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Anti-aging Activity, *In Silico* Modeling and Molecular Docking from *Sonneratia Caseolaris*

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Abstract

BACKGROUND: Anti-aging agents contribute to the prevention and control of skin photoaging. Antioxidant containing cosmetic has anti-aging therapy that can inhibit free radical formation. Sonneratia caseolaris leaf extract has robust antioxidant activity.

AIM: This study aimed to determine the anti-aging activity in-silico and in-vitro.

METHODS: In vitro antioxidant potential was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-Azino-bis-(3-ethylbenzothiazoline-6-sulfonate) cation (ABTS+) radical scavenging and FRAP. Investigation of in-silico docking activity was done for ROS (3ZBF), collagenase (966C), hyaluronidase (1FCV) receptors. Metabolomics analysis were conducted through HR-LCMS on the extract Sonneratia caseolaris. To explore the use value of antiaging, we analyzed the molecular docking of metabolites profiling Sonneratia caseolaris.

RESULTS: The result of metabolite profiling on the HR-LCMS from Sonneratia caseolaris extract are Luteolin, Betaine, and Choline. Molecular docking involves the exploration of protein or nucleotide, 3D structural modeling, and binding energy calculation. DPPH method showed IC50 28.214±0.809 ppm. The ABTS method showed IC50 1.528±0.042 ppm and FRAP is 345,125±4,196 mM/g sample. The compound luteolin had the Lowest binding energy scores with most of the target proteins: ROS (-8,3), collagenase (-11), and hyaluronidase (-6,8), according to molecular docking results.

CONCLUSION: It concluded that the study indicates extract Sonneratia caseolaris has the potential to be developed as a new drug for antiaging.

Introduction

Pidada merah (*Sonneratia caseolaris*) is a woody mangrove species widely distributed in tropical coastal areas [1] *S. caseolaris* isolate contains luteolin and luteolin 7-O-β glucoside compounds that provide antioxidant, antibacterial, and anti-cancer benefits [2] Research [3] found that the plant contains compounds of alkaloids, flavonoids, glycosides, saponins, and phenols. In this Sonneratia species, secondary metabolites are found, called (-)-(R)-nyasol, (-)-(R)-4'-O-methylnyasol, and maslinic acid that showed cytotoxic activity against glioma rat cell lines. C6 with IC_{50} values of 19.02, 20.21, and 31.71 ppm, respectively [4] According to [5] of the 62 compounds in the ethanol extract of Pidada merah leaves, which are thought to be efficacious, they are choline, betaine, and luteolin. *S. caseolaris* used as a traditional cosmetic product by the Dayak tribe (Native people in Borneo Island, Indonesia) called "bedak dingin." It is sold in traditional markets in Samarinda and Balikpapan. It contains some herbal medicines for skin care, but unfortunately, scientific evidence about it has not been known yet. Therefore, in this study, we focus on the leaves of *S. caseolaris* for its property as skin care through melanin biosynthesis and antioxidant assays. UV radiation generates reactive oxygen species (ROS). Antioxidants act as anti-aging (premature aging) because of their ability to maintain homeostasis in ROS in cells [6]. Based on that circumstance, the study aims to investigate the potential new anti-aging medicine from *S. caseolaris* leaves.

Materials and Methods

Plant materials and sample preparation

Pidada merah (*S. caseolaris*) leaves were collected from Sanga-sanga, Kutai Kartanegara, East Borneo, Indonesia during January 2022. It identified in Department Biology, Faculty of Math and Natural Science, Universitas Mulawarman, Indonesia.

The leaves were cleaned, separated, and then dried. After that, it is ground into a fine powder. The ground samples were sieved to get uniform particle size, then kept in an air-tight container and stored until further analysis.

Extraction

As many as 100 g of powdered *S. caseolaris* leaves were put in a maceration container and added the ethanol 95% until the simplicia was submerged. Put aside it for 24 h and stir occasionally. The simplicia filtered and separated from the dregs. Furthermore, the dregs were macerated again using a new ethanol filter. It was conducted for 3 consecutive days. The ethanol 95% extract of sansevieria leaves was concentrated by a rotary evaporator [7].

Determination of total phenolics contents

Total phenolics content was determined using Folin–Ciocalteu reagent adapted from [8] with slight modifications. The Folin–Ciocalteu reagent (previously diluted 10-fold with distilled water) 2.25 ml was mixed with 300 ml of extract and set aside at room temperature for 5 min, then added 2.25 ml of sodium carbonate (60 g/l) solution. After 90 min, the absorbance was measured at 725 nm using a spectrophotometer. The results were expressed as equivalent of mg gallic acid in 1 g of dried sample (mg GAE/g).

Physicochemical examination

Physicochemical examination of the standardized extract was identified using the high resonance mass spectrophotometer (HRMS).

Diphenyl-2-picrylhydrazyl (DPPH) free-radical scavenging assay

The antioxidant activity was estimated using 1,1-DPPH as a free radical model. This method was adapted from [9]. The antioxidant activity test was conducted by making a 40 ppm DPPH solution, in which 0.004 g of DPPH added ethanol to 100 ml, then from 2 ml of the 40 ppm DPPH solution, the absorption was observed in the range of 450–600 nm to determine the maximum wavelength. The ascorbic acid standard was used as a positive control using several concentrations ranging from 5 to 15 ppm. Preparation of standardized extracts obtained concentrations of 10–50 ppm, set aside for 30 min to observe the absorption at each concentration. Then determine the absorbance by adding DPPH, that is, from each concentration series, 2 ml pipetted, and 4 ml of 40 ppm DPPH solution. Set aside it for 30 min to observe the absorption at each concentration, and then calculate the free radical inhibitory activity using the formula:

Inhibition percentages (%)=[(blank absorbance-sample absorbance)/blank absorbance]×100

Furthermore, in calculating the percentage value of inhibition, a linear equation was made to determine the IC_{50} value, which was the radical inhibitory activity. The result is the IC_{50} value which is then categorized.

ABTS (2,2'- azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

To begin with, 7.4 mM ABTS solution was made with 203 mg of ABTS powder and then dissolved with distilled water to 50 mL. Afterward, prepare a solution by weighing 64.8 mg of potassium persulfate powder dissolved in 50 mL of distilled water. Mix it well while homogenizing, then store the solution to avoid light. The solution that has been formed is the ABTS stock solution [10]. The dilution was performed 50 times by taking 200 μL of a mixture of ABTS stock solution and potassium persulfate and then diluting with ethanol to 10 mL. This solution is called the ABTS control. The absorbance of the ABTS control was read, while the ABTS control blank was ethanol. Prepared sample solution and sample blank by dissolving 25 μL of sample into a test tube and then adding 4975 μL of ABTS control. Homogenization was conducted with a vortex for 1 min, and then incubated for 17 min. The sample blank used was 25 μL of sample added to 4975 μL of ethanol, then homogenized with a vortex for 1 min. Let it stand for 30 min to observe the absorption that occurred at each concentration, and then the radical inhibitory activity was calculated. The final result was calculated as IC_{50}.

FRAP (Ferric reducing/antioxidant power) assay

The procedure was adopted from [11]. To begin, prepare a 10:1:1 mixture of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution, and 20 mM FeCl3–6H2O in a water bath and heat to 37°C. A total of 3.0 mL FRAP reagent was added to a test tube, and a blank reading was acquired with a spectrophotometer at 593 nm. The cuvette was filled with 100 L of selected plant extracts and 300 L of distilled water. After 90 min of incubation at 37°C in a water bath, a second reading at 593 nm was performed after adding the sample to the FRAP reagent. The change in absorbance after 90 min from the initial blank was compared with the standard curve. Standard concentrations of Fe (II) were known using several concentrations ranging from 100 to 1000 M. A standard curve was then developed by plotting the FRAP value of each standard versus its concentration. The result expressed the concentration of antioxidants that can reduce iron in a 1 gram sample (μM/g).

In silico validation

Three distinct proteins — collagenase (966C), ROS (3ZBF), and hyaluronidase (1FCV) — are associated with anti-aging received from the protein database, used to create the 3-D crystal structure of the protein. Protein data bases are three-dimensional structural data repositories for biological substances. The active site or receptor binding is determined using an online server. They are visual datasets that show the different molecules that make up their structure and a diagram of how those molecules are connected. The primary ID of the protein database from the protein structure was input into the home search box.

The protein preparation wizard is used to create complex protein structures. Hydrogen ions are added automatically, while the structure is refined and minimized. From the PubChem database [12], the available structures of Luteolin, Betaine, and Choline are taken. All ligands were prepared for docking studies using AutoDock Vina. Tautomers were developed and optimized for each ligand. The force field is used to calculate the partial atomic charge.

Molecular docking

The XP (extra precision) option was used to link inflexible protein structures with elastic ligands using Schrodinger Glide-v 7.4 created 100 poses for each docking calculation. To determine the potential for non-polarity elements of proteins and ligands, the Van Der Waals (VDW) bonds were adjusted to 1.0 with a net atomic charge cutoff of 0.25 sub-units. In contrast, other elements bonds VDW were not adjusted. Glide docking uses a conventional cluster-based method to find the best ligand-binding region in a particular receptor lattice plane. The ligand with the lowest score has the strongest binding affinity for the enzyme [13], [14]. Molecular docking studies were performed for three target proteins (collagenase (966C), ROS (3ZBF), and hyaluronidase (1FCV)) using Autodock Vina software.

Results and Discussion

HRMS results show structure A (Formula: C15 H10 O6, Molecular Weight: 286,047, RT [min]: 7,717, Area (Max.): 40366144,37, mz Cloud Best Match: 99,7 is Luteolin), B (Formula: C5 H11 N O2, Molecular Weight: 117,079, RT [min]: 0,903, Area (Max.): 143657529,38, mz Cloud Best Match: 96,7 is Betaine), C (Formula: C5 H11 N O2, Molecular Weight: 117,079, RT [min]: 0,903, Area (Max.): 143657529,38, mz Cloud Best Match: 96,7 is Choline)

S. caseolaris Leaves extract contains sterol hydrocarbons, fatty acid compounds, luteolin, betain, and choline. It also includes two flavonoids called luteolin andluteolin 7-O-β glucoside [1]. Figure 1, showed metabolite profiling on the HR-LCMS from s. caseolaris extract are Luteolin, Betaine, and Choline. *S. caseolaris* leaves inhibitory concentration 50 (IC_{50}) values of shoot leave extract, young leaves extract, and mature leaves extract are 12.0013 ppm, 13.9915 ppm, and 14.6613 ppm, respectively [15]. They are all referred to as antioxidants, which have potent effects. About 96% solvent ethanol was used to extract the phytochemical component with the highest concentration from shoot leaves.

The extract showed strong antioxidant capacity in the DPPH and ABTS assay. It exhibited more robust antioxidant activity and ascorbic acid as a positive control for antioxidant assays. Specifically, the ABTS assay is based on the generation of a blue/green ABTS+ reduced by antioxidants. In contrast, the DPPH assist assay based on reducing the purple DPPH to 1,1-diphenyl-2-picryl hydrazine. Both assays are convenient and most popular, but they are also limited because they use non-physiological radicals.

Table 1 showed that antioxidant capacity by ABTS assay was stronger than DPPH assay, independent sample t-test ($p < 0.001$). Compared with the DPPH assay's antioxidant capacity measured by the ABTS assay showed a significant correlation ($r = 0.878$; $p < 0.001$). Some studies of *S. caseolaris* Leaves [16] with DPPH showed an antioxidant effect IC_{50} value in methanol extract of 21.62 ppm, n-hexane fraction 82.36 ppm, ethyl acetate fraction 13.41 ppm, and the n-butanol fraction of 13.04 ppm. It means this plant has potent antioxidant activities.

Table 1: IC_{50} for DPPH and ABTS

Sample	DPPH (ppm)	ABTS (ppm)
Ascorbic Acid	11.524 ± 0.016	0.722 ± 0.003
Extract	28.214 ± 0.809	1.528 ± 0.042

DPPH: Diphenyl-1-picrylhydrazyl, ABTS: Azino-bis-(3-ethylbenzothiazoline-6-sulfonate.

Although the mechanism of action of DPPH (cation radical scavenging) and FRAP (iron ion reduction) is different, the results of these two assays correlated significantly in all samples tested. The FRAP assay is the only test that directly measures antioxidants or reducing agents in a sample. It measures the reducing ability of antioxidants that react with ferric tripyridyltriazine complex (Fe3+–TPTZ) and produce ferrous (Fe2+–TPTZ) [11], [17]. The results are expressed as the combined concentrations of all electron-donating reducing agents occurring in the sample in the various sample plants. The ability to reduce the tested extract from *S. caseolaris* leaves as shown in Table 2. It significantly correlated with phenolic ($p < 0.05$). Antioxidant capacity by both tests was more strongly correlated with total phenolics [18], [19]. Studies from Kim *et al*. [19] found that the antioxidant capacity measured by the ABTS test was highly significantly correlated with total phenolics. These findings indicate that phenolic compounds are significant contributors to antioxidants.

Table 3: The docking score, number of H-bonds, interacting residues, and bond length of the selected compounds, ROS (3ZBF), Collagenase (966 C), and hyaluronidase (1FCV)

ROS (3ZBF)				
	Skor docking	Number interaction	Residu Interaction	Bond Length (Å)
Native	–8.4	7	LEU1951	3.77
			MET2029	3.31
				3.70
			ALA1978	3.89
			LEU2086	3.86
				3.86
			ARG2083	3.11
			LYS1980	4.05
			VAL1959	4.24
Betain	–2.9	1	MET2029	1.96
Choline	–3.5	2	GLU2027	3.36
			MET2029	1.99
Luteolin	–8.3	7	LYS1980	5.37
			ALA1978	4.73
			LEU2026	5.43
			LEU2086	4.80
			VAL1959	5.06
				5.18
			MET2029	2.00
				1.93
			LEU1951	3.54
Collagenase (966 C)				
Native	–9.6	6	VAL215	5.33
			ASN180	3.05
			LEU181	1.96
				5.24
			ALA182	2.74
				1.88
			GLU219	2.42
				2.37
			HIS228	2.07
Betain	–3.9	4	GLU219	5.24
			LEU181	2.56
			ALA182	2.29
			HIS218	3.87
				4.78
Kolin	–4.6	4	ALA182	2.61
			SER239	3.47
			TYR237	3.42
				3.65
			HIS218	3.87
				3.99
				3.75
Luteolin	–11.0	5	LEU235	2.50
			SER239	4.07
			VAL215	5.06
			HIS218	4.41
			LEU181	5.14
Hyaluronidase (1FCV)				
Native	–8.0	8	SER304	1.88
				2.57
			GLN271	3.35
			GLU113	3.05
			TYR184	3.10
				3.73
			ASP111	2.98
			TYR227	1.85
			TRP301	3.74
			TYR55	3.54
Betain	–3.0	2	SER304	1.94
			TYR55	4.30
				3.60
Kolin	–3.5	4	TYR184	3.71
			TRP301	3.45
				4.36
			GLU113	4.52
				3.59
			ASP111	4.95
				3.65
				3.62
Luteolin	–6.8	3	ASP111	3.96
			GLU113	3.57
			TYR55	3.75
				3.87

ROS: Reactive oxygen species.

Molecular docking was used to simulate the potential binding mechanism of phytochemicals from S. caseolaris to a protein associated with aging.

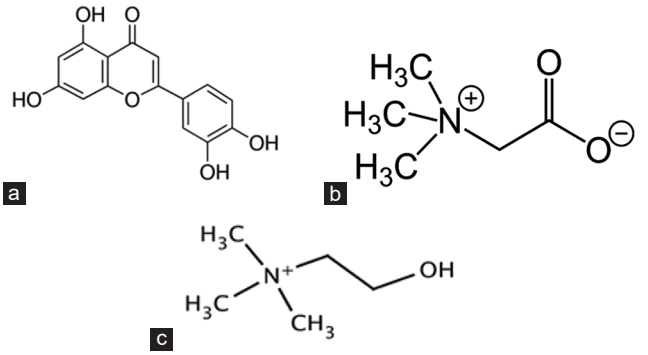

Figure 1: (a) Luteolin, (b) Betaine, (c) Choline

Collagenase (966 C), matrix ROS (3ZBF), and hyaluronidase (1FCV) all have their three-dimensional structures taken from the RCSB protein data bank.

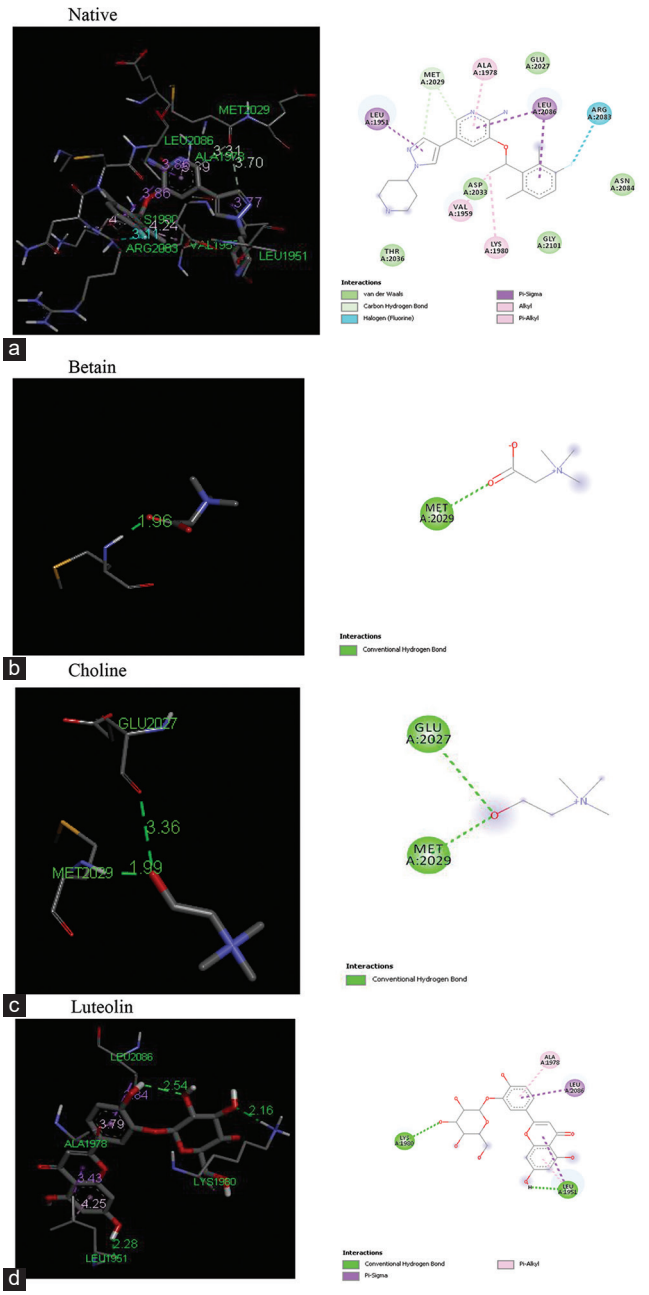

Figure 2: Molecular docking of ROS (3ZBF) with (a) Native, (b) Betain, (c) Choline, (d) Luteolin

Table 2: FRAP and Total Phenolic

Sample	Rep	FRAP		Total Phenolic	
		Concentration (mM/g sample)	Average ± SD	Concentration (mg GAE/g)	Average ± SD
Extract	1	349.425	345.125 ± 4.196	206.0632	204.3408 ± 1.7224
	2	339.433		200.896	
	3	346.517		206.0632	

FRAP: Ferric reducing/antioxidant power.

The data were then cleaned up by removing any co-crystallized ligand and crystallographic water. Molecular docking simulation was carried out with AutoDock Vina's default settings (Vina). The best-docked conformation determined by vina scoring was employed for the visual analysis [20]. Pose View, a program available through Protein PDB, was used to infer the intermolecular interactions of the protein-ligand combination.

To propose a molecular-level explanation of ROS, collagenase, and hyaluronidase inhibition enzymes by the best inhibitors (luteolin, docking scores are –8,3, –11, and –6,8). Luteolin principally attributed to the hydrogen bond interactions related to the residues LYS1980, ALA1978, LEU2026, LEU2086, VAL1959, MET2029, and LEU1951 in ROS enzyme, LEU235, SER239, VAL215, HIS218, and LEU181 in collagenase and ASP111, GLU1113 and Tyr 55 for hyaluronidase. A molecular docking study was conducted to assume the model where the protein and ligand were considered rigid and flexible during the docking procedure [21]. Unfortunately, the sameness in the estimated docking score, number of H-bonds, interacting residues, and bond length are shown in Table 3. The reason there why there is a limitation of the docking model for how the compounds could arrive at the active site. The procedure starts with the active ligand at the site in all the molecular docking experiments.

The molecular docking of phytochemical compounds showed in Figure 2: ROS (3ZBF), Figure 3: Collagenase (966 C) and Figure 4: Hyaluronidase

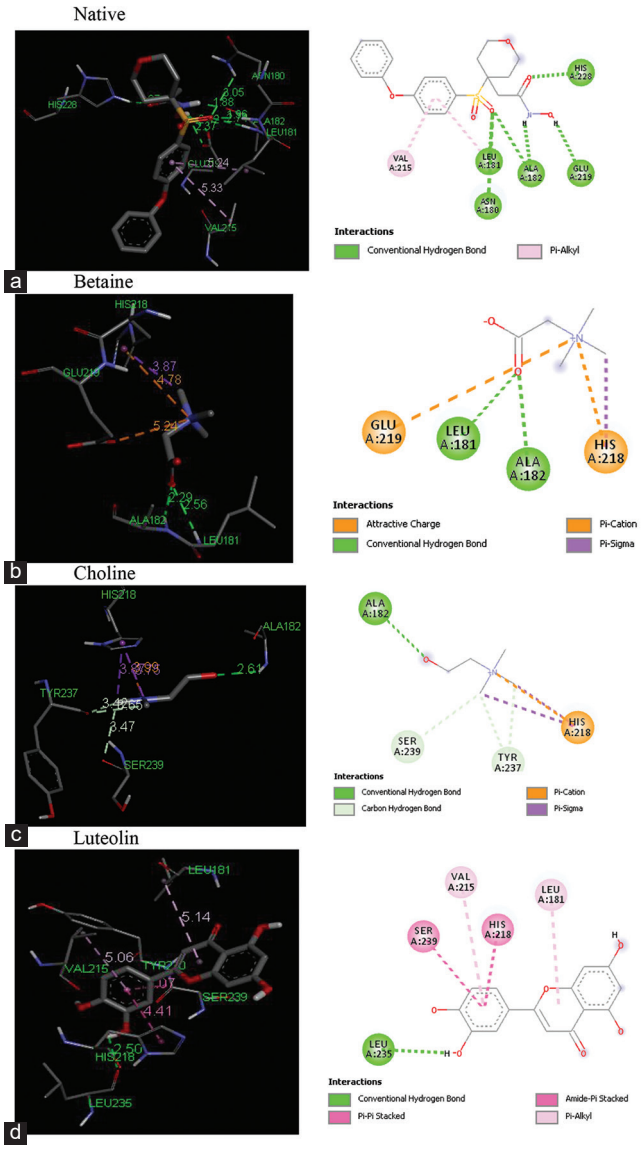

Figure 3: Molecular docking of Collagenase (966C) with (a) Native, (b) Betain, (c) Choline, (d) Luteolin

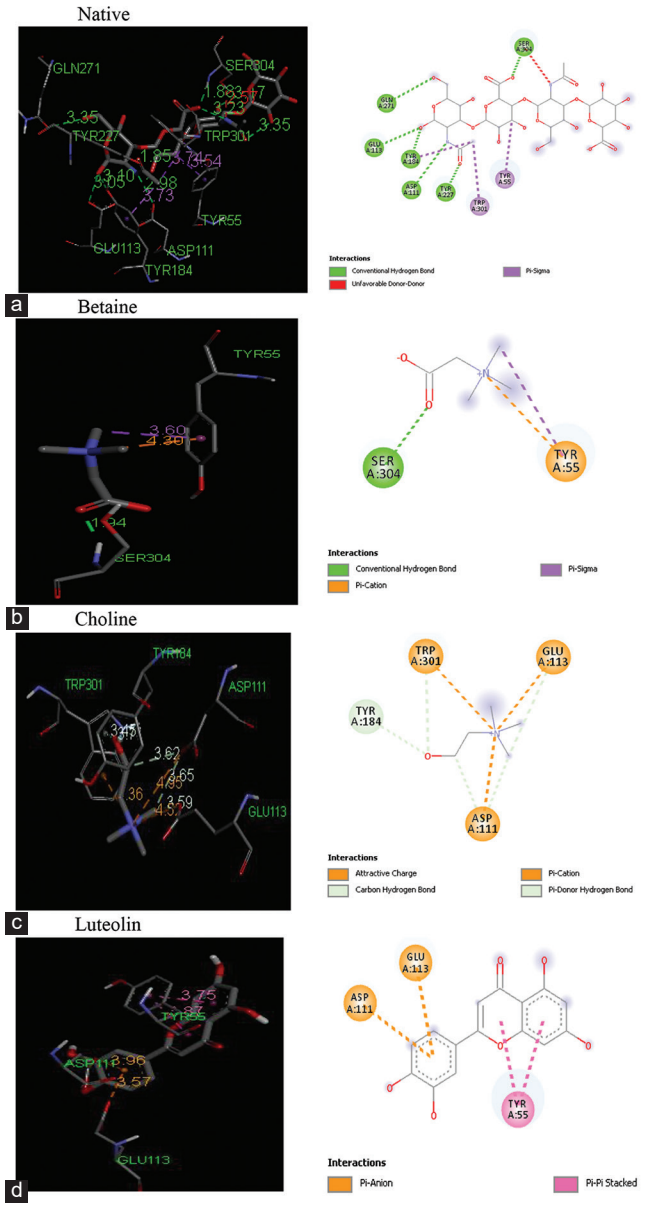

Figure 4: Molecular docking of Hyaluronidase (1FCV) with (a) Native, (b) Betain, (c) Choline, (d) Luteolin

(1FCV) a. Native, b. Betain, c. Choline, d. Luteolin. The protein showed as surface representation. The ligand showed as a stick representation. Aging is a natural process affecting various body organs. It is often shown by ROS build-up in cells [22]. In a normal situation, ROS holds a vital role in various biological processes, such as immune response, but radical homeostasis is impaired through various stimulus. Increased cytoplasmic ROS can induce the synthesis related to the degradation of the extracellular matrix, causing tissue structural diminishment that manifests as the formation of wrinkles and sagging elasticity [23]. The antioxidant phytochemical compound helps decrease ROS-induced skin damage [24]. Not only relieves the oxidative stress, but it also decreased collagen degrading enzyme activity [25]. The present study found luteolin from Extract *S. caseolaris* has strong antioxidant potency based on its measured reductive capacity. Thus, Extract *S. caseolaris* may alleviate skin cell damage caused by oxidative stress. Extracting *S. caseolaris* may also indirectly attenuate enzyme-related activity in the degradation of the extracellular matrix.

Their potent inhibition of collagenase, ROS, and hyaluronidase activities, particularly with the potent antioxidant activity. Skin is one of the major targets of oxidative stress caused by reactive species (RS), including ROS and reactive nitrogen species. RS are major and significant contributors to skin hyperpigmentation and aging [26]. It is believed that antioxidant agents show anti-aging, whitening, and anti-inflammatory activities [27]. By considering the results of antioxidant activities against DPPH, ABTS, and FRAP, luteolin should be a good candidate for skin-whitening and antioxidant medication.

Conclusion

In this study, extract of *S. caseolaris* leaves demonstrated the inhibitory activities for anti-aging. The extract showed very strong antioxidant capacity in the DPPH, ABTS, and FRAP assay. The findings of this studies *in silico* model revealed that luteolin has already been approved for anti-aging. Luteolin docked for each target ROS (3ZBF), Collagenase (966 C), Hyaluronidase (1FCV), and observed to be strongest docked have shown significant binding energy, a significant percentage of hydrogen bonds, and hydrophobic interactions with their selected molecular targets. Thus, Luteolin from *S. caseolaris* leaves extract can be a potential new anti-aging drug.

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