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A Computational Insights of *Ocimum basilicum* Flavonoid and Essential Oils Interaction in the Targeting Keap1/SIRT1/NFKB Signaling Pathway

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ABSTRACT

Type 2 diabetes mellitus (T2DM) is a metabolic disorder that has a negative relationship with male reproduction. The imbalance between endogenous antioxidants and inflammatory mediators would initiate inflammation development, further accelerating tissue aging. This study aimed to investigate the flavonoids and essential oils from *Ocimum basilicum* involved in Keap1/SIRT1/NFκB. *O. basilicum* compounds used were flavonoid (apigenin, rutin, and quercetin) and essential oils (α-bergamotene, α-cadinol, methyl cinnamate, and methyl eugenol), which were then evaluated for toxicity by Protox II and pharmacokinetic properties by ADMET. The protein network was built by STRING. The molecular docking was performed by PyRx on NFκB, SIRT1, and Nrf2. The result demonstrated that apigenin, rutin, α-bergamotene, α-cadinol, and methyl cinnamate have low toxicity. The pharmacokinetics study showed that *O. basilicum* was primarily absorbed in the human intestine. The protein network analysis revealed that NFκB and Nrf2 were involved in inflammatory response, regulation of stress response, and insulin resistance pathways. SIRT1 and Nrf2 have pivotal roles in insulin resistance-induced gonadal disease. Rutin has the strongest binding affinity for Keap1 (4IQK), whereas α-bergamotene and α-cadinol have the strongest binding affinity for NFκB (3DO7) and SIRT1 (4I5I), respectively. The flavonoid contents might be beneficial to activate Nrf2, whereas the essential oils of *O. basilicum* inhibit NFκB and activate SIRT1. These preliminary findings suggested that *O. basilicum* bioactive compounds might provide a promising candidate for restoring the imbalance in T2DM through the Keap1/SIRT1/NFκB signaling pathways.

Keywords: antioxidant, essential oil, flavonoid, *Ocimum basilicum*, inflammation

Introduction

Diabetes mellitus (DM) is a chronic and significant health problem that substantially influences the overall quality of life and welfare of individuals, families, and society globally. Regarding the previous report, the DM prevalence is predicted to rise by 578 million (10.2%) by 2030 and 700 million (10.9%) by 2045, dominated by type 2 DM (T2DM).[1] Insulin resistance was the hallmark of T2DM, which further initiated chronic low-grade inflammation.[2,3] Recently, T2DM has been known to negatively correlate with the male reproductive tract, including fertility and poor sperm characteristics.[4] Testicular oxidative stress and inflammation have been linked to decreased fertility rates in both experimental and clinical studies.[5,6] The up-regulation of mediator inflammation, nuclear factor kappa B (NFκB), and the down-regulation of endogenous antioxidant factor, nuclear factor-erythroid-2-related factor 2 (NFE2L2) or Nrf2, are key signals in DM-mediated inflammation development.[7]

Mitochondria produces reactive oxygen species (ROS) and superoxide by the p450 reaction.

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An altered balance between oxidants and antioxidants, specifically in Leydig cells in the testis, might decrease the protective effect of antioxidants.[8,9] Moreover, excessive ROS might damage spermatozoa, which further disturbs their function and leads to infertility.[10] A previous study reported that testosterone did not change in 8-month-old knocked-out mice but decreased significantly by age (21-24 months), which further characterized the aging cells.[11] Sirtuin-1 (SIRT1) is a nicotinamide adenosine dinucleotide (NAD)-dependent histone deacetylase that belongs to the sirtuin family of deacetylases. SIRT1 plays many critical physiological functions, including gene expression control, metabolism, and aging. Interestingly, male mice lacking SIRT1 have reproductive dysfunction due to poor spermatogenesis and abnormal sperm maturation.[12] In diabetic nephropathy, SIRT1 activated the Keap1/Nrf2/ARE signaling pathway, down-regulated fibronectin and TGF-β1, and increased the transcriptional activity of Nrf2 in the nucleus.[8,13,14] Another study reported that the SIRT1/NFκB/miR-29/Keap1/Nrf2 signaling pathway was potentially a therapeutic target in DM-induced renal injury.[15] In contrast, SIRT1 activation suppressed NFκB activation through AMPK activation in DM rats.[16] Indeed, SIRT1 activity might benefit the male reproductive system by restoring the balance of inflammatory mediators and endogenous antioxidants.

Basil (*Ocimum basilicum* L.) belongs to the *Lamiaceae* family and is considered more than just a flavorful herb to enhance the taste of dishes. *O. basilicum* contains bioactive compounds essential in traditional and modern medicine. *O. basilicum* is a rich source of essential oils, flavonoids, polyphenols, and other biologically active substances that have gained growing interest in food science, nutrition, and pharmacology. The previous study reported that *O. basilicum* has antifungal,[17] anti-inflammatory,[18] antiviral,[19] anticancer,[20,21] and antioxidant properties.[22] A previous study reported that the dominant essential oil compounds in *O. basilicum* were chavicol (81.82%), β-(E)-

ocimene (2.93%), and α-(E)-bergamothene (2.45), whereas quercetin, rutin, apigenin, chlorogenic acid, and p-hydroxybenzoic were considered the most important antioxidants in *O. basilicum*.[23,24] However, although *O. basilicum* contains many phytochemicals, there are limited studies comparing their essential oils and flavonoids. The essential oils of *O. basilicum* were influenced by cultivars, growth location, agronomic management, seasonal variation, harvesting, drying, and processing methods[24]. Additionally, it would be intriguing to conduct a comparative analysis to determine whether essential oils or flavonoids play a beneficial role in the Nrf2/Keap1/ARE or SIRT1/NFKB signaling pathways. *In silico* approaches have gained interest in recent years for pharmacological screening due to improved cost and time efficiency, a limited error rate, and the materials used.[25] Molecular docking is a computational technique used in structural biology and drug discovery to predict and analyze the interactions between small molecules (ligands) and a target protein (receptor).[26] It is a valuable tool for understanding ligands' binding modes to receptors and screening potential drug candidates.[27] Active compounds found in *O. basilicum* are essential to explore, especially their function to restore reproductive dysfunction due to gonadal dysfunction due to an imbalance between the inflammatory and stress responses with antioxidants.

Materials and Methods

Preparation of Ligand Molecule

The ligand from the *O. basilicum* active compound and control drug were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in .sdf format. The ligand structure was then converted into a .pdb file using PyMOL software (Schrödinger Inc., LLC). The list of ligands in this study is presented in Table 1.

Toxicity evaluation

Oral toxicity evaluation was predicted by ProTox-II (https://tox-new.charite.de/protox_II/index.php?site=home) online web server.[28,29] Toxicity evaluation is essential for drug-like candidates to validate the safety of products or active compounds and was classified into six classes according to the previous study.[25]

Pharmacokinetic properties

The investigation of pharmacokinetic properties seeks to ascertain the potential of a molecule to be a drug candidate based on its adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties to avoid its toxicity or other unwanted effects.[30,31] The pharmacokinetics of compounds, including ADMET, were evaluated using pkCSM online tool (https://biosig.lab.uq.edu.au/pkcsm/prediction),[32] whereas AMES test predicted toxicity.

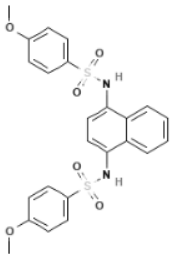
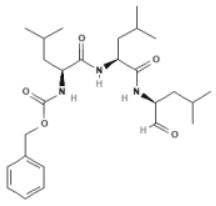
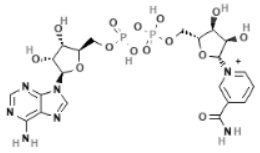
Protein Network Analysis

Some proteins involved in particular pathways were identified using a protein network analysis. The online web server for the STRING database was used to analyze the protein network containing Keap1, SIRT1, and NFκB. (http:/string-db.org/).[33] The STITCH online web server built the ligand and protein interactions (http://stitch.embl.de/cgi/input.pl).

Docking Preparation

Preparation for the docking study included choosing the target protein, cleaning the protein target from ligands and water molecules, and determining the target protein's active site. The target protein for this study was downloaded from the Protein Data Bank (PDB) online webserver (https://www/rcsb.org/). This research study used Keap1 (PDB ID 4IQK), NFκB (3DO7), and SIRT1 (PDB ID 4I5I). The target proteins were then cleaned from ligand and water molecules using PyMOL software. The determination of the active site for each protein used reverse docking methods. The reverse docking examined the active site based on a known ligand or control drug. The active site of each protein is provided in Table 2.

Table 1: List of ligand molecules of molecular docking study

Ligand name	CID Number	Structure
Drug control (known ligand)		
N,N'-naphthalene-1,4-diylbis(4-methoxybenzene-sulfonamide) as KEAP1 inhibitor	1073725	
MG132 as NFκB inhibitor	462382	
Nicotinamide-Adenine-Dinuclotide (NAD) as SIRT1 activator	5893	
The flavonoid compound of O. basilicum		

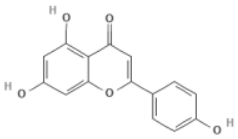
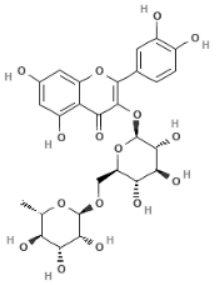
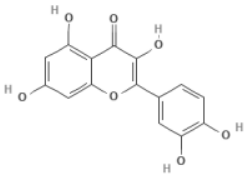
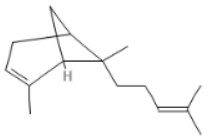
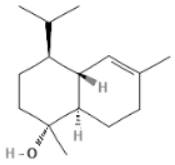
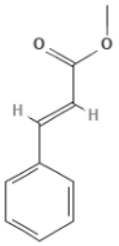
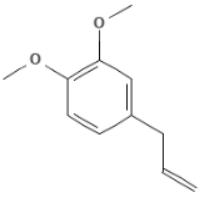
Apigenin	5280443	
Rutin	5280805	
Quercetin	5280343	
Essential oil compound of O. basilicum		
Alpha-bergamotene	86608	
Alpha-cadinol	10398656	
Methyl Cinnamate	637520	
Methyl eugenol	7127	

Table 2: Grid center and dimension for molecular docking study

Protein	Grid center			Dimensions (Å)		
	X	Y	Z	X	Y	Z
Keap1	-45.8985	4.9769	-9.8627	20.0005	20.6159	20.3690
NFκB	27.9170	61.2406	75.3277	20.6716	20.8264	20.2799
SIRT1	30.6408	-19.2598	27.9530	20.6935	20.8346	20.1092

Docking Simulation

A molecular docking simulation was performed using PyRx – Virtual Screening Tool v.0.8 (https://pyrx.source forge.io).[34,35] All ligands were minimized by the Open Babel GUI before docking. The target protein was uploaded as a macromolecule. Molecular docking between protein and ligand was performed according to the active site that has been evaluated using the control drug (Table 2). Flavonoid compounds of *O. basilicum* were docked with the Keap1 protein, while essential oil compounds of *O. basilicum* were docked with NFκB and SIRT1. NFκB and SIRT1 are nuclear proteins located in the nucleus.[36,37] Essential oil is a lipid-based compound that could pass the phospholipid bilayer membrane, be directly bound to nuclear factor, and induce specific cellular mechanisms.[38,39] Visualization and analysis of amino acid residues used BIOVIA Discovery Studio.[40]

Results and Discussion

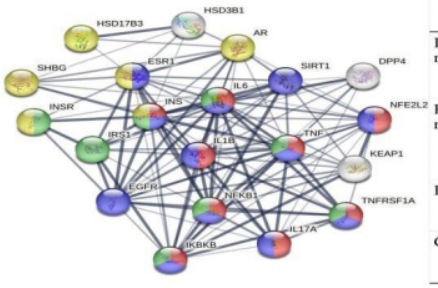
Pharmacokinetic properties, including adsorption, distribution, metabolism, excretion, and toxicity (ADMET), are essential in drug discovery research. Pharmacokinetic properties evaluate the efficacy and safety of a drug or drug-like candidate compound.[41] Adsorption of molecules in pharmacokinetic properties evaluated the initial transport of molecules along the gastrointestinal tract membrane. Caco-2 cells were used to predict adsorption in the human intestinal mucosa. High permeability to Caco-2 cells has a predicted value > 0.90.[32] Based on the results, it was demonstrated that only rutin and quercetin have a predicted value < 0.9. Meanwhile, the other compounds from *O. basilicum* have a predicted value > 0.9. If a molecule has a heavy molecular weight, it might be more difficult to be absorbed by the body.[31] However, the result indicated that *O. basilicum* active compounds were mostly absorbed completely in the human intestine. The percentage absorption of molecules is also in line with Caco-2 permeability, where apigenin, α-bergamotene, α-cadinol, methyl cinnamate, and methyl eugenol were absorbed > 90%. These findings could be considered evidence that enterocytes could easily absorb *O. basilicum's* active compound in the body.

The volume of distribution at steady state (VDss) represents the distribution of a drug or compound in the plasma and/or tissue. The VDss value is indicated as low if below log VDss < -0.15, while it is indicated as high if above log VDss > 0.45.[32] Major compounds from *O. basilicum* showed log VDss > 0.45, such as apigenin, rutin, quercetin, and alpha-bergamotene. Meanwhile, alpha-cadinol, methyl cinnamate, and methyl eugenol have a moderate value of VDss. Molecules that have high VDs are categorized as lipophilic molecules. Lipophilic molecules easily pass through the phospholipid bilayer membrane; thus, they leave the bloodstream and then distribute in the tissue, especially high-lipid-density tissue.[42] this finding suggested that *O. basilicum* active compounds are primarily distributed in the tissue. The BBB permeability value showed that flavonoid content of *O. basilicum* has BBB permeability with logBB < 0.3 while essential oil compound of *O. basilicum* has BBB permeability with logBB > 0.3. Molecules with logBB > 0.3 are categorized as readily to cross the blood-brain-barrier through active uptake.[43] These results indicated that the flavonoid contents in *O. basilicum* were distributed in the peripheral tissue, while the essential oil compounds in *O. basilicum* could directly interact with the CNS. This information might be beneficial for developing a CNS-targeted medicine because a compound that effectively penetrates the BBB is still the main challenge for new drug development.[44]

The AMES test for excretion and toxicity showed that none of the compounds affect the organic cation-transported 2 (OCT2) substrate or are toxic. OCT2 has a function in the disposition and renal clearance of organic cations. Thus, the remaining drug components can be eliminated in the body.[45] AMES toxicity assesses potential compounds that can cause mutagens to lead to carcinogens. AMES toxicity used *Salmonella enterica* serovar Typhimurium in the in vitro research.[46] ADMET analysis indicated that active compounds from *O. basilicum* are safe to use as drug-like candidates for stress-induced inflammation treatment..

Based on oral toxicity prediction, it was revealed that N,N'-naphthalene1,4-diylbis, as a Keap1 inhibitor drug, has a hepatotoxicity risk or can cause liver damage while consumed at a certain amount. Furthermore, all ligand compounds show no cytotoxicity risk. Apigenin, rutin, alpha-bergamotene, alpha-cadinol, and methyl cinnamate are categorized at class 5 of toxicity, while methyl eugenol is at class 4 and quercetin is at class 3 (Table 3).

Protein network analysis showed that NFκB1, TNF, TNFRSF1A, and INS are responsible for developing the inflammatory response, regulating the stress response, and insulin resistance pathway (Figure 1). Furthermore, NFE2L2 is also present in the inflammatory response and regulation of the response to stress pathways. These results suggested that activation of NFκB1 due to inflammation and response to stress-induced insulin resistance, which led to gonadal disease. SIRT1 and NFE2L2 are also responsible for the development of insulin resistance-induced gonadal disease.



Pathway description	False discovery rate	Proteins
Inflammatory response	5.08×10^{-07}	NFKB1, NFE2L2, IKBKB, TNF, TNFRSF1A, IL6, IL1B, IL17A, INS
Regulation of response to stress	4.58×10^{-07}	SIRT1, NFKB1, NFE2L2, IKBKB, EGFR, TNF, IL1B, TNFRSF1A, IL6, IL17A, INS
Insulin resistance	1.94×10^{-11}	NFKB1, IKBKB, TNF, TNFRSF1A, IL6, INS, IRS1, INSR
Gonadal disease	4.32×10^{-06}	INS, INSR, ESR1, SHBG, AR, HSD17B3

Figure 1: Protein network analysis using STRING at various pathways, namely inflammatory response pathway (red ball), regulation of response to stress pathway (blue ball), insulin resistance pathway (green ball), and gonadal disease pathway (yellow ball).

Table 3: Oral toxicity prediction

Compounds	LD50 (mg/kg)	Class	Hepato-toxicity	Carcinogenicity	Immuno-toxicity	Mutage-nicity	Cytoto-xicity
N,N'-naphthalene-1,4-diylbis(4-methoxybenzene-sulfonamide)	1190	4	(+)	(-)	(+)	(-)	(-)
MG132	2025	5	(-)	(-)	(-)	(-)	(-)
Nicotinamide-Adenin-Dinuclotide (NAD)	11250	6	(-)	(-)	(+)	(-)	(-)
Apigenin	2500	5	(-)	(-)	(-)	(-)	(-)
Rutin	5000	5	(-)	(-)	(+)	(-)	(-)
Quercetin	159	3	(-)	(+)	(-)	(+)	(-)
Alpha-bergamotene	3700	5	(-)	(-)	(-)	(-)	(-)
Alpha-cadinol	2830	5	(-)	(-)	(-)	(+)	(-)
Methyl cinnamate	2610	5	(-)	(-)	(-)	(-)	(-)
Methyl eugenol	810	4	(-)	(+)	(-)	(-)	(-)

Further analysis using STITCH revealed that rutin, apigenin, and quercetin were involved in regulating the response to stress pathways (Figure 2). Surprisingly, the essential oils of *O. basilicum*, such as alpha-bergamotene, methyl cinnamate, and methyl eugenol, did not have enough evidence to be involved in the regulation of the response to stress pathways (Figure 2). Chronic or excessive stress can lead to dysregulation of the inflammatory response, including activation of NFκB.[47]

Prolonged stress increases NFκB and leads to the overproduction of inflammatory molecules, called stress-induced inflammation.[48] During inflammation, immune cells release cytokines, which are signaling molecules that regulate the immune response. In chronic inflammation, excessive release of pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and IL-1β, can lead to tissue damage and systemic effects.[49,50] STRING analysis demonstrated that the development of inflammatory and stress responses included NFκB, TNFα, IL6, and IL1β within the mechanism. On the other hand, response to stress was also marked by SIRT1 and NFE2L2 (Nrf2). SIRT1 modulated various physiological processes, including the inflammatory response, DNA repair, apoptosis, cancer, and stress.[51] SIRT1 and Nrf2 control cellular responses during inflammation through their antioxidant defense systems. A recent study revealed that SIRT1 plays a role in regulating the Nrf2-KEAP1 pathway. Under unstressed conditions, KEAP1 binds to the Nrf2 protein and ubiquitinates Nrf2 by KEAP1-CUL3 ligase. KEAP1 also degraded the Nrf2 protein through the proteasome pathway.[52] SIRT1 can deacetylate, preventing the degradation of Nrf2, thus activating Nrf2..[53,54] By activating Nrf2, SIRT1 contributes to the upregulation of antioxidant and detoxification genes, which can help protect cells from stress-induced inflammation. The docking result demonstrated that Rutin (–10.2) (Figure 3) has the strongest binding affinity with Keap1 than apigenin (–8.5) and quercetin (–8.4) (Table 5). Rutin also has a lower binding affinity value compared with drug control, which means rutin has a stronger binding affinity to Keap1 than drug control. Gly364 amino acid residues also appeared in all Keap1-ligand dockings. This result suggested that Gly364 might play a crucial role in the inhibition mechanism of the Keap1 protein. The KEAP1-Nrf2 complex is commonly present in the cytoplasm, and Nrf2 will separate from KEAP1 under stress stimuli and then bind to the transcription factor of multiple antioxidant enzymes.[55] Molecular docking between KEAP1 and the ligand Gly364 is an amino acid residue at selected flavonoid content from *O. basilicum*. Gly364 was an amino acid residue that interacted with all complexes. Gly364 induces the Keap1-dependent mechanism to activate Nrf2 through interaction with H-bonds and H-benzene.[56] Gly364 is also located near Ser363, which is potentially involved in interaction with Glu82 at the ETGE motif of Neh2 in Nrf2.[57] The change of glycine at 354 position with other residues will be sterically unfavorable and affect the conformation of Ser363 residue, thus disrupting the interaction between Ser363 from KEAP1 and Glu-82 from the Nrf2 protein.[58] Gęgotek et al. reported that Rutin stimulated the Nrf2 pathway after UVA and UVB radiation in skin keratinocytes and fibroblasts, which further restored antioxidant enzyme activity and suppressed proinflammatory cytokines.[59]

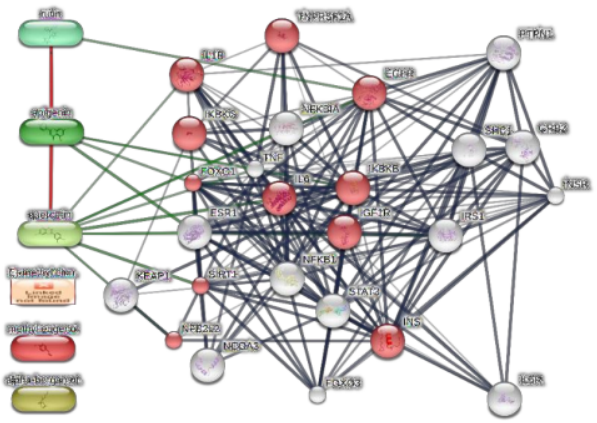


Figure 2: Protein and ligands interact in regulating response to stress pathway (red ball) with 9.63×10^{5} false discovery rates.

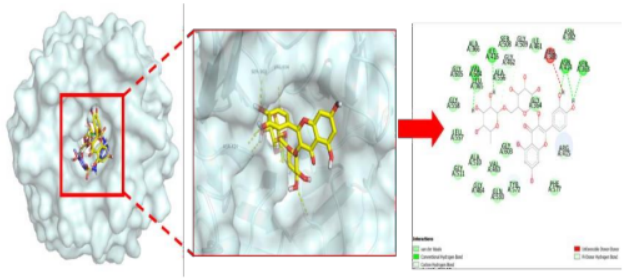


Figure 3: Visualization from molecular docking between Keap1 protein (blue) with small molecules. The yellow ligand indicated as Rutin following with the list of amino acid residues by BIOVIA discovery analysis.

Table 4: Evaluation of pharmacokinetics properties for each ligand

Parameters		Compounds									
		N,N'-naphthalene-1,4-diylbis	**MG132**	**NAD**	**Apigenin**	**Rutin**	**Quercetin**	**Alpha-bergamotene**	**Alpha-cadinol**	**Methyl cinnamate**	**Methyl eugenol**
Absorption	Caco-2 permeability (log Papp in 10^{-6} cm/s)	-0.354	0.77	-0.625	1.007	-0.949	-0.299	1.395	1.479	1.442	1.358
	Intestinal absorption (human) (% Absorbed)	89.182	64.418	10.114	93.25	23.446	77.207	96.229	94.296	97.453	94.532
Distribution	VDss (human) (log L/kg)	-1.716	0.424	0.451	0.822	1.663	1.559	0.861	0.42	-0.001	0.265
	BBB permeability (log BB)	-0.327	-0.955	-2.603	-0.734	-1.899	-1.098	0.86	0.596	0.238	0.422
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No
	CYP3A4 substrate	Yes	Yes	No	No	No	No	Yes	No	No	Yes
	CYP1A2 inhibitor	No	No	No	Yes	No	Yes	No	No	Yes	Yes
	CYP2C19 inhibitor	Yes	Yes	No	Yes	No	No	No	No	No	No
	CYP2C9 inhibitor	Yes	No	No	No	No	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No
	CYP3A4 inhibitor	Yes	Yes	No	No	No	No	No	No	No	No
Excretion	Total Clearance (log ml/min/kg))	0.375	1.14	0.07	0.566	-0.369	0.407	1.176	1.085	0.814	0.388
	Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No
Toxicity	AMES toxicity	No	No	No	No	No	No	No	No	No	No

Table 5: The binding affinity and the amino acid interaction between selected *O. basilicum* bioactive compounds with Keap1

Protein	Compounds	Binding Affinity (kcal/mol)	Interaction	
			Hydrogen	Van der Waals
Keap1 (4IQK)	N,N'-naphthalene- 1,4-diylbis(4-methoxybenzene-sulfonamide)	–9.4	Gln530, Ser508	Gly364, Gly462, Gly509, Gly603, Ile461, Phe577, Ser555, Ser602, Tyr334, Tyr572
	Apigenin	–8.5	Val463	Ala510, Gly364, Gly367, Gly462, Gly464, Gly509, Gly603, Gly605, Leu365, Val465, Val604
	Rutin	–10.2	Asn414, Ile416, Ser363, Val604	Ala366, Ala510, Asn382, Gln530, Gly364, Gly464, Gly511, Gly558, Gly603, Gly605, Ile461, Leu365, Leu557, Phe577, Ser508, Tyr572, Val463
	Quercetin	–8.4	Gly364, Ser508	Ala366, Arg483, Gly417, Gly462, Gly603, Ile416, Leu365, Leu557, Phe478, Tyr525

Table 6: The binding affinity and the amino acid residues interaction between selected *O. basilicum* bioactive compounds with NFKB

Protein	Compounds	Binding Affinity (kcal/mol)	Interaction	
			Hydrogen	Van der Waals
NFKB (3DO7)	MG-132	–6.7	–	Arg52, Lys221
	Alpha-bergamotene	–4.9	–	Arg52, Glu58, Ser222
	Alpha-cadinol	–4.8	–	Gln284, Lys252
	Methyl Cinnamate	–4.8	Ser188	Arg52, Asp219, His140, Phe53
	Methyl Eugenol	–4.8	Ser188	Arg52, Asp219, His140, Phe53, Ser222

In another study, Ji et al. demonstrated that flavonoid quercetin prevents hepatotoxicity by inhibiting Keap1 and Nrf2, thus increasing antioxidative genes.[60]

The docking result demonstrated that alpha-bergamotene (–4.2) (Figure 4) has the strongest binding affinity with NFκB than alpha-cadinol (–4.8), methyl cinnamate (–4.8), and methyl eugenol (–4.8). Two-dimensional (2D) interaction also found that amino acid residue Arg52 appears in molecular docking between NFκB with MG-132, alpha-bergamotene, methyl cinnamate, and methyl eugenol (Table 6). The selected essential oil from *O. basilicum* bound to NFκB at amino acid residues with pharmacophore regions. Arg52, Arg54, and Glu58 from loop L1 and Lys 221 from the linker are responsible for base-specific interactions at NFκB. In contrast to the hydrophobic carbons and rings of DNA bases, Glu58 interacted polarly with the core of flanking CCC:GGG sequences. Ser222 was the interdomain linker that made water-mediated contact with the DNA backbone.[56] Alpha-bergamotene showed Arg52 and Glu58 as amino acid residues, while methyl cinnamate and methyl eugenol also bound to Arg52 of NFκB; thus, these findings indicated that *O. basilicum* essential oil bound to an important region of NFκB.

Molecular docking results showed that alpha-cadinol (Figure 5) has the strongest binding affinity (–5.9) to SIRT1 compared with alpha-bergamotene (–5.0), methyl cinnamate (–5.4), and methyl eugenol (–5.5). Gln361, Gly364, and Ser365 are also found mostly in drug control and essential oil compounds of *O. basilicum* (Table 7). Furthermore, SIRT1-ligand docking demonstrated that there are several amino acid residues similar to those of drug control and the essential oil of *O. basilicum*, namely Gln361, Gly364, and Ser365. Gly364 was reported as one of the interactive sites in SIRT1, further increasing SIRT1 deacetylase activity to promote its function to regulate metabolism and gene expression.[56]

Conclusion

The present study demonstrated that *O. basilicum*'s active compounds, both flavonoid and essential oil content, could be used to treat stress-induced inflammation via the KEAP1/NFκB/SIRT1 signaling pathway. Flavonoid content, especially rutin, has proven its ability as an inhibitor of KEAP1 greater than KEAP1 drug control. Essential oil from O. *basilicum* also might play a direct role as an NFκB inhibitor and SIRT1 activator as a nuclear factor due to its lipid-based structure, which directly passes through the phospholipid bilayer. However, further investigation is necessary to determine the role of the *O. basilicum* active compound through in vitro and in vivo studies.

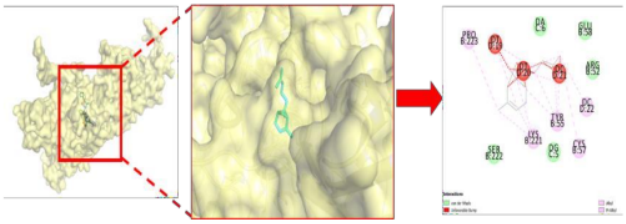


Figure 4: Visualization of NFκB (brown) with small molecules (ligands). The blue ligand as alpha-bergamotene followed with list of amino acid residues analysis by BIOVIA Discovery Studio.

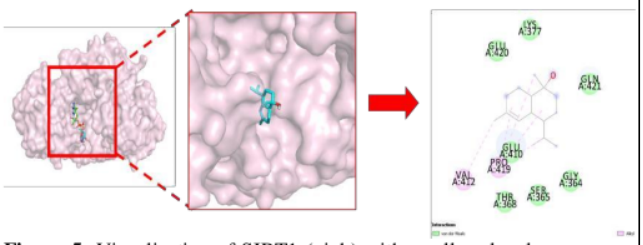


Figure 5: Visualization of SIRT1 (pink) with small molecules (ligands). The blue ligand as alpha cadinol followed with list of amino acid residues by BIOVIA Discovery Studio.

Table 7: The binding affinity and the amino acid interaction between selected *O. basilicum* bioactive compounds with SIRT1

Protein	Compounds	Binding Affinity (kcal/mol)	Interaction	
			Hydrogen	Van der Waals
SIRT1 (4I5I)	NAD	–9.6	Gln352, Gln361, Gln421, Gu410, Ile356, Ser365, Tyr376	Asn417, Gln357, Gly364, Ile359, Ile360, Leu418, Lys377, Pro419, Thr368, Val378, Val412
	Alpha-Bergamotene	–5.0	–	Gln352, Gln357, Gln361, Gln421
	Alpha-Cadinol	–5.9	–	Glu410, Glu420, Glu421, Ser365, Thr368
	Methyl_Cinnamate	–5.4	Ser365	Ala367, Asn417, Gln361, Gly364, Val412
	Methyl Eugenol	–5.5	Ser365, Thr368	Ala367, Asn417, Gln361, Gly364, Val412

A Computational Insights of Ocimum basilicum Flavonoid and Essential Oils Interaction in the Targeting Keap1/SIRT1/NFKB Signaling Pathway

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