This text depicts the complex interplay between genes, proteins, and metabolites in diseases and drugs. This is achieved by integrating various multidisciplinary concepts such as biochemistry, bioinformatics, and systematic biology [20]. These concepts align with the multifaceted characteristics of natural products [21]. The integration of network pharmacological prediction and molecular docking verification has emerged as a crucial approach to elucidate the primary molecular targets and underlying mechanisms of action of herbal medicines before conducting in vitro and in vivo experiments [22]. The aim of this study was to elucidate the molecular mechanisms underlying the anti-breast cancer activity of aporphine alkaloids from Seroja using network pharmacology and molecular docking techniques.

MATERIALS AND METHODS

Compound database

The identification of aporphine alkaloid compounds derived from Seroja was accomplished through a comprehensive review of the relevant literature. Three aporphine alkaloid compounds, nuciferine,
nor-nuciferine, and roemerine, were acquired. The database was queried using the simplified molecular input line entry (SMILE) notation of the compounds sourced from the PubChem database, namely "nuciferine, nor-nuciferine, and roemerine," as the search terms.

**Pharmacokinetic prediction**

The molecular weight, log P, H-bond acceptor, H-bond donors, rotatable bond, % intestinal absorption, Blood-Brain Barrier (BBB), toxicity, hepatotoxicity, and maximum tolerated dose (mg/kg/day) pharmacokinetic parameters of nuciferine, nor-nuciferine, and roemerine were acquired using the pkCSM tools, which can be accessed via https://biosig.unimelb.edu.au/pkcsm/[23].

**Compound-target identification**

The canonical SMILE of the compounds was uploaded to the Swiss Target Prediction database (http://www.swisstargetprediction.ch/)[24]. The compound-target network was visualized using Cytoscape [3.9.0][25].

**Breast cancer target identification**

Breast cancer targets were retrieved by searching the GeneCards database (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/) were integrated using VENNY (https://bioinfogp.cnb.csic.es/tools/venny_old/index.html), and the intersecting targets were presented in a Venn diagram [26].

**Construction of protein-PPI network**

The Protein–protein interaction (PPI) network was constructed by uploading the genes to the STRING v_11.0 database (https://string-db.org/) [27]. The settings for building the PPI network were established in accordance with the "Homo sapiens" model, and the confidence level of the interaction between the targets was set at 0.9 [26]. The network nodes represent proteins, and the edges reflect the protein-protein interactions. For core target protein identification, the Cytoscape data were sorted using these parameters, including degree, closeness centrality, betweenness centrality, and clustering coefficient [26].

**Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis**

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were analyzed using the ShinyGO database (http://bioinformatics.sdstate.edu/go/) with "homo sapiens" as a species with a False Discovery Rate (FDR) cutoff of 0.05 [29, 30].

**Molecular docking**

The possible interactions between the compounds nuciferine, nor-nuciferine, and roemerine with the targets, including Epidermal Growth Factor Receptor (EGFR, PDB ID 5hg8), Mitogen-Activated Protein Kinase 8 (MAPK8, PDB ID 3elj), Janus Kinase 2 (JAK2, PDB ID 5aep), and Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB, PDB ID 4kik) were modelled. Each protein was retrieved from the RCSB Protein Data Bank (https://www.rcsb.org/pages/policies). The interaction between each compound and its target was predicted using PyRx Virtual Screening Tools (version 0.8). All interactions between the target proteins and compounds were constructed using PyMOL 2.5 in the PDBQT format file. Finally, 2-dimensional visualization of the interaction between compounds and target proteins was investigated using Discovery Studio Visualizer 2021 [31, 32].
RESULTS

Pharmacokinetic parameters of compounds

The pharmacokinetic parameters of nuciferine, nornuciferine, and roemerine were determined using the pkCSM tools. This investigation was used to screen for interactions of the compounds with the human body. Ten parameters were used, as listed in table 1. These results indicate that nuciferine, nornuciferine, and roemerine are suitable compounds for consumption.

Identification of drug targets in breast cancer

The experimental procedure is illustrated in fig. 1. Targets in breast cancer were searched in the GeneCards Human database and DisGeNet using "breast cancer" as keywords. After removing duplicates among these databases, 18467 breast cancer targets were identified based on the relevance score (fig. 2). The targets of each aporphine alkaloid were obtained from Swiss Target Prediction. The identified predicted targets included 100 targets of nuciferine, nornuciferine, and roemerine. The 55 intersecting targets of aporphine alkaloids and breast cancer are shown in a Venn diagram (fig. 2) and listed in table 1. Furthermore, a compound-target network plot was constructed using Cytoscape 3.9.0 (fig. 3).

Analysis of target protein-protein interaction (PPI) network

The 55 intersecting targets were imported into the STRING database, and the potential relationships among them were investigated. A PPI network with a confidence score of 0.9 was constructed (fig. 4). STRING database analysis revealed that the average node degree, defined as the average number of interactions of a protein in a network, was 3.93, and the local clustering coefficient, defined as the wellness of the connected nodes in a network, was 0.651. The interactions between the targets comprised 30 nodes and 59 edges, with each edge representing an association between nodes. Cytoscape analysis revealed one main cluster associated with the PPI network (fig. 4). According to the scores of degrees, closeness centrality, betweenness centrality, and clustering centrality, signal transducer and activator of Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB), and Protein Kinase C Epsilon (PRKCE) were identified as the top five intersecting targets of aporphine alkaloid and breast cancer interaction (fig. 4).
### Table 1: Pharmacokinetic parameters of nuciferine, normuciferine, and roemerine

<table>
<thead>
<tr>
<th>No</th>
<th>Parameters</th>
<th>Compounds</th>
<th>Nuciferine</th>
<th>Normuciferine</th>
<th>Roemerine</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Molecular weight</td>
<td></td>
<td>295.382</td>
<td>281.355</td>
<td>279.339</td>
</tr>
<tr>
<td>2</td>
<td>Log P</td>
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<td>3.1674</td>
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<tr>
<td>3</td>
<td>H-bond acceptor</td>
<td></td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>H-bond donors</td>
<td></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Rotatable bond</td>
<td></td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>% Intestinal absorption</td>
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<td>96.604</td>
<td>92.767</td>
<td>96.771</td>
</tr>
<tr>
<td>7</td>
<td>BBB (log BB)</td>
<td></td>
<td>0.418</td>
<td>0.743</td>
<td>0.321</td>
</tr>
<tr>
<td>8</td>
<td>Ames toxicity</td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>Hepatotoxicity</td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>Max tolerated dose (mg/kg/day)</td>
<td></td>
<td>-0.23</td>
<td>-0.575</td>
<td>-0.456</td>
</tr>
</tbody>
</table>

Fig. 5: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The biological processes (A), cellular component (B), molecular function (C), and KEGG terms (D) distributed in the ordinate and the degree of enrichment was analyzed. The size of the dots represents the gene count. The blue-to-red represents the low-to-high value of the enrichment.

**Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis**

GO enrichment analysis was conducted to analyze the impact of the targets in breast cancer. The classification of GO was based on three criteria: biological processes, GO molecular function, and GO subcellular localization (fig. 5). First, with the candidate target and KEGG pathway analysis were interpreted using the ShinyGO database. The top seven biological processes of aporphine alkaloids were identified by false discovery rate (FDR) and sorted by fold enrichment, including positive regulation of mucus secretion, positive regulation of peptide hormone secretion, peptidyl-serine phosphorylation, positive regulation of cellular protein localization, peptidyl-serine modification, Mitogen-Activated Protein Kinase (MAPK) cascade, and peptidyl-amino acid modification (fig. 5). Molecular function was mainly enriched in protein serine/threonine kinase activity, protein kinase activity, protein kinase activity, phosphotransferase activity, alcohol group as acceptor, kinase activity, transferase activity transferring phosphorus-containing groups, protein kinase binding, adenosine 5’-triphosphate (ATP) binding, adeny1 ribonucleotide binding, and adeny1 nucleotide binding (fig. 5). Finally, subcellular localization includes multivesicular body internal vesicles, basal dendrites, Cluster of Differentiation 40 (CD40) receptor complex, I kappaB kinase complex, membrane raft, membrane microdomain, receptor complex, focal adhesion, cell-substrate junction, and synapses (fig. 5). Furthermore, the KEGG pathway analysis suggested that the predicted targets were components of the pathways involved in oncogenesis, particularly in the Programmed Death Ligand 1 (PD-L1) expression and Programmed Death 1 (PD-1) checkpoint pathway in cancer, Forkhead Box O (FoxO) signaling pathway, microRNAs in cancer, Ras signaling pathways, and pathways in cancer (fig. 5).

**Compounds-target interaction analysis by molecular docking**

Among the 55 targets of nuciferine, normuciferine, and roemerine in breast cancer, four potential targets, including EGFR, MAPK8, JAK2, and IKBKB, were investigated for possible interactions with nuciferine, normuciferine, and roemerine. The aporphine alkaloids were molecularly docked using PyRx Virtual Screening Tool, and their interactions with the highest affinity for each target are presented in fig. 6, and table 2. The major binding interactions included hydrogen bonding and van der Waals interactions. The binding energy score during docking indicates the affinity of a component for the target protein. Here, all the binding energy scores analyzed were less than 0, suggesting high-affinity interactions among the targets and the aporphine alkaloids (nuciferine, normuciferine, and roemerine) [33, 34].

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Fig. 5: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. The biological processes (A), cellular component (B), molecular function (C), and KEGG terms (D) distributed in the ordinate and the degree of enrichment were analyzed. The size of the dots represents the gene count. The blue-to-red represents the low-to-high value of the enrichment.
DISCUSSION

The utilization of network pharmacology-based evaluations facilitates comprehensive investigations of the interactions between potential drug candidates and diverse network factors [35]. The integration of multidisciplinary concepts from biological systems and polypharmacological models has led to the emergence of potent tools for drug target identification [36]. In addition to identifying novel drugs and their corresponding targets, this tool facilitates the investigation of potential target regions and repurposing of established drugs for a variety of ailments [37]. The present study delineates the molecular mechanism underlying the interaction between nuciferine, nornuciferine, and roemerine, which are aporphine alkaloids derived from Seroja. A previous study demonstrated that aporphine alkaloids, namely nuciferine, nornuciferine, and roemerine, exhibit antiproliferative effects in AGS and DU-145 cells [15]. Nuciferine inhibited the growth of MDA-MB-231 and MCF-7 cells in the context of breast cancer by inducing apoptosis and inhibiting proliferation through cell cycle arrest [38]. However, the molecular mechanisms underlying this effect remain unknown. Elucidating the molecular mechanisms of aporphine alkaloids can enhance their anti-breast cancer activity. This study identified potential targets of compounds that overlap with breast cancer targets, such as Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), and Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB). Based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, these targets are important for the advancement of breast cancer [39-42].

EGFR expression has been detected in a range of 14-91% of individuals diagnosed with breast cancer and has been associated with an unfavorable prognosis [43]. Activation of EGFR triggers the activation of various pathways, such as Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), and Inhibitor of Nuclear Factor Kappa B Kinase Subunit Beta (IKBKB). Based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses, these targets are important for the advancement of breast cancer [39-42].

EGFR expression has been detected in a range of 14-91% of individuals diagnosed with breast cancer and has been associated with an unfavorable prognosis [43]. Activation of EGFR triggers the activation of various pathways, such as RAS/RAF/ERK, PI3K/AKT, and STAT3 [44]. These pathways have been found to play a crucial role in regulating the growth and progression of cancer [45, 46]. The MAPK8 pathway is crucial in breast cancer and is similar to the EGFR protein [47]. MAPK8, also called c-Jun NH-2 terminal kinase-1 (JNK1), belongs to the MAPK family [48]. The pathway above has been documented to be active in the process of apoptosis in cases of breast cancer [49]. JAK2 is a member of the MAPK family.
and has been detected in breast cancer [50]. Proteins involved in the JAK-STAT pathway have been shown to undergo modifications in breast cancer. These alterations can occur through various mechanisms, such as downregulation of phosphoryrosine-specific phosphatases, elevation of the JAK/STAT activating ligand IL-6, activation of other upstream oncogenic pathways such as ERβ1 or PI3K/mTOR, and downregulation of STAT3 negative regulators such as suppressor of cytokine signaling 3 [51-53]. The most recent protein identified as a target of nuciferine, nor-nuciferine, and roemarine was IKKβ. IKKβ has been identified as a constituent of NF-κB signaling, and its heightened activity has been observed in breast cancer [54]. In certain instances, upregulation of IKKβ has been observed to result in a reduction in the responsiveness of cancer cells to chemotherapy [55].

The present study employed molecular docking analysis to investigate potential targets for their interactions with nuciferine, nor-nuciferine, and roemarine. The results of our study support our initial hypothesis, as the compounds in question exhibited strong binding affinity for the designated targets. It is probable that these compounds effectively hindered the functions of EGFR, MAPK8, JAK2, and IKKβ, which are known to promote tumor growth [56-58]. Furthermore, there is a dearth of information pertaining to aporphine alkaloids, namely, nuciferine, nor-nuciferine, and roemarine, in various databases. Thus, the present findings represent an inaugural report on this subject. Further clarification is required regarding the interactions between drugs and their respective targets, and it is imperative to validate the pharmacokinetic profile, safety, and efficacy of drugs. This study has provided scientific insights into this compound, indicating its potential for use in anti-cancer drug research and development for clinical purposes.

CONCLUSION

The present study described the potential activity of aporphine alkaloids from Seroja as anti-breast cancer agents through network pharmacology and molecular docking tests, which illustrated that these compounds are suitable for development as oral drugs because the parameters were correct, including toxicity tests. Taken together, using a combination of network pharmacology and molecular docking, we demonstrated that nuciferine, nor-nuciferine, and roemarine as aporphine alkaloids can suppress breast cancer via modulation of Epidermal Growth Factor Receptor (EGFR), Mitogen-Activated Protein Kinase 8 (MAPK8), Janus Kinase 2 (JAK2), and IKKβ, which are known to promote tumor growth [56-58]. Furthermore, there is a dearth of information pertaining to aporphine alkaloids, namely, nuciferine, nor-nuciferine, and roemarine, in various databases. Thus, the present findings represent an inaugural report on this subject. Further clarification is required regarding the interactions between drugs and their respective targets, and it is imperative to validate the pharmacokinetic profile, safety, and efficacy of drugs. This study has provided scientific insights into this compound, indicating its potential for use in anti-cancer drug research and development for clinical purposes.

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AUTHORS CONTRIBUTIONS

Muhammad Fauzan Lubis: Conceptualization, Supervision, Methodology, Data curation, Validation, Formal analysis, Investigation, Funding acquisition, Writing–original draft. Adrian: Data curation, Validation, Formal analysis, Writing–original draft. Roni Abdi Syahputra: Data curation, Validation, Formal analysis, Writing–original draft. Ririn Astyk: Resources, Data curation, Conceptualization, Methodology, Formal analysis, Writing–original draft. Sumaiyah Sumajah: Project administration, Software, Formal analysis, Writing–original draft. Muhammad Andika Yuval Harahan: Data curation, Validation, Formal analysis, Writing–original draft. Zaharatul Aini: Project administration, Software, Formal analysis, Writing--original draft.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

REFERENCES


25. El有人说，网络药学和分子对接可以发现药物的潜在靶点。


