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The Potential of Clove (*Syzygium aromaticum*) Essential Oil as Sunscreen and Anti-Aging Agents: An *In Vitro* and *In Silico* Study

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Abstract

The earth thermal conditions increase exposure to sunlight, triggering skin damage. The skin has defense mechanisms but needs additional protection to prevent photoaging. Using sunscreen is an effort to reduce the harmful effects of sun exposure. Natural ingredients with antioxidant activity can be developed into sunscreen and antiaging products, including cloves. This study aims to assess the antioxidant, sun protection, and anti-aging properties of essential oils derived from clove leaves and buds. The antioxidant activity was assessed by the DPPH system and then formulated into 5 and 10 % clove leaves cream, as well as 5 and 10 % clove bud cream. The SPF values were assessed using a spectrophotometer and the Mansur formula. The antiaging potential was assessed using the *in silico* method with MMP-1 protein employing the Auto Dock Vina software, followed by analysis and visualization using BIOVIA Discovery Studio. The essential oils from clove leaves and buds have DPPH IC_{50} values of 23.09 and 30.31 ppm. The SPF values of clove leaves essential oil, 5 % leaves cream, and 10 % leaves cream are 38.61, 14.32, and 22.15. Meanwhile, the SPF values of clove bud essential oil, 5 % bud cream, and 10 % bud cream are 38.19, 9.32, and 18.16. The *in silico* test revealed that caryophyllene, eugenol, humulene, and phenol, 2-methoxy-4-(2-propenyl), acetate) have strong interactions because they can interact with the active site of proteins using hydrogen, hydrophobic, and van der Waals bonds. The essential oils from clove leaves and buds exhibit very strong antioxidant activity and offer ultra-protection as a sunscreen. The cream formulations still exhibit sunscreen activity, albeit at a lower level compared to pure essential oil. Caryophyllene, eugenol, humulene, and phenol, 2-methoxy-4-(2-propenyl)-, acetate), have potential as antiaging agents, as indicated by their binding affinity and chemical bond interactions observed in the *in silico* test.

Keywords: Antiaging, Antioxidant, Clove (*Syzygium aromaticum*), Molecular docking, Sunscreen, SPF

Introduction

Indonesia is a tropical country where most of the population works outdoors. This situation increases the skin's exposure to direct sunlight [1]. UV radiation acts as an external factor prompting the generation of reactive oxygen species (ROS), initiating oxidative stress. Oxidative stress damages the structure and

function of the skin, resulting in an aging skin phenotype such as wrinkles, rough skin surface, telangiectasia, and uneven skin color [2]. The application of substances that can protect the skin against the dangers of UV rays can prevent the initiation of premature aging due to UV. Using sunscreen is an effort to prevent the harmful effects of UV rays.

The sunscreen's general mechanism of action is to reflect or absorb UV rays. Several natural ingredients have been proven to exhibit sunscreen activity. The sunscreen activity of natural ingredients is related to the active compounds in these plants. Generally, the plants used also have antioxidant activity. Some plants that have been proven to contain sunscreen-active ingredients include aloe vera, grapes, green tea, tomatoes, cucumbers, and almonds. Clove (*Syzygium aromaticum*) is a plant with antioxidant activity [2-4].

Clove plants contain saponins, tannins, alkaloids, glycosides, and flavonoids [5]. Wahyuningsih *et al*. [3] showed that cloves contain p-amino benzoic acid (PABA), octyl salicylate, octyl cinnamate, anthranilate, and benzophenone, which have the potential to be sunscreen agents. Other studies stated that clove leaf extract contains flavonoids with high antioxidant activity [6-9]. The eugenol content in cloves could prevent the aging process through oxidative processes and induction of collagen synthesis [10].

The antiaging mechanism works by inhibiting the enzyme system involved in the aging pathway, one of which is the matrix metalloproteinase-1 (MMP-1) enzyme. The enzymes that play a role in MMP are collagenase, tyrosinase, and elastase [2]. The antiaging activity test is conducted by assessing the inhibitory activity of MMP enzymes, both via *in vitro* and *in silico* methods. Mohtar *et al*. [6] reported that the eugenol ligand, an identity compound in cloves, has an *in silico* potential as an anticancer agent against ER-α, ER-β, and Her-2 receptors in breast cancer. A previous research by Dewi *et al*. determined the potential of ellagic acid as an anti-photoaging agent through a molecular mechanism targeting the MMP-1 enzyme *in silico*. The study proved that ellagic acid has anti-photoaging activity because it has an affinity with the target protein, namely the MMP-1 enzyme [7]. Currently, there is limited data proving clove compounds' antiaging effects. Based on the background provided, the authors aim to investigate the potential of clove essential oil as a sunscreen and antiaging agent using *in vitro* and *in silico* methods.

Materials and methods

This experimental laboratory research employed both *in vitro* and *in silico* approaches, utilizing clove leaves and bud essential oils. The samples utilized in this study were essential oils derived from clove leaves and buds obtained from the the Lhoknga plantation, Aceh Besar district, Indonesia specificallay located at 5°28'51.5"N 95°15'15.7"E coordinate. The essential oil extracted from both clove leaves and buds through the steam distillation method. These oils were formulated into creams containing 5 and 10 % clove leaves essential oil, as well as 5 and 10 % clove bud essential oil. Some of the essential oils were underwent phytochemical analyzed qualitative [11] and semi-quantitatively with GC-MS. The 5 most abundant compounds from the GC-MS results became ligands for the *in silico* testing.

Gas chromatography and mass spectroscopy (GC-MS)

The phytochemical analysis semi-quantitatively of each clove essential oils was performed using the Shimadzu - QP2010 Ultra (Shimadzu Corporation, Kyoto, Japan). Helium was used as the carrier gas, passing through the gas chromatography column Rtx-5MS (Shimadzu Corporation, Kyoto, Japan) at a flow rate of 1.2 mL/min. The oven temperature ascended from 30 to 300 °C at a rate of 10 °C/min, with the injector port kept at 270 °C. 1 µL of the mixture was injected into the GC-MS instrument after 90 µL of extract and 10 µL of trimethyl silane hydrogen (TMSH) were mixed [12].

Research procedure

The extracts in the form of essential oils from clove leaves and buds were made into 5 and 10 % cream preparations, which were used as test substances in this study. **Table 1** displays the cream formulation [13].

Table 1 Cream formulations of 5 and 10 % clove cream and base cream.

Ingredient	Amount (g)		
	5 % Clove Cream	**10 % Clove Cream**	**Cream Base**
Stearic acid	6	6	6
Cetyl alcohol	6	6	6
Vaseline	39	39	39
Distilled water	61.5	56.5	66.5
Glycerine	15	15	15
Triethanolamine	3	3	3
Phenoxyethanol	0.5	0.5	0.5
Clove essential oil	5	10	0

Antioxidant activity test

DPPH radical scavenging activities were undertaken based on a modified method by Kikuzaki *et al*. [14] using a 96-well microplate. Briefly, 50 µL of clove essential oil was obtained from leaves and flowers was serially diluted to concentration 5 - 250 ppm. These concentrations were subsequently added to each well of a 96-well microplate containing 100 µL of 0.4 mM DPPH (dissolved in methanol). After vortex mixing and incubation for 30 min, the absorbance at 517 nm was measured using. the ELISA microplate reader. The antioxidant activity is determined based on the IC_{50} value that calculated using the formula: Percent (%) radical capture = [(A0-A1)] ÷ A0×100 %. A0 is the absorbance of the control and A1 is the absorbance of the sample.

Sunscreen activity test

The sunscreen activity was tested by determining the SPF value using UV-Vis spectrophotometry. Clove leaves and bud essential oils, 5 and 10 % clove leaves creams, and 5 and 10 % clove bud creams were prepared at concentrations of 600, 400, and 200 ppm using analytical-grade ethanol. Each sample was put into a 100 mL volumetric flask and then diluted with 70 % ethanol. Ultrasonication was subsequently carried out for 5 min, followed by centrifugation for 5 min. The absorbance of each cream sample was read at wavelengths between 290 - 320 nm with 5 nm intervals. The blank sample was analytical-grade ethanol. The absorbance results were used to calculate the SPF value of the cream compared to pure essential oils and cream base using the following formula:

$$SPF_{spectrophotometry} = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda) \quad (1)$$

where:

EE : *Erythemal effect spectrum*

I : *Solar Intensity Spectrum*

Abs : *Absorbance of sunscreen product*

CF : *Correction Factor (10)*

In silico antiaging potential test

The 3 most abundant compounds obtained from the GC-MS results became the ligands for antiaging testing utilizing a method adapted from Salas *et al*. [15]. The test began with the preparation of the ligand structure in the form of compounds from clove essential oil, as well as 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid as the control ligand. The test and control ligands were obtained from the PubChem database downloaded in *sdf format. The ligands were then optimized using AutoDock Vina, which is integrated into the PyRx software 0.9 and saved in *pdbqt format. Next, the receptor structure was prepared, namely the matrix metalloproteinase-1 (MMP1) protein, obtained via http://www.rscb.org page with the collagenase code 1SU3 and downloaded in *pdb format. The molecular docking using Auto Dock Vina integrated into PyRx software was carried out between collagenase and the prepared essential oil compounds. Interaction sites were analyzed based on ligand-residue interactions and 3D structure. Compounds with the lowest binding energy or equivalent to the positive control in kcal/mol were visualized using BIOVIA Discovery Studio software 2021. Finally, energy and chemical bond analyses were conducted by selecting a model with the lowest ΔG and the 3D visualization closest to the receptor area. The receptor-control ligand interaction model was incorporated using Discovery Studio. The merging results were saved in PDB format and analyzed for energy, hydrogen bonds, and hydrophobic interactions using BIOVIA Discovery studio software. The analysis was carried out by comparing the visualization of the ligand docking area on the receptor to that of the control ligand. The antiaging activity was predicted based on the ability of the ligand (a compound in the extract) to bind to the collagenase receptor to form a stable complex, which is assessed based on the affinity energy and inhibition constant of the test ligand.

Statistical analysis

Antioxidant activity are presented in IC_{50} values which are calculated based on the radical capture percentage formula using the regression equation curve (in ppm). The SPF value is computed following the respective formula. Subsequently, the categories are determined as minimum, medium, extra, and ultra-activities. The antiaging activity data were predicted based on the ability of the ligand (a compound in the extract) to bind to the collagenase receptor to form a stable complex, which is assessed based on the affinity energy and inhibition constant of the test ligand.

Results and discussion

Study result

The phytochemical screening test results discovered that the essential oil in clove leaves in this study contained secondary metabolite compounds, namely alkaloids, tannins, and triterpenes. Meanwhile, clove buds contain alkaloids, polyphenols, and triterpenes.

Figure 1 and **Table 2** present the results of the semiquantitative analysis of clove leaves essential oil. The GC-MS spectra shows ten components in the clove leaves essential oil with eugenol (50.40 %), caryophyllene (37 %), and humulene (7.18 %) being the most abundant components.

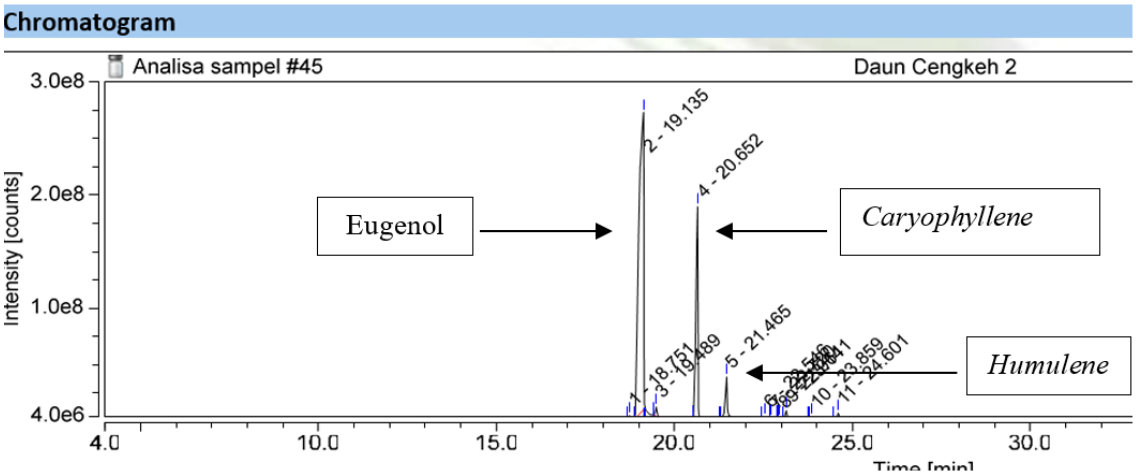

Figure 1 Chromatogram of clove leaves essential oil using GC-MS.

Table 2 The chemical composition of clove leaves essential oil.

No.	Compounds	Percentage (%)
1	a-Cubebene	0.82
2	**Eugenol**	**50.40**
3	(1S,4aR,8aS)-1-Isopropyl-7-methyl-4- methylene-1,2,3,4,4a,5,6,8a- octahydronaphthalene	1.59
4	**Caryophyllene**	**37**
5	**Humulene**	**7.18**
6	Benzene, 1,2-dimethoxy-4-(1-propenyl)-, (E)-	0.52
7	Phenol, 3,5-bis(1-methylethyl)-	0.34
8	3-Oxabicyclo[4.2.0]oct-5-ene, endo-8- methyl-exo-8-(2-propenyl)-	0.22
9	1-Isopropyl-4,7-dimethyl-1,2,3,5,6,8a- hexahydronaphthalene	1.47
10	1H-Indene, 1-ethylideneoctahydro-7a- methyl-, cis-	0.46

The results of the chemical composition analysis of clove bud essential oil are presented in **Figure 2** and **Table 3**. The GC-MS spectra shows 32 components in the clove bud essential oil with eugenol (57.16 %), caryophyllene (16.35 %), and 2 phenol, 2-methoxy-4-(2-propenyl)-, acetate (10.21 %), as the most abundant components.

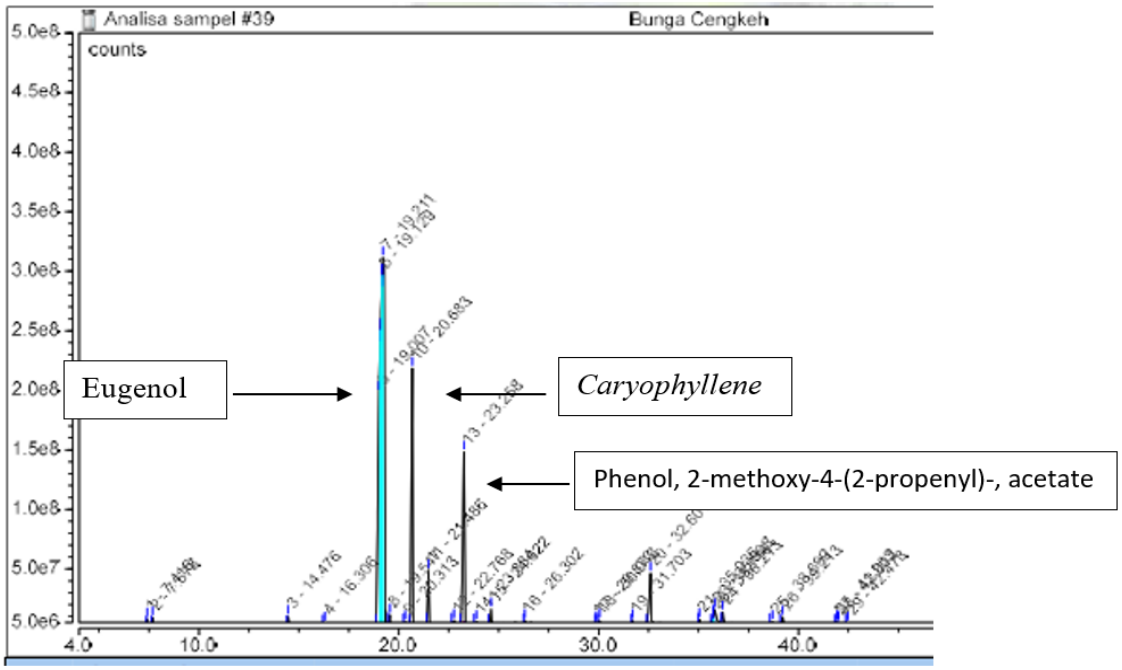

Figure 2 Chromatogram of clove bud essential oil using GC-MS.

Table 3 The chemical composition of clove bud essential oil.

No.	Compounds	Percentage (%)
1	Ethanone, 1-(3-ethyloxiranyl)-	0.48
2	Hexane, 1-(3-butenyloxy)-	0.55
3	Methyl salicylate	0.59
4	Phenol4-(2-propenyl)-	0.16
5	**Eugenol**	**57.16**
6	Copaene	0.56
7	1H-Indene, 1-ethylideneoctahydro-7amethyl-, cis	0.17
8	**Caryophyllene**	**16.35**
9	1,4,7,-Cycloundecatriene, 1,5,9,9tetramethyl-,Z,Z,Z	3.25
10	2H-Benzo[f]oxireno[2,3-E]benzofuran-8(9H)one, 9-[[[2(dimethylamino)ethyl]amino]methyl]octahydro-2,5a-dimethyl-	0.20
11	**Phenol, 2-methoxy-4-(2-propenyl)-, acetate**	**10.21**
12	trans-Z-a-Bisabolene epoxide	0.20
13	Isoaromadendrene epoxide	0.27
14	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.18
15	2-Pentadecanone, 6,10,14-trimethyl-	0.30
16	Hexadecanoic acid, methyl ester	0.28
17	n-Hexadecanoic acid	3.08

No.	Compounds	Percentage (%)
18	Cyclopropaneoctanoic acid, 2-[[2 [(2ethylcyclopropyl)methyl]cyclopropyl]methyl]-,Methyl ester	0.30
19	(Z)-18-Octadec-9-enolide	0.50
20	cis-Vaccenic acid	0.89
21	Octadecanoic acid	0.67
22	2H-Cyclohepta[b]furan-2-one, 6-[1(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8ahexahydro-7-methyl-3-methylene-	0.17
23	2H-Cyclohepta[b]furan-2-one, 6-[1(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8ahexahydro-7-methyl-3-methylene-	0.48
24	2H-Cyclohepta[b]furan-2-one, 6-[1(acetyloxy)-3-oxobutyl]-3,3a,4,7,8,8ahexahydro-7-methyl-3-methylene-	0.21
25	cis-Z-a-Bisabolene epoxide	0.19
26	3',8,8'-Trimethoxy-3-piperidyl-2,2'binaphthalene-1,1',4,4'-tetrone	0.15
27	1-Heptatriacotanol	0.24
28	Stigmastan-6,22-dien, 3,5-dedihydro-	0.13
29	1-Heptatriacotanol	0.30
30	Stigmasta-3,5-diene	0.71
31	Stigmasterol	0.24
32	ß-Sitosterol	0.80

The results of the antioxidant activity test of clove leaves and bud essential oils are shown in **Table 4**. In this study, the IC_{50} of clove leaves essential oil and clove bud essential oil are 23.09 and 30.31 ppm, respectively. A compound is considered a powerful antioxidant if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is between 50 - 100 ppm, moderate if the IC_{50} value is between 100 - 150 ppm, and weak if the IC_{50} value is between 151 - 200 ppm.

Table 4 Antioxidant activity of clove leaves and bud essential oils.

Parameters	IC_{50} (ppm)
Clove leave	23.09 ± 0.36
Clove buds	30.31 ± 0.66

Sunscreen preparations can protect against UV B radiation if their SPF value is between 2 and 100. The Food and Drug Administration (FDA) classifies sunscreen strength based on SPF values: 2-4 (minimum), 4 - 6 (medium), 6 - 8 (extra), 8 - 15 (maximum), and > 15 (ultra). **Table 5** shows the UV-Vis spectrophotometric SPF values of creams prepared from clove leaves and bud essential oils.

Table 5 SPF values of clove leaves and bud essential oils and cream preparations.

Sample	SPF Value	Category
5 % clove leaves cream	14.32	Maximum
5 % clove bud cream	9.32	Maximum
10 % clove leaves cream	22.15	Ultra
10 % clove bud cream	18.16	Ultra
Clove leaves essential oil	38.61	Ultra
Clove bud essential oil	38.19	Ultra
Cream base	0.23	No activity

Table 5 shows that clove leaves and bud essential oils can be used as active sunscreen ingredients because they produce an SPF value higher than 2. Pure clove leaves and bud essential oils have the highest SPF value, namely 38. The essential oil cream formulation also has sunscreen activity although having a lower SPF value than pure essential oils, i.e., the 5 % leaves cream shows an SPF of 14 and the 5 % bud cream has an SPF of 9. Higher oil concentrations produce higher SPF values: 10 % leaves cream exhibits an SPF of 22 and 10 % bud cream has an SPF of 18.

Variations in clove oil concentration significantly affect the SPF value of each sunscreen cream. A 5 % cream preparation has a lower SPF value than a 10 % cream preparation. Essential oil preparations from clove leaves and buds have the highest SPF value.

Based on the GC-MS results, the 3 most abundant compounds in clove buds were tested using an *in silico* method on MMP-1 as the target protein with 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid as the control. The docking results between protein and the 3 most abundant ligands from GC-MS results of the clove buds show binding free energy values that are lower or close to the control (**Table 6**). Meanwhile, the docking results between protein and the 5 most abundant ligands from GC-MS results of the clove leaves show binding free energy values that are lower or close to the control.

Table 6 The binding free energy of the MMP-1 (protein target) and ligand (from clove leaves and bud) complex.

	Ligand	GCMS level (%)	Binding energy (kkal/mol)
Clove leaves	Caryophyllene	37.00	−6.8
	Humulene	7.18	−6.5
	Eugenol	50.40	−5.3
Clove bud	Caryophyllene	16.35	−6.8
	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	10.21	−5.9
	Eugenol	57.16	−5.3
Control	4-(2-Hydroxyethyl)-1-piperazine ethane sulfonic acid		−5.6
	Kojic acid		−4.9

Caryophyllene has the lowest binding energy compared to 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid as the control, namely −6.8 kcal/mol. Meanwhile, the binding energy of eugenol, the characteristic compound in cloves, is −5.3 kcal/mol, greater than the control. The molecular bond stability,

indicated by the free bond energy value, is essential in a molecular complex. A lower free bond value results in a stronger molecular interaction, creating a more stable molecular complex.

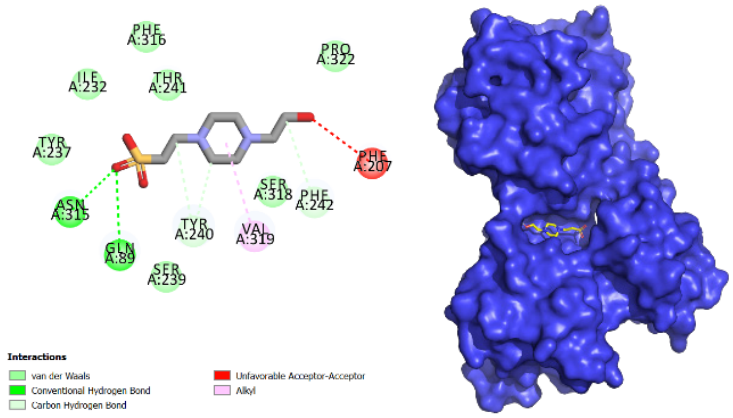

Figure 3 The compound *4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid* (control) on the MMP-1 (target protein).

Figure 3 shows the visualization of the native ligand or control ligand from the MMP-1 protein, which shows several types of interactions, such as van der Waals bonds, conventional hydrogen bonds, carbon-hydrogen bonds, and alkyl bonds. Control ligands were obtained in the protein complex from the database; therefore, they are also called native ligands. The binding affinity between the control ligand and the MMP-1 protein is −5.6 kcal/mol.

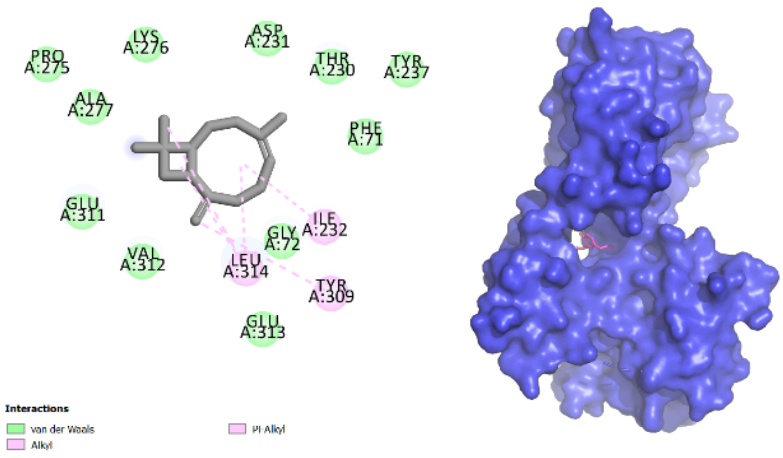

Figure 4 Caryophyllene on the MMP-1.

The visualization between caryophyllene, the ligand from the clove bud with the lowest binding affinity, and the MMP-1 receptor shows several types of interactions, such as van der Waals, alkyl, and pi-alkyl bonds, as seen in **Figure 4**. The binding affinity between caryophyllene and the MMP-1 protein is -6.8 kcal/mol. This result shows that the caryophyllene ligand has a lower binding affinity than the control, thereby having a better interaction than the control.

Table 7 shows the interactions between the amino acid residues from the MMP-1 protein and the ligands, the most abundant compound in clove.

Table 7 The bonds formed between the amino acid residue from compounds in clove on MMP-1 (target protein).

Senyawa	Ikatan Hidrogen	Ikatan Hidrofobik	Van der Waals
Caryophyllene		Leu; 314, Ile: 232, Tyr: 309	Pro: 275, Ala: 277, Lys: 276, Asp: 231, Thr: 230, Tyr: 237, Phe: 71, Gly: 72, Glu: 313, Val: 312, Glu: 311
Phenol, 2-methoxy-4-(2-propenyl)-, acetate	Glu: 311, Val: 312	Ile: 232, Tyr: 309, Leu: 314	Tyr: 237, Phe: 71, Gly: 72, Pro: 310
Eugenol	Phe: 242, Phe: 316, Val: 319, Tyr: 240	Pro: 322, Tyr: 240	Phe: 207, Glu: 209, Phe: 242, Ser: 318, Thr: 241, Val: 319
Humulene		Tyr: 309, Lys: 276, Leu: 314	Ala: 277, Pro: 275, Asp: 231, Thr: 230, Ile: 232, Tyr: 237, Phe: 71, Gly: 72, Glu: 313, Val: 312, Glu: 311
4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid (kontrol)	Asn: 315, Gln: 89, Tyr: 240, Phe: 242	Val: 319	Ser: 239, Ser: 318, Tyr: 237, Ile: 232, Phe: 316, Thr: 241, Pro: 322

The caryophyllene ligand has 3 hydrophobic bonds and 11 van der Waals interactions. The eugenol ligand has 4 hydrogen bonds, 2 hydrophobic bonds, and 6 van der Waals interactions. Meanwhile, the control ligand, 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid, has 4 hydrogen bonds, 1 hydrophobic bond, and 7 van der Waals interactions.

The docking results of the 5 most abundant compounds in clove leaves show that the caryophyllene ligand has the lowest binding affinity, namely −6.8 kcal/mol. This result means that the caryophyllene ligand has a strong potential to become an antiaging candidate. A binding affinity value that is lower than the control indicates a better interaction than the control. Based on the bond formed, the caryophyllene ligand can potentially inhibit the MMP-1 protein in slowing down aging based on the binding free energy value and chemical bond interactions. Previous studies reported that caryophyllene, apart from acting as an anti-inflammatory and antioxidant agent, also has a role in remodeling mechanisms and increases collagen content.

Discussion

Based on the phytochemical analysis qualitatively, the clove leaves (*Syzygium aromaticum*) contain secondary metabolite, including tannins, polyphenols, alkaloids, and triterpenes. These results agree with previous study by Suhendar and Fathurrahman [16], which identified similar compounds in clove's leaves methanol extract. Additionally, Azizah *et al*. [17] and Taher *et al*. [18] stated that reported comparable findings about the phytochemical compounds of clove buds and leaves that contain tannins, saponins,

flavonoids, phenols, and alkaloids. Meanwhile, clove buds, stalks, and leaves contain similar compounds but with different concentrations, i.e., 36.43, 88.93 and 91.18 %, respectively [18,19]. The variation of secondary metabolite differences in the clove extract based on the differences in the type of solvent used and extraction methods. Each solvent has a different polarity, thus affecting the content of secondary metabolite compounds in the extract [18,19]. The geographical factors, such as climate and altitude, plant temperature, the variation in of the area where clove leaves are cultivated, water availability, and disturbances in physiological processes and plant growth stages also contribute to variations in phytochemical composition [20,21].

The GC-MS results revealed eugenol as the most abundant compound, followed by caryophyllene. This result aligning with previous studies by Bhuiyan [22], that reported that the main ingredients in clove essential oil are eugenol (49.7 %), caryophyllene (18.9 %), 1-ethyl-3-nitro benzene (11.1 %), and 3-1-methyl ethyl benzoate acid (8.9 %). Eugenol, the main component in clove oil exhibits various pharmacological activities, including analgesic, anti-inflammatory, antimicrobial, antiviral, antifungal, antiseptic, antispasmodic, antiemetic, stimulant, and local anesthetic. Consequently, this compound finds extensive application within the pharmaceutical sector [23].

Eugenol, as the main compound in clove leaves, exhibits antioxidant activity. In this study, antioxidant testing yielded an IC_{50} of 23.09 ppm for essential oil from clove's leaves and 30.31 ppm for clove bud. Previous study by Afrendi *et al*. [24] reported that clove essential oil contains good antioxidant activity with an IC_{50} value of 9.29 ppm. Ongoing research on natural antioxidants, involves medicinal plants, particularly those which are rich in antioxidant compounds. This constituent can counteract or reduce the negative effects of oxidants in the body by acting as electron donors for oxidized compounds, thereby inhibiting the activity of the oxidized compounds. Natural compounds included as antioxidants are phenolic compounds such as gallic acid and flavonoids. Phenolic and flavonoid compounds have hydroxyl groups that provide hydrogen atoms, enabling them to neutralize free radicals to become more stable [25,26]. Nam and Kim [27] reported eugenol's potent antioxidant effect that counteracts various free radicals within cells. Apart from eugenol, caryophyllene in cloves also demonstrated antioxidant properties [28,29].

This study found that clove leaves essential oil can be used as an active ingredient in sunscreen because it produces an SPF value higher than 2. Pure clove leaves essential oil has the highest SPF value, namely 38. The essential oil cream formulation also has an SPF value, although lower than pure essential oil. The 5 % cream has an SPF value of 14 and the 10 % cream has an SPF value of 22. Meanwhile, clove bud essential oil has the highest SPF value, namely 38. The essential oil cream formulation also has an SPF value, although lower than pure essential oil. The 5 % cream has an SPF value of 9 and the 10 % cream has an SPF value of 18. Variations in the clove oil concentration significantly affect the SPF value of each sunscreen cream. A 5 % cream preparation has a lower SPF value than a 10 % cream preparation [30]. These results agree with the study by Fedia [31], which reported that clove oil cream can be used as a sunscreen with a mean SPF value of 5.91 in the 5 % cream formulation, 6.46 in the 7.5 % cream, and 6.68 in the 10 % cream. This result indicates that the higher the concentration of clove essential oil in the cream, the higher the SPF value of the sunscreen cream will be. Similar research by Rahmasari [32] reported that clove essential oil could be used as sunscreen with an average SPF value of 37.30.

Based on the GC-MS results, 32 compounds were obtained from clove buds and ten from clove leaves. The 5 most abundant compounds from the leaves and bud GC-MS results were tested *in silico* against the MMP-1 protein target with 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid as the control. The docking results between the protein and the 5 most abundant ligands from GC-MS results from clove show

binding free energy values that are lower or close to the control. Out of the 5 most abundant ligands from clove, the lowest binding energy, which is close to the binding energy of 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid as a control is caryophyllene, showing a binding energy of -6.8 kcal/mol. The molecular bond stability, indicated by the free bond energy value, is essential in a molecular complex. A lower free bond value results in a stronger molecular interaction, creating a more stable molecular complex.

This study discovered that ligands from clove have strong potential because they can interact with the MMP-1 protein through several interactions. The caryophyllene ligand has 3 hydrophobic bonds and 11 van der Waals interactions. The phenol, 2-methoxy-4-(2-propenyl)-, acetate ligand has 2 hydrogen bonds, 3 hydrophobic bonds, and 4 van der Waals interactions. The 3-allyl-6-methoxyphenol ligand has 2 hydrogen bonds, 1 hydrophobic bond, and 8 van der Waals interactions. The eugenol ligand has 4 hydrogen bonds, 2 hydrophobic bonds, and 6 van der Waals interactions. Meanwhile, the control ligand, 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid, has 4 hydrogen bonds, 1 hydrophobic bond, and 7 van der Waals interactions. The eugenol ligand has 4 hydrogen bonds, 2 hydrophobic bonds, and 6 van der Waals interactions. Meanwhile, the control ligand, 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid, has 4 hydrogen bonds, 1 hydrophobic bond, and 7 van der Waals interactions.

The molecular bond stability, indicated by the free bond energy value, is essential in a molecular complex. A lower free bond value results in a stronger molecular interaction, creating a more stable molecular complex. The free binding energy of the caryophyllene and MMP-1 complex is −6.8 kcal/mol. This free bond energy is relatively good, even better than the free bond energy value of the control complex, 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid with MMP-1, which is −5.6 kcal/mol. This study shows that caryophyllene can form a stable bond with the MMP-1 receptor and has an inhibitory effect on this receptor, even better than 4-(2-Hydroxyethyl)-1-piperazine ethanesulfonic acid.

The matrix metalloproteinase-1 (MMP-1) is a collagen-degrading enzyme whose activity is triggered by ultraviolet (UV) light. MMP-1 has a direct effect on photoaging; therefore, inhibitors are required to prevent skin aging. Caryophyllene and eugenol in cloves are known to contain active compounds that have the potential to inhibit skin aging. A similar study by Gupta and Phulara [33] also reported that caryophyllene has antiaging and neuroprotective activity in experiments conducted on animal models. Likewise, some studies discovered that caryophyllene and eugenol could bind to the MMP receptor in which the compounds have anticancer activity, including being pro-apoptotic by downregulating E2F1/surviving [34], and having antiproliferative and pro-apoptotic properties through the formation of ROS and activation of p53, PARP and caspase 3, as well as reducing MMP levels which cause aging [35]. Another study by Kim [36] also reported similar results, namely that the eugenol content significantly reduced the amount of MMP-1 induced by TNF.

Caryophyllene is associated with increased levels of IL-10 and GPx and decreased levels of pro-inflammatory molecules, including TNF-α, IFN-γ, IL-1β, and IL-6. Immunohistochemical testing showed increased re-epithelialization through increased immunolabeling of laminin-γ2 and desmoglein-3. Caryophyllene also acts in the remodeling mechanism, as well as increases collagen content, which inhibits the aging process [37]. Eugenol also plays a vital role in antiaging activity. Eugenol reduces UVB damage to skin cells, including oxidative stress damage and degradation of the extracellular matrix (ECM). Eugenol reduces aging by repairing the skin barrier in the skin, triggering skin tissue regeneration, modulating the skin microenvironment, and changing the skin's gene expression profile [38]. The study shows that eugenol can bind to the regulator of the oxidative stress response pathway, the proteins sty1 and ctt1. Further research is required to clarify the effect of eugenol on specific stress responses. Overall, various studies have demonstrated eugenol's potential antiaging activity [39].

Conclusions

This study concludes that the clove essential oil contains secondary metabolites: alkaloids, polyphenols, tannins, and triterpenes. The GC-MS analysis on clove leaves and buds shows that eugenol, caryophyllene, humulene, and phenol, 2-methoxy-4-(2-propenyl)-, acetate) are the most abundant compounds. The essential oils from clove leaves and buds exhibit very strong antioxidant activity, along with sunscreen activity with SPF values of 38.61 and 38.19. The formulation of clove essential oil into cream. The compounds in clove essential oil exhibit antiaging potential based on binding affinity values and chemical bond interactions observed during *in silico* testing.

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