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Original Article

Anti-inflammatory and antioxidant potential of *Syzygium polyanthum* (Wight) Walp. bioactive compounds in polycystic ovary syndrome: An *in silico* study

[Potencial anti-inflamatorio y antioxidante de compuestos bioactivos de *Syzygium polyanthum* (Wight) Walp. en el síndrome de ovario poliquístico: Un estudio *in silico*]

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Abstract

Context: Polycystic ovary syndrome (PCOS) is significantly associated with inflammation and oxidative stress. *Syzygium polyanthum* is a plant rich in pharmacological properties. *Aims*: To evaluate the anti-inflammation and antioxidant potential of *S. polyanthum* bioactive compounds using *in silico* approach.

Methods: The *S. polyanthum* was extracted using the ultrasound-assisted extraction (UAE) method, and the bioactive compounds were screened using Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis. This study predicted the biological activity of *S. polyanthum* compounds using PASS Online server. Before docking, we analyzed the protein-protein interactions (PPIs) network of TNFα, NF-κB, SOD, and KEAP1. The molecular docking was done using Autodock Vina in PyRx software and visualized using Discovery Studio. Probability to be active (Pa) was determined.

Results: The bioactive compounds found in *S. polyanthum* and used in this study were deoxyphomalone, NCGC00169066-01, and phloretin with retention times [min] of 0.886, 0.907, and 8.323, respectively. The predicted biological activity of compounds and controls were anti-inflammatory, immunosuppressant, TNF expression inhibitor, immunomodulatory and HIF1α expression inhibitor (Pa>0.5 for all *S. polyanthum* compounds and Pa<0.5 for SPD304, MG-132, and MDF). Based on PPIs network analysis, TNFα, NF-κB, SOD, and KEAP1 are associated. The molecular docking analysis showed that deoxyphomalone, NCGC00169066-01, and phloretin had inhibition potential against TNFα and NF-κB, and activation potential against SOD, due to several residues involved in the interaction of compounds-protein was the same as the interaction of inhibitor (SPD-304 and MG-132) and activator (gallic acid) control against the protein. The residues may have the same inhibition or activation mechanism as the control. However, *S. polyanthum* bioactive compounds may still have inhibition potential against KEAP1 through Ala548 residue that is also involved in the interaction of DMF-KEAP1.

Conclusions: The bioactive compounds of *S. polyanthum* showed anti-inflammation and antioxidant potential, which may have a good effect in the treatment of PCOS, yet still need to be confirmed *in vitro* or *in vivo* research.

Keywords: antioxidant; inflammation; molecular docking; polycystic ovary syndrome; *Syzygium polyanthum*.

Resumen

Contexto: El síndrome de ovario poliquístico (SOP) está significativamente asociado con la inflamación y el estrés oxidativo. *Syzygium polyanthum* es una planta rica en propiedades farmacológicas. *Objetivos*: Evaluar el potencial anti-inflamatorio y antioxidante de los compuestos bioactivos de *S. polyanthum* utilizando un enfoque *in silico*.

Métodos: S. polyanthum se extrajo mediante el método de extracción asistida por ultrasonido (UAE), y los compuestos bioactivos se seleccionaron mediante análisis de cromatografía líquida-espectrometría de masas de alta resolución (LC-HRMS). Este estudio predijo la actividad biológica de los compuestos de *S. polyanthum* utilizando el servidor PASS Online. Antes del acoplamiento, analizamos la red de interacciones proteína-proteína (PPI) de TNFα, NF-κB, SOD y KEAP1. El acoplamiento molecular se realizó con Autodock Vina en el software PyRx y se visualizó con Discovery Studio. Se determinó la probabilidad de estar activo (Pa).

Resultados: Los compuestos bioactivos encontrados en *S. polyanthum* y utilizados en este estudio fueron desoxifomalona, NCGC00169066-01 y floretina con tiempos de retención [min] de 0,886; 0,907 y 8,323, respectivamente. La actividad biológica predicha de los compuestos y controles fue anti-inflamatoria, inmunosupresora, inhibidora de la expresión de TNF, inmunomoduladora e inhibidora de la expresión de HIF1α (Pa>0,5 para todos los compuestos de *S. polyanthum* y Pa<0,5 para SPD304, MG-132 y MDF). Según el análisis de red de PPI, se asocian TNFα, NF-κB, SOD y KEAP1. El análisis de acoplamiento molecular mostró que la desoxifomalona, NCGC00169066-01 y la floretina tenían potencial de inhibición contra TNFα y NF-κB, y potencial de activación contra SOD, debido a que varios residuos involucrados en la interacción de compuestos-proteína eran los mismos que la interacción del inhibidor (SPD-304 y MG-132) y activador (ácido gálico) controlan contra la proteína. Los residuos pueden tener el mismo mecanismo de inhibición o activación que el control. Sin embargo, los compuestos bioactivos de *S. polyanthum* aún pueden tener un potencial de inhibición contra KEAP1 a través del residuo Ala548 que también está involucrado en la interacción de DMF-KEAP1.

Conclusiones: Los compuestos bioactivos de *S. polyanthum* mostraron potencial anti-inflamatorio y antioxidante, lo que puede tener un buen efecto en el tratamiento del SOP, pero aún debe confirmarse en investigaciones *in vitro* o *in vivo*.

Palabras Clave: acoplamiento molecular; antioxidante; inflamación; síndrome de ovario poliquistico; *Syzygium polyanthum*.

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INTRODUCTION

The most common endocrine disorder found in fertile age of women is polycystic ovary syndrome (PCOS), with a prevalence of 6-20% (Witchel et al., 2019). The development of PCOS occurs when the number of androgens, testosterone, and androstenedione are excessively produced by the ovary (Sever et al., 2019). PCOS is found to be characterized by the occurrence of chronic anovulation, biochemical and clinical hyperandrogenism, and the morphology of polycystic ovarian (Rudnicka et al., 2021). There are four diagnostic criteria for PCOS: classic PCOS, essential NIH Criteria, ovulatory PCOS, and non-hyperandrogenic PCOS (Rosenfield and Ehrmann, 2016).

This syndrome's pathophysiology is complex, involving the interaction of genetic and epigenetic alteration, aberrations of ovarian primer, changes in neuroendocrine and endocrine, and metabolic modifiers (Ibáñez et al., 2017). Besides, inflammation is also reported as the hallmark and consequence of this syndrome. The endocrine process regulates immunity and inflammatory response, production, and secretion of pro-inflammatory cytokines (Tosatti et al., 2020). Inflammation, as well as oxidative stress, is reported to be associated with PCOS pathogenesis. The enhancement of reactive oxygen species (ROS) production is caused by peripheral blood leukocytes, pro-inflammatory transcription factor nuclear *kappa* B (NF-κB), and the increase of pro-inflammatory cytokines and C-reactive protein (Victor et al., 2016). Studies reported that the high level of ROS becomes pathogenesis inducement potential in PCOS due to significant oxidative stress circulating markers that are mostly found in PCOS patients compared to normal (Mohammadi, 2019).

Based on the urgency of inflammation and stress oxidative mechanism in PCOS, anti-inflammatory and the antioxidant agent show significant interest as a strategy for PCOS treatment. The inhibition of these two from herbal medicine shows a promising effect with minimum side effects. Furthermore, several bioactive compounds contained in the herb were found to have a curative effect on PCOS, such as flavonoids and polyphenols (Rani et al., 2022). In addition, Amini et al. (2015) reported that antioxidants and vitamins positively affect the management of women with PCOS.

Syzygium polyanthum (Wight) Walp. (family *Myrtaceae*) also known as bay leaves, one medicinal plant found in Indonesia. *S. polyanthum* is rich in pharmacological potentials, such as anti-cholesterol (Hartanti et al., 2019), anti-tumor, anti-diabetic, anti-microbial, anti-cancer, antioxidant, and anti-inflammatory activities (Ismail and Ahmad, 2019). The anti-inflammatory and antioxidant mechanisms of *S. polyanthum* bioactive compounds remain unclear. So, this study aims to evaluate the anti-inflammatory and antioxidant potential of *S. polyanthum* bioactive compounds through *in silico* studies.

MATERIAL AND METHODS

Plant material

The plant of *S. polyanthum* was obtained from UPT. Balai Materia Medika, East Java, Indonesia (7°52'01.2"S and 112°31'13.2"E). UPT Balai Materia Medika deposited the taxonomic identification with *a* determination number of 074/629/102.7-A/2021. The plant part used in this study was *S. polyanthum* leaf, which was then air-dried at room temperature and powdered.

Plant extraction

The extraction of *S. polyanthum* leaf bioactive compounds was done using the ultrasound-assisted extraction (UAE) method using SONICA Ultrasonic Cleaner, model SONICA® 2400EP S3 (Soltec Soluzioni Technologiche, Italy). First, the leaf powder of *S. polyanthum* was soaked in 96% ethanol (1:10, m:v), which was then extracted using UAE for 30 m at room temperature and stirred for every 10 m. The mixture was then filtered using filter paper and evaporated using a rotary evaporator (50°C, 70 rpm). The result of evaporation was then heated at 40°C in the oven until dry. The extract was then stored in a 4°C refrigerator until being used.

Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis

The bioactive compounds of *S. polyanthum* leaf extract were screened using LC-HRMS. First, the sample was prepared by diluting the extract according to the solvent with a final volume of 1500 µL. Next, the sample was mixed using vortex at 2000 rpm for 2 m and spindown at 6000 rpm for 2 m. The supernatant was taken and filtered using a syringe filter of 0.22 µm and placed into a vial. The sample was then inserted into the auto-sampler and ready to be injected into the LC-HRMS.

The LC-HRMS instrument was Thermo Scientific Dionex Ultimate 3000 RSLCnano with a microflow meter. The analytical column was Hypersil GOLD aQ 50 × 1 mm × 1.9 u particle size, and the analytical rate was 40 µL/min. The flow gradient was 30 m of run times and 30°C of column oven. The High-Resolution

Table 1. Ligands details.

No	Name	PubChem CID	Molecular formula	SMILES
	Compound			
1	Deoxyphomalone	26470515	$C_{13}H_{18}O_4$	CCCC(=O)C1=C(C=C(C(=C1O)CC)O)OC
2	NCGC00169066-01	10065950	$C_{16}H_{22}O_8$	CC1CC(=O)OC(C(C=CC(=O)OC(C(C=CC(=O)O1)O)C)O)C
3	Phloretin	4788	$C_{15}H_{14}O_5$	C1=CC(=CC=C1CCC(=O)C2=C(C=C(C=C2O)O)O)O
	Control			
1	SPD-304	5327044	$C_{32}H_{32}F_3N_3O_2$	CC1=CC2=C(C=C1C)OC=C(C2=O)CN(C)CCN(C)CC3=CN(C4=CC=CC=C43)C5=CC=CC(=C5)C(F)(F)F
2	MG-132	462382	$C_{26}H_{41}N_3O_5$	CC(C)CC(C=O)NC(=O)C(CC(C)C)NC(=O)C(CC(C)C)NC(=O)OCC1=CC=CC=C1
3	Gallic acid	370	$C_7H_6O_5$	C1=C(C=C(C(=C1O)O)O)O)C(=O)O
4	Dimethylformamide	12215	$C_6H_8O_4$	COC(=O)C=CC(=O)OC

Mass Spectrometer was Thermo Scientific Q Exactive with a full scan at 70,000 resolutions, data-dependent MS2 at 17,500 resolution, 30 m of run times, and positive and or negative polarity. The processing data software was Compound Discoverer with mzCloud MS/MS Library.

Data retrieval

The bioactive compounds of *S. polyanthum* leaf extract used in this study were deoxyphomalone, NCGC00169066-01, and phloretin. The controls used in this study for molecular docking analysis were SPD-304 (TNFα inhibitor), MG-132 (NF-κB inhibitor), gallic acid/GA (SOD activator), and dimethylfumarate/DMF (Nrf2 activator). The SMILES and 3D structure of the ligands (compounds and controls) were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/) (Table 1). Besides, the protein target (TNFα, NF-κB, SOD, and KEAP1) for molecular docking analysis were retrieved from RSCB Protein Data Bank (https://www.rcsb.org/) with ID of 2AZ5, 3DO7, 1CB4, and 4ZY3, respectively.

Biological activity analysis

The prediction of the biological activity of the compounds was made using a webserver of PASS Online (http://way2drug.com/passonline/index.php). This server provided over 4000 kinds of biological activity and was used to predict and screen the potential of the bioactive compounds in drug discovery. The compound's SMILES or mol.file was inserted to predict the biological activities, and the probability to be active (Pa) cut-off value was set for Pa > 0.5. The higher the Pa value, the more accurate the prediction results (Nafisah et al., 2021).

Protein-protein interactions (PPIs) analysis

The analysis of protein-protein interactions was done through STRING webserver (https://string-db.org/). This analysis was done to evaluate the interaction of each protein. The medium confidence (0.400) with no more than five interactions was set in this analysis, where the thickness of the line indicated the strength of data support.

Molecular docking analysis

The preparation of protein and ligand was done before docking. The protein was prepared using Discovery Studio Software to delete the water molecule and unnecessary ligand. In addition, the ligands were prepared by minimizing the energy using open babel at PyRx Software. The molecular docking was specific, using AutoDock Vina at PyRx and visualized using Discovery Studio Software. The binding affinities, interactions, and residues of protein-ligand interaction were obtained in this analysis.

RESULTS

Liquid Chromatography–High Resolution Mass Spectrometry (LC-HRMS) analysis

Based on LC-HRMS analysis, we found at least three bioactive compounds in *S. polyanthum* extract, which was then used for further analysis in this study. The bioactive compounds were deoxyphomalone, NCGC00169066-01, and phloretin. Deoxyphomalone IUPAC name is 1-(3-ethyl-2,4-dihydroxy-6-methoxyphenyl)butan-1-one. Deoxyphomalone is an aromatic ketone with a molecular formula of $C_{13}H_{18}O_4$ (Table 1). The retention time (RT) of this compound was 0.886, and m/z as $[M + H - H_2O]^{+1}$ was 239.129

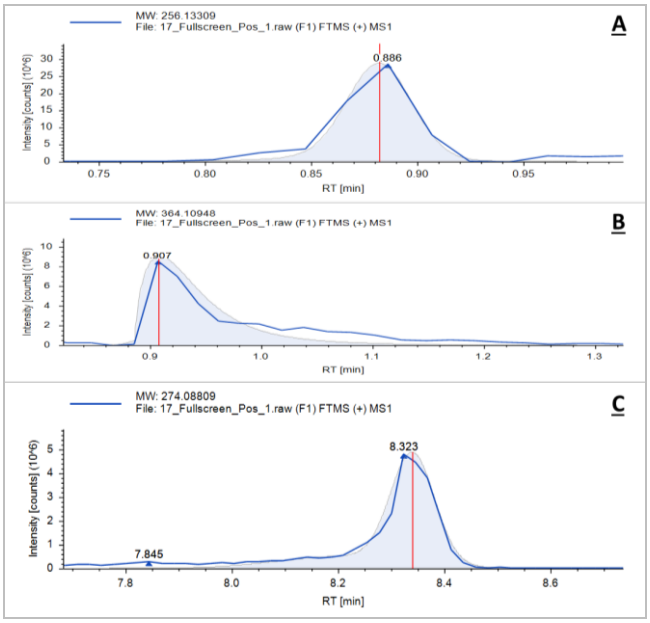

Figure 1. Retention time (RT) of investigated bioactive compounds obtained from LC-HRMS analysis of *S. polyanthum* leaf extract, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin.

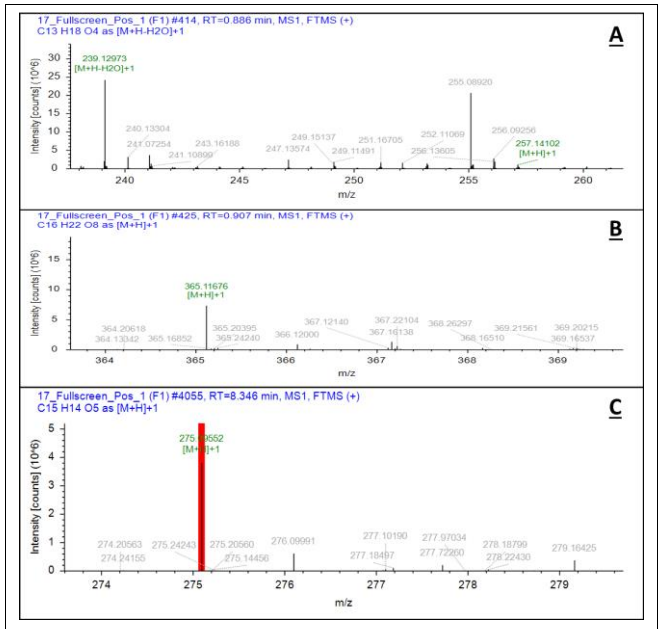

Figure 2. Mass to charge ratio (m/z) value of investigated bioactive compounds obtained from LC-HRMS analysis of *S. polyanthum* leaf extract, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin.

(Figs. 1A, 2A). Besides, NCGC00169066-01 was the other name of 4,10,16-Trimethyl-9,15-dihydroxy-1,5,11-trioxa-7,13-cyclohexadecadiene-2,6,12-trione with molecular formula of $C_{16}H_{22}O_8$ (Table 1). The RT and m/z of NCGC00169066-01 was 0.907 and 365.116 as $[M + H]^{+1}$ (Figs. 1B, 2B). This study also found a natural dihydrochalcone as the group of ketones known as phloretin. The molecular formula of this compound was $C_{15}H_{14}O_5$ (Table 1), with RT and m/z values were 8.323 and 275.095 as $[M + H]^{+1}$ (Figs. 1C, 2C).

Biological activities of *S. polyanthum* bioactive compounds

The compound's biological activity was predicted using PASS Online based on drug-like compounds. The probability activity was shown by the Pa value (to be active), where Pi value was the probability to be inactive. The value can vary from 0 to 1; the higher the Pa value, the higher the prediction accuracy (Lagunin et al., 2018). Several activities were obtained from the analysis correlated with anti-inflammation activities (Table 2). Deoxyphomalone showed an anti-inflammatory activity with Pa > Pi value of 0.648 > 0.023 and TNF expression inhibitor with 0.509>0.028. Besides that, HIF1α expression inhibitor and immunosuppressant were the activities of NCGC00169066-01 with values 0.657 > 0.025 and 0.654 > 0.023, respectively. The activity of HIF1α expression inhibitor and anti-inflammatory also has been predicted in phloretin with 0.673 > 0.023 and 0.573 > 0.038 values.

The anti-inflammatory activity of compound control, including SPD-304, MG-132, GA, and DMF also evaluated using PASS Online (Table 2). The results showed similar activity compared to *S. polyanthum* bioactive compounds. SPD-304 also showed HIF1α expression inhibitor with a lower Pa value (0.403) compared to GA (0.712) and DMF (0.562). MG-132 showed an immunomodulatory activity as DMF with Pa values of 0.320 and 0.335, respectively. GA showed several activities with higher Pa values than the other controls, such as anti-inflammatory, TNF expression inhibitor, and immunosuppressant (Pa values of 0.640, 0.560, and 0.351, respectively). Besides, DMF also showed TNF expression inhibitor and immunosuppressant with Pa value 0.562 and 0.342, respectively. Based on this study, the anti-inflammatory activity of controls (SPD-304, MG-132, and DMF) demonstrated a lower Pa value compared to *S. polyanthum* bioactive compounds. Therefore, it can be assumed that the anti-inflammation activities prediction of *S. polyanthum* bioactive compounds was higher than controls.

The analysis showed several anti-inflammatory-related activities, including anti-inflammatory, TNF expression inhibitor, immunosuppressant, and HIF1α expression inhibitor, which were assumed to be linked. The activity of immunosuppressant, TNF and HIF1α expression inhibitors were widely reported to have a role in the anti-inflammation mechanism. Immunosuppressant is the activity of anti-inflammatory cytokines, which may inhibit the activity of cytokine pro-inflammatory or regulate inflammation through the mediation of cytokines on cellular activities (Shao

Table 2. Anti-inflammatory activities prediction using PASS online.

Compound	Biological activity	Pa	Pi
Deoxyphomalone	Anti-inflammatory	0.648	0.023
	TNF expression inhibitor	0.509	0.028
NCGC00169066-01	HIF1α expression inhibitor	0.657	0.025
	Immunosuppressant	0.654	0.023
Phloretin	HIF1α expression inhibitor	0.673	0.023
	Anti-inflammatory	0.573	0.038
SPD-304	HIF1α expression inhibitor	0.403	0.099
MG-132	Immunomodulator	0.320	0.060
Gallic acid	HIF1α expression inhibitor	0.712	0.019
	Anti-inflammatory	0.640	0.003
	TNF expression inhibitor	0.560	0.018
	Immunosuppressant	0.351	0.084
Dimethylformamide	TNF expression inhibitor	0.562	0.024
	HIF1α expression inhibitor	0.412	0.094
	Immunosuppressant	0.342	0.090
	Immunomodulator	0.335	0.053

et al., 2014). The inhibition of TNF expression potential of bioactive compounds as well as HIF1α expression inhibition is linked to treatment strategies for various inflammatory diseases, including PCOS. TNF is an inflammatory cytokine that can induce inflammation through inflammatory mediator induction, inflammatory cell recruitment, and endothelial cell activation (Kalliolias and Ivashkiv, 2016). In addition, HIF1α induces inflammation through its crosstalk with NF-κB and regulates inflammation function on inflammatory cells (Palazon et al., 2014).

Protein-protein interactions (PPIs) analysis

We analyzed the PPIs network of TNFα, NF-κB, SOD, and Nrf2 to reveal the association of inflammatory and antioxidant-related molecules. The PPIs network analysis was done using STRING webserver. The analysis showed the interaction of each protein and the interaction against other molecules. TNFα and NF-κB were linked to each other and associated with NFKBIA (NF-*kappa*-B inhibitor alpha), TNFRSF1A (tumor necrosis factor receptor superfamily member 1A), TRADD (tumor necrosis factor receptor type 1-associated DEATH domain protein), and RELB (Relb proto-oncogene, NF-κB subunit) (Fig. 3). TNFα was also linked to antioxidant-related molecules, SOD, Nrf2, and KEAP11 (Kelch-like ECH-associated protein 1). In addition, SOD and Nrf2 were also linked to KEAP11 (Fig. 3).

Antioxidants and inflammation have been widely reported to be linked to each other. Inflammation can be caused by the imbalance of natural antioxidants, which induce soluble inflammatory mediators of cells, such as cytokines, arachidonic acid, and chemokines (Arulselvan et al., 2016). During inflammation, TNFα was reported to be the first cytokine released systematically (Suzuki et al., 2020). NF-κB regulates the production of TNFα from M1 macrophages as a transcription factor, leading to an inflammatory response (Liu et al., 2017). The inflammation regulation of NF-κB occurs directly or indirectly by inducing the production of inflammatory cytokines or cell proliferation, apoptosis, and differentiation (Liu et al., 2017).

Molecular docking analysis

TNFa and S. polyanthum bioactive compounds molecular docking

The molecular docking analysis was done to evaluate the inhibition potential of the compounds against inflammatory molecules TNFα and NF-κB, as well as the activation against SOD and Nrf2 as antioxidant-related molecules. This analysis used SPD-304 as a control of the TNFα inhibitor. SPD-304 is a small molecule inhibitor that has been widely reported to inhibit TNFα activity through protein trimerization disruption (Mascret et al., 2021). From this molecular docking analysis, SPD-304 bind to TNFα with a binding

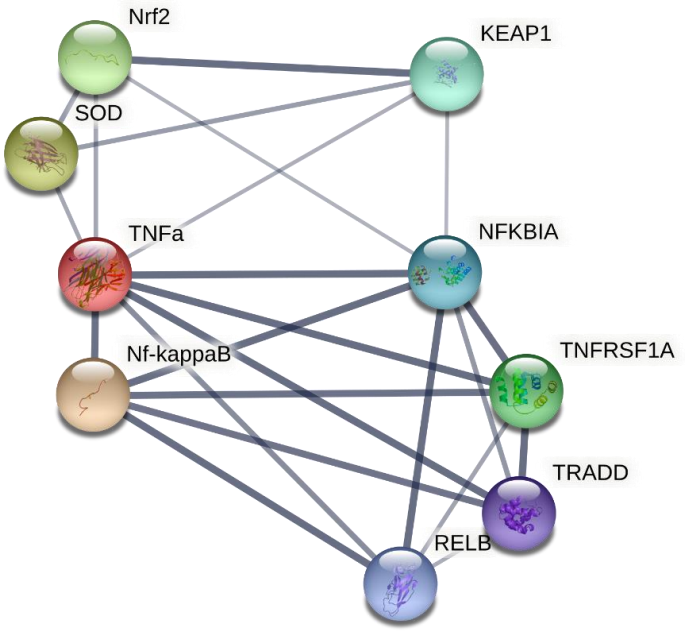

Figure 3. Protein-protein interactions (PPIs) network of TNFα, NF-κB, SOD, and Nrf2

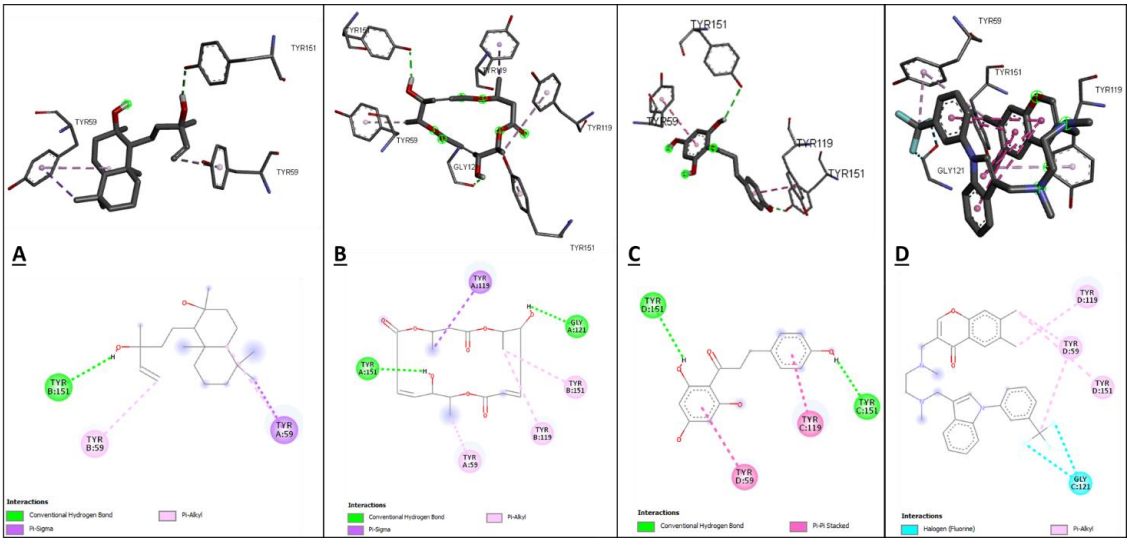

Figure 4. The interaction of *S. polyanthum* bioactive compounds against TNFα was shown in 3D (above) and 2D (below) diagrams, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin; and **(D)** SPD-304.

affinity of -9.1 kcal/mol (Table 3). The binding sites of SPD-304 and TNFα were Gly121 as halogen interaction and Tyr59, Tyr119, and Tyr151 as hydrophobic interaction (Table 3, Fig. 4D). Besides, the interaction of *S. polyanthum* bioactive compounds against TNFα showed higher binding affinity compared to SPD-304. The binding affinity of deoxyphomalone, NCGC 00169066-01, and phloretin was -7.4, -8.2, and -7.1 kcal/mol, respectively (Table 3). NCGC00169066-01 has the lowest binding affinity among the other bioactive compounds. The complex of NCGC00169066-01 and TNFα involved Gly121 and Tyr151 in the form of hydrogen bonds and hydrophobic interactions through Tyr119, Tyr59, and Tyr151 residues (Table 3, Fig. 4B), which were reported as SPD-304 binding sites against TNFα in this study. Although the binding affinities of two other compounds were higher, the binding site was also the same as SPD-304. Based on these results, the bioactive compounds were expected to have inhibition potential as SPD-304 against TNFα activity.

NF-κB and S. polyanthum bioactive compounds molecular docking

A molecular docking study analyzed the inhibition potential of NF-κB by *S. polyanthum* bioactive compounds. This study used MG-132, NF-κB small mole-

Table 3. Binding affinity and interaction of protein-ligand.

Protein	Ligand	Binding affinity (kcal/mol)	Interaction	Residue
TNFα	Deoxyphomalone	-7.4	Hydrogen bond	**Tyr151**
			Hydrophobic	**Tyr59**
	NCGC00169066-01	-8.2	Hydrogen bond	**Gly121, Tyr151**
			Hydrophobic	**Tyr119, Tyr59, Tyr151**
	Phloretin	-7.1	Hydrogen bond	**Tyr151**
			Hydrophobic	**Tyr59, Tyr119**
	SPD-304	-9.1	Halogen	**Gly121**
			Hydrophobic	**Tyr59, Tyr119, Tyr151**
NF-κB	Deoxyphomalone	-5.6	Hydrophobic	**Lys305, Lys273,** Lys335, **Lys283, Tyr285**
	NCGC00169066-01	-6.8	Hydrogen bond	**Lys305**
			Hydrophobic	**Lys305, Lys273**
	Phloretin	-6.7	Hydrogen bond	Ser279
			Hydrophobic	**Lys305,** Lys274, **Lys273,** Glu280
	MG-132	-6.7	Hydrogen bond	**Lys273, Asp304, Lys305**
			Hydrophobic	**Lys283, Tyr285, Lys305**
SOD	Deoxyphomalone	-7.4	Hydrogen bond	**Val7, Val146**
			Hydrophobic	Lys9, **Val146**
	NCGC00169066-01	-7.8	Hydrogen bond	**Val7, Asn51,** Gly145
			Hydrophobic	Lys9, **Val146, Val7**
	Phloretin	-7.6	Hydrogen bond	**Asn51, Val146, Val7,** Gly145, Gly54
			Hydrophobic	**Val146, Val7**
	Gallic acid	-6.1	Hydrogen bond	**Val7, Val146, Asn51**
			Hydrophobic	**Val146**
KEAP1	Deoxyphomalone	-7.2	Hydrophobic	Pro549, Lys565, Cys583, Val594, **Ala548,** Pro549
	NCGC00169066-01	-7.7	Hydrogen bond	Asp589, Tyr567
			Hydrophobic	Arg551, **Ala548,** Pro549
	Phloretin	-7.0	Hydrogen bond	Trp591, Asp589
			Hydrophobic	**Ala548,** Pro549, Lys565, Cys583, Arg551
	Dimethylformamide	-4.5	Hydrogen bond	**Asp573**
			Hydrophobic	**Ala548, Trp591**

cule inhibitor, as a control and compared it to the investigated bioactive compounds. MG-132 is a well-known NF-κB inhibitor through the ubiquitin-proteasome system (UPS), inhibiting the degradation of IκB-α (Zhang et al., 2018). This study found the interaction of MG-132 against NF-κB was through Lys273, Asp304, and Lys305 as hydrogen bonds, and Lys283, Tyr285, and Lys305 as hydrophobic interaction (Table 3, Fig. 5D). The binding affinity of MG-132 was the same as phloretin against NF-κB (-6.7 kcal/mol), higher than NCGC00169066-01 (-6.8 kcal/mol), but lower than deoxyphomalone (-5.6 kcal/mol) (Table 3). The interaction of NCGC00169066-01 and NF-κB was through the binding sites of MG-132/NF-κB, such as Lys305 as hydrogen bond and Lys305 and Lys273 as hydrophobic interaction (Table 3. Fig. 5B). Deoxyphomalone and phloretin also involved MG-132 amino acid binding sites but in the form of hydrophobic interaction. So, it was assumed that these two compounds' interaction

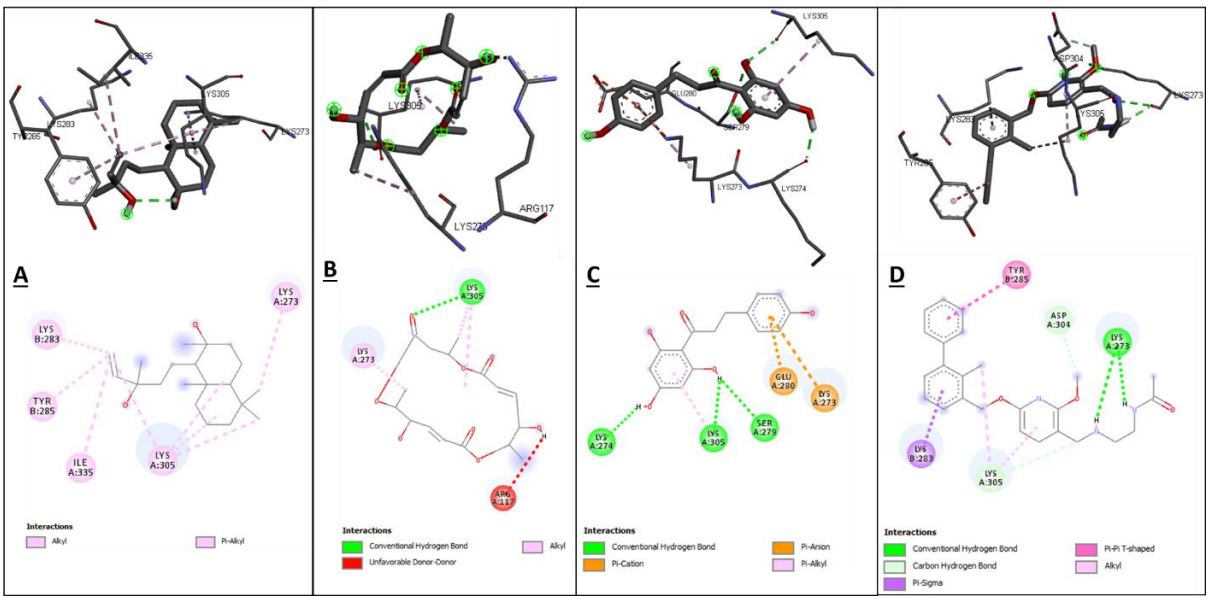

Figure 5. The interaction of *S. polyanthum* bioactive compounds against NF-κB shown in 3D (above) and 2D (below) diagram, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin; and **(D)** MG-132.

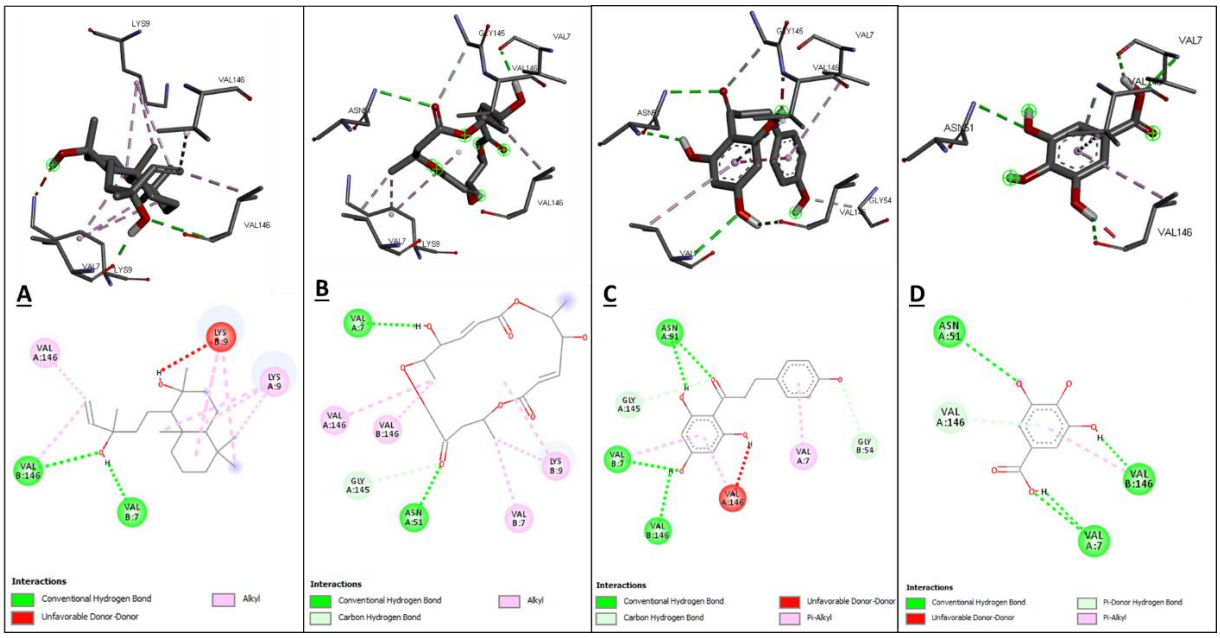

Figure 6. The interaction of *S. polyanthum* bioactive compounds against SOD was shown in 3D (above) and 2D (below) diagram, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin; and **(D)** Gallic acid.

was weaker compared to NCGC00169066-01 interaction against NF-κB.

SOD and S. polyanthum bioactive compounds molecular docking

Through a molecular docking analysis, this study evaluated the potential of *S. polyanthum* activation on SOD, an antioxidant enzyme. The molecular docking was done by comparing a control, gallic acid (GA), and SOD activator and has scavenging activity against free radicals (Pandey et al., 2019). Based on molecular docking analysis, the binding site of GA and SOD were Val7, Val146, Asn51 through hydrogen bonds, and Val146 through hydrophobic with a binding affinity of -6.1 kcal/mol (Table 3, Fig. 6D). Comparing the binding affinity, the three investigated bioactive compounds showed lower binding affinity, such as -7.8 kcal/mol (NCGC00169066-01), and -7.6 kcal/mol (phloretin), and -7.4 kcal/mol (deoxyphomalone). The lower binding affinity needed, the stronger the interaction. The binding site of *S. polyanthum* bioactive compounds interaction against SOD also involved the residue of GA/SOD binding sites,

such as in NCGC00169066-01/SOD interacted through Val7, Asn51 (hydrogen bond) and Val146, Val7 (hydrophobic); phlortein/SOD were Asn51, Val146, Val7 (hydrogen bond) and Val146, Val7 (hydrophobic); and deoxyphomalone/SOD were Val7, Val146 (hydrogen bond) and Val146 as hydrophobic (Table 3, Fig. 6).

KEAP1 and S. polyanthum bioactive compounds molecular docking

The potential of Nrf2 activator from *S. polyanthum* evaluation was done by a molecular docking analysis of investigated bioactive compounds against KEAP1 as a regulator of Nrf2. The inhibition of KEAP1 lead to the activation of Nrf2 and its function in oxidative stress response (Li et al., 2019). This study used DMF as a control, Nrf2 activator through KEAP1-Nrf2 interaction disruption, and has been marketed (Ma et al., 2020). This study found that DMF and KEAP1 interaction's binding affinity was higher than *S. polyanthum* bioactive compounds. The binding affinities from lowest to highest were NCGC00169066-01 < Deoxyphomalone < phloretin < DMF; -7.7 < -7.2 < -7.0 < 4.5 (kcal/mol), respectively (Table 3). This study found that the binding sites of DMF-KEAP1 were Asp573 as a hydrogen bond and Ala548, and Trp591 as hydrophobic interaction (Table 3, Fig. 7D). The bioactive compounds of *S. polyanthum* have different binding sites compared to DMF, except residue Ala548. However, the involvement of Ala548 in KEAP1 and *S. polyanthum* bioactive compounds interaction was expected to disturb the interaction of KEAP1-Nrf2 protein-protein interaction so that the Nrf2 could be activated.

DISCUSSION

The pathogenesis of PCOS has been widely reported to be significantly related to chronic inflammation and oxidative stress and is considered a chronic systematic disease (Zuo et al., 2016). Chronic inflammation in PCOS is characterized by permanently producing excessive pro-inflammatory cytokines such as IL-18, TNFα, and CRP (Regidor et al., 2020). The induction of oxidative stress also leads to the activation of NF-κB, which causes the promotion of TNFα and IL-6 (González, 2012). In this regard, this study evaluated the anti-inflammatory and antioxidant potential of *S. polyanthum* bioactive compounds.

The alteration of steroidogenesis in the ovaries in PCOS leads to the production of oxidative stress and increased androgen levels, disturbs the development of follicular, and causes infertility (Sulaiman et al., 2018). Oxidative stress is the condition of excessive oxidant presence in the imbalanced antioxidant defenses (Wang et al., 2020). SOD is an enzymatic antioxidant that plays a crucial role in defenses against oxidative stress. The low level of SOD and other antioxidant parameters is found in a patient with PCOS and the increased oxidative stress (Uçkan et al., 2022).

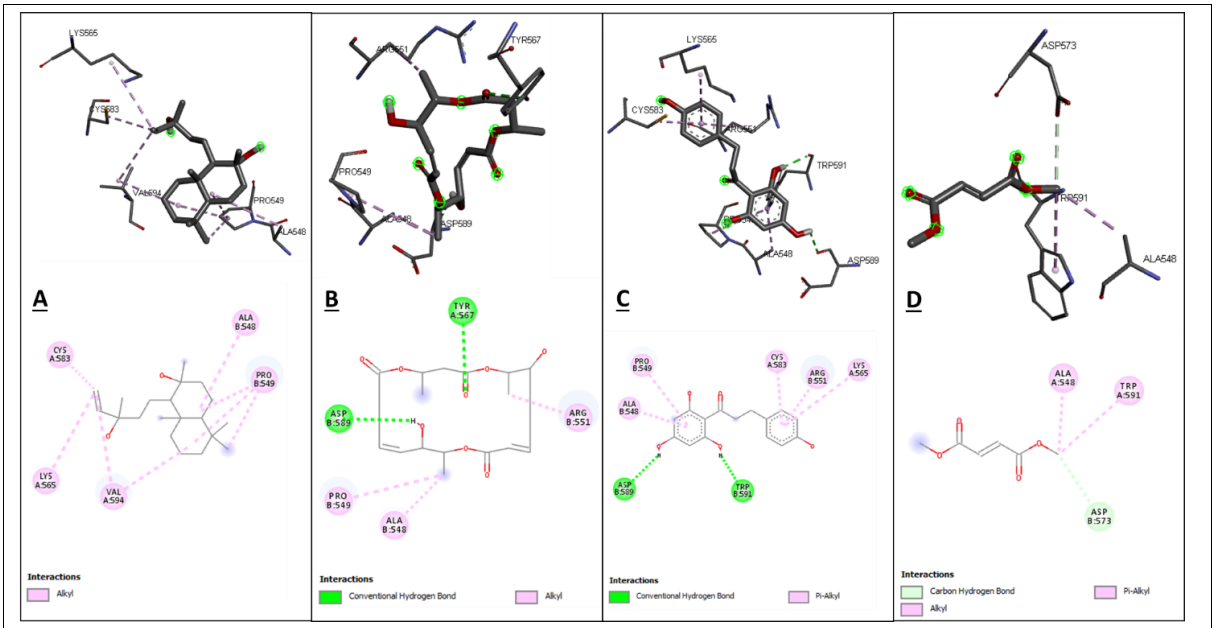

Figure 7. The interaction of *S. polyanthum* bioactive compounds against KEAP1 was shown in 3D (above) and 2D (below) diagrams, **(A)** Deoxyphomalone; **(B)** NCGC00169066-01; **(C)** Phloretin; and **(D)** Dimethylformamide**.**

SOD is activated during the response against the stress through the KEAP1/Nrf2 pathway; Nrf2 detaches from KEAP1 molecules, translocates to the nucleus, and activates several antioxidant genes (Wang et al., 2020). This study found that *S. polyanthum* bioactive compounds interacted with SOD and KEAP1 through several binding sites. Compared to GA, as a control or SOD activator, *S. polyanthum* bioactive compounds had stronger (lower binding affinity) interactions against SOD. The activation mechanism was predicted to occur through the same mechanism as GA, shown by the involvement of binding site residues in *S. polyanthum* compounds and SOD interaction. Besides, the molecular docking against KEAP1 showed *that S. polyanthum* bioactive compounds had stronger interaction than control (DMF). This study expected that the interaction of *S. polyanthum* compounds against KEAP1 could disturb the KEAP1/Nrf2 pathway so Nrf2 can be activated.

The increase in ROS production in PCOS patients is positively correlated with the activity of the immune system and pro-inflammatory cytokines that cause inflammation (Victor et al., 2016). The researcher suggested that inflammation in PCOS patients is stimulated by hyperandrogenism, which might be linked to insulin resistance, adipose tissue content enhancement, and visceral fat related to metabolic disorder (Prabhu et al., 2021). Studies reported that TNFα is elevated in PCOS patients, and it becomes a useful biomarker for PCOS diagnosis and is directly linked to insulin resistance and high androgen level (Gao et al., 2016). During the inflammation, TNFα is controlled by the NF-κB pathway through positive feedback (He et al., 2021). NF-κB is a transcription factor that regulates various genes involved in the immune system and inflammatory response process. This study evaluated the anti-inflammatory potential of *S. polyanthum* bioactive compounds by inhibiting TNFα and NF-κB. The study found that the investigated bioactive compounds had inhibition potential against TNFα and NF-κB through the inhibition mechanism of control, SPD-304, and MG-132, respectively. The inhibition mechanism was predicted by the involvement of key residues in the control binding sites that the bioactive compounds also owned.

CONCLUSION

This study found the potential activity of *S. polyanthum* bioactive compounds as anti-inflammatory and antioxidant. The prediction of PASS online showed all the compounds had anti-inflammatory activity, which then docked with inflammation and antioxidant-related molecules, TNFα, NF-κB, SOD, and Nrf2. Each molecule was related based on the analysis of the PPIs network using STRING. Our molecular docking analysis found that deoxyphomalone, NCGC00169066-01, and phloretin inhibited TNFα and NF-κB through similar binding sites (inhibition mechanism) as the control. Our study also found activation potential against SOD, which was stronger than control, shown by lower binding affinity through the same residues as the binding site of control. However, KEAP1 may also inhibit through Ala548 residue with very low binding affinity compared to control. This study expected this prediction potential of *S. polyanthum* as anti-inflammatory and antioxidant can be an alternative to treat PCOS. Indeed, further analysis needs to be done, such as *in vitro* or *in vivo* studies.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Aditya R	Santoso B	Widjiati W
Concepts or ideas		x	
Design	x		
Definition of intellectual content			x
Literature search		x	x
Experimental studies	x		
Data acquisition	x		
Data analysis	x		
Statistical analysis			
Manuscript preparation	x		
Manuscript editing		x	x
Manuscript review	x	x	x

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