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Formulation of Sunscreen of Gaharu Leaf Ethanol Extract (*Aquilaria malaccensis* Lam.)

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Abstract

Excessive sun exposure can have a bad impact on the skin and cause various skin problems. Agarwood leaf extract *(Aquilaira malaccensis* Lam.) contains flavonoid compounds that can protect the skin from the adverse effects of UV rays. This research aims to formulate a cream from ethanol extract of agarwood leaves *(Aquilaira malaccensis* Lam.) can be used as sunscreen. In this study, a cream formula was made from agarwood leaf ethanol extract with 3 variations of different extract concentrations, namely F1 2% agarwood leaf extract, F2 4% agarwood leaf extract, and F3 8% agarwood leaf extract. The three formulas were then carried out organoleptic test, homogeneity test, pH test, dispersion test, adhesion test and SPF test. The results showed that Formula 3 (F2) with an 8% agarwood extract concentration had the highest maximum protection with an SPF value of 42.42.

Keywords: Extract, Gaharu leaf, *Aquilaria malaccensis* Lam, Sunscreen, Formulation

1 Introduction

The skin, as the outermost layer, protects the internal organs from damage or interference from the outside. Excessive exposure to sunrays can negatively impact skin health and cause various skin problems, so the skin needs to be protected to prevent damage that can cause dangerous diseases such as melanoma (Hutasoit *et al*.,2021).

Melanoma is the rarest type of skin cancer but is the cause of 75% of deaths due to skin cancer. Melanoma is malignant and aggressive; sun exposure is the main cause of melanoma (80%) (Pasaribu E. *et al*., 2019). The incidence of melanoma over the past few decades has shown a significant increase, where in Indonesia in 2020 there were 1690 cases with a death rate of 699 and a prevalence of 4331 cases in the next 5 years (WHO, 2020). The form of prevention against melanoma that can be done is the application of sunscreen (Autier and Doré, 2020).

Sunscreen is a product that contains chemical compounds that can absorb, disperse or reflect UV rays so that it can be used to protect the function and structure of human skin from the negative effects of UV rays. Sunscreen products usually contain synthetic substances as active ingredients such as oxybenzone, avobenzone, and PABA derivatives (*p-aminobenzoic acid*), where excessive use of synthetic compounds often causes allergic effects and hypersensitivity (Putra, 2023). Sunscreen products made from natural

ingridients need to be developed to minimize side effects at a more affordable price. The phytochemical content such as flavonoids, phenolic compounds, and terpenoids in plants shows high antioxidant activity and has been proven to protect against UV radiation (Mansuri *et al.*,2021).

A natural ingredient that can act as a sunscreen is the agarwood plant (*Aquilaria malaccensis* Lam.). agarwood plants are widely distributed in Sumatra and Kalimantan and are known to contain secondary metabolite compounds such as polyphenols (flavonoid dan tannin) and steroids that have very strong antioxidant activity (IC< 50 ppm) that can act as photoprotective agents (Halim *et al*., 2022). Testing the utilization of agarwood leaves (*Aquilaria malaccensis* Lam.) was carried out based on chemical compounds contained in it in the form of flavonoid groups, namely flavonoids, flavonols and isoflavones. Some sources explain that flavonoids, terpenoids and phenols act as antri-free radicals are distributed in different parts of the plant such as wood, bark, roots, fruits, flowers, seeds and leaves, where the leaves have an abundant source of antioxidants. Agarwood (*Aquilaria malaccensis* Lam.) has the potential as an antioxidant to produce photoprotective properties, but there has been no research related to the formulation of preparations from agarwood leaves (*Aquilaria malaccensis* Lam.) (Halim *et al*.,2022).

2 Materials and Methods

This type of research is experimental, namely, research conducted in the laboratory by making a sunscreen cream formulation from agarwood leaf extract. The tools used in this research include analytical balance (Ohaus), UV-Vis Spectrophotometer (Shimadzu, 1280), oven (Mammert), micropipette (Scilogex), water bath, porcelain cup 250 mL, Becker glass 100 mL (pyrex), Erlenmeyer 250 mL (Pyrex), and measuring cylinder glass 100 mL (Pyrex). The material used is agarwood leaves obtained on Telaga Dewa 4 street No. 26, Pagar Dewa, Selebar District, Bengkulu City.

Methods

2.1. Extraction Preparation

Agarwood leaf samples (*Aquilaria malaccensis* Lam.) had previously been verified at the Indonesian Institute of Sciences (LIPI) in Cibinong 2020 and it was found that the species used was *Aquilaria malaccensis* Lam.

The agarwood leaves obtained are then sorted wet, after which the leaves are washed and then cut and dried by drying in direct sunlight until dried simplicial is obtained. Dried agarwood leaves are mashed with a blender and weighed to obtain aa simplicial yield. A total of 500g of agarwood leaf powder was extracted using the maceration method with 70% ethanol solvent for 3x24 hours and occasionally stirred. The maserat is then filtered and remaged until a clear filtrate is obtained. The filtrate is then concentrated with a rotay evaporator at a temperature of 50^{o}C at a speed of 70 rpm until a thick extract is obtained (Harliatika et al., 2021).

2.2.Cream Preparation Formulation

The concentration of agarwood leaf (*Aquilaria malaccensis* Lam) extract used 2%, 4%, and 8% and was made as much as 30 g for each formula with 3 replications of each formula. The formula of the preparation in the form of precentages used is presented in Table 1 below:

Tabel 1. Cream Formulation

Materials	Concentration %				Usability
	F0	F1	F2	F3	
Agarwood Leaf Ethanol Extract 70%	-	2	4	8	Active Substances
Stearicid Acid	12	12	12	12	Emulsifier
Cetyl Alcohol	0.5	0.5	0.5	0.5	Thickener
Triethanolamine	1	1	1	1	Emulsifier
Methly Paraben	0.1	0.1	0.1	0.1	Preservatives
Paraben Proply	0.05	0.05	0.05	0.05	Preservatives
Glycerin	2	2	2	2	Moisturizer
Oleum Rosae	Qs	Qs	Qs	Qs	Fragrance
Aquadest	Ad 100	Ad 100	Ad 100	Ad 100	Solvent

Source: Safitri, *et al.* 2016 Development of Formulation and Physical Evaluation of Cream Preparation of Ethanol Extract 70% Chayote Leaf *(Sechium edule (Jacq.)Swatz).* Farmagazine.

The preparation begins with weighing all the necessary ingredients. The materials that are the oil phase such as stearic acid, cetyl alcohol and properly paraben are put into a porcelain cup and then melted using a water bath with a temperature of 70°C. Then materials that are the water phase such as TEA, glycerin, methyl paraben and Aqua distilate are put into Erlenmeyer after that heated to a temperature of 70°C in the water bath. After everything is melted, the oil phase is put into hot pot and the water phase is added little by little until a cream base is formed. Agarwood leaf extract is then added to the cream base. Finally, add Rosae oleum to taste as a frgarance. After the preparation is finished, then a physical evaluation of the preparation is carried out (Milutinov *et al*., 2023).

2.3. Evaluation of Cream Preparations

1. Organoleptic Test

The organoleptic test is carried out by direct observation of the color and smell of the emulgel produced. A total of 1g of the preparation was placed on parchment paper and then observed for its smell, color, and texture. The color of the preparation of ethanol extract of agarwood leaves (*Aquilaria malaccensis* Lam.) is good green according to the color of the extract preparation. A good emulgel smell of agarwood leaf ethanol extract (*Aquilaria malaccensis* Lam.) has a distinctive odor of agarwood leaves (*Aquilaria malaccensis* Lam.) and a good emulgel preparation texture, which is semi-solid (Istiqomah *et al*., 2021).

2. Physical Homogeneity Test

A total of 0.5 g of cream was applied to a piece of transparent glass and then observed whether it showed a homogeneous arrangemen and no coarse grains were seen. If the color is even and there are no fine grains in the cream preparation, then the cream is said to be homogeneous (Larasati, *et al,* 2023).

3. pH Test

The pH measurement of cream uses the Ohaus Starter 3100 brand pH meter. The tool is calibrated first using a solution of pH 4, pH 7 and pH 10. The pH check is carried

out by dipping the electrode into a glass containing 1 gram of cream diluted with aquadest up to 10 mL. Strengthen the pH of the preparation by inserting electrodes into the cream preparation and looking at the number indicated on the device (Istiqomah *et al*, 2021). The pH value of leather products following SNI 16-4399-1996 ranges from 4.5-8.

4. Adhesion test

As much as 0.5 g of cream placed on a glass with a size of 10x10 cm, and cover it again with the same glass. Then, an additional load of 50, 100, and 150 g is placed and left for 1 minute, then it is calculated how long it takes for the two glasses to come off. Good emulgel adhesion is more than 1 second (Istiqomah *et al*., 2021).

5. Spread Power test

A total of 0.5 g of emulgel is placed on glass with a size of 10x10 cm and covered again with the same glass. Then, an additional load weighing 50, 100, 150 g is placed and left for 1 minute and then the diameter is measured. Good emulgel spread between 5-7 cm (Sari *et al*., 2015).

6. SPF (*Sun Protection Factor*) Test

The sunscreen effectiveness test was carried out in vitro using a UV-Vis spectrophotometer. Cream sample with ethanol extract of agarwood leaf (*Aquilaria malaccensis* Lam.) with a concentration of 2%; 4%; 6% and 8%, as well as 0.5 g of extract-free blank cream dissolved in 25 mL of 90% ethanol (20,000 ppm). Each sample was measured for absorption with a UV-Vis spectrophotometer. The absorbance spectrum obtained in the range of 290-320 nm at every 5 nm interval was replicated 3 times. The SPF value is determined using Equation 1 (Dutra *et al*., 2004; Sayre *et al*., 1979). The value of EE x 1 is a value constant of wavelengths every 5 nm difference from 250-350 nm and can be seen in Table 2. This capability will be compared to the sunscreen's effectiveness value based on the SPF value in Table 3.

The calculation of the SPF value mathematically according to Sayre 1979 is:

$$SPF_{spectrophotometric} = CF \times \Sigma EE \times I \times Abs(\lambda)$$

$$= 10 \times Abs(\lambda)$$

Information:
EE = Erythema effect spectrum
I = Spectrum intensitas
sinar A = Absorbance
CF = Correction factor of correction actors that already have a fixed value of 10 (Sayre *et al*., 1979)

Table 2. EE x I values at 290-320 nm

Wavelength (nm)	EE x I
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Source: Pratiwi, 2016 Determination of Spf (*Sun Protection Factor*) Value Levels Using Uv-Vis Spectrophotometry in Brightening Creams Containing Sunscreen Circulating in the City of Bandung.

Table 3. Sunscreen effectiveness based on SPF value (WHO, 2003)

SPF	Category Synthesis Phrase Surya
1-2	Weak Protection
3-4	Medium Protection
8-10	Strong Protection
≥11	Maximum Protection

Source: WHO, 2003 *Sun Protection A Primary Teaching Resource.*

Information:
1-2: No protection
3-7: Required protection
8 ≥ 11: Have over/extra coverage

Discussion

1. Organoleptic Test

From the results of the organoleptic test, color and odor characteristics were obtained in several formulas, as listed in Table 4.

Table 4. Organoleptic Test Results

	Information	
Formula	**Color**	**Aroma**
F0	White	Fragrance of Oleum Rosae
F1	Light Green	Fragrance of Oleum Rosae
F2	Green	Fragrance of Oleum Rosae
F3	Hijau Kehitaman	Fragrance of Oleum Rosae

Information:
F0 = Cream formula 0 is the cream base
F1 = Cream formula 1 contains 2% agarwood leaf extract
F2 = Cream formula 2 contains 4% agarwood leaf extract
F3 = Cream formula 3 contains 8% agarwood leaf extract

The organoleptis test is a direct observation of preparations through the five senses such as smell, color, and texture which aims to determine the physical characteristics of the preparations produced. Observations made on the emulgel preparation of ethanol extract of agarwood leaf (*Aquilaria malaccensis* Lam.) includes the smell, color, and texture of the preparation. The results of the organoleptis test of emulgel preparations of ethanol extract of agarwood leaves (*Aquilaria malaccensis* Lam.) can be seen in Table 4.

The results of organoleptis testing on the color of the agarwood leaf ethanol extract cream preparation (*Aquilaria malaccensis* Lam.) at F0 (base) obtained a preparation with a white color in accordance with the color of the preparation base because there was no addition of extract to the base formula. However, the other three formulas, namely F1 (2%), F2 (4%) and F3 (8%), showed that there were color variations in the preparations. All three formulas showed a cream with a darker green color as the concentration of the extract in each formula increased.

The results of the aroma observation on the preparation showed that all formulas had the aroma of oleum rosae due to the addition of oleum rosae as a fragrance in each formula. However, the aroma at F0 (base) is more pronounced, while at F1 it has a slightly distinctive aroma from the concentration of the added extract. The aroma smelled at F2 and F3 also increasingly has a distinctive aroma of extract and the aroma

of eloum rosa is reduced. This shows that the higher the concentration of the extract added to the formula, the more pronounced the characteristic aroma of the extract and the less oleum rosae aroma it is.

The results of the observation of the texture of the preparation at F0 are denser when compared to the other three formulas, namely F1 (2%), F2 (4%), and F3 (8%). If observed from the three preparations, it still has a semi-dense texture, but F1 has a denser texture when compared to F2 and F3. The three formulations showed similar dosage forms, only there were differences in the color concentration, aroma intensity, and texture of the preparations produced. The higher the concentration of the extract, the more concentrated the color and viscosity of the preparation, as well as the more distinctive the aroma of the preparation produced.

2. Homogeneity Test

From the results of the homogeneity test, homogeneity characteristics are obtained in several formulas, as listed in Table 5.

Table 5. Homogeneity Test Results

Formula	Test Results
F0	Homogeneous
F1	Homogeneous
F2	Homogeneous
F3	Homogeneous

Information:
F0 = Cream formula 0 is the cream base
F1 = Cream formula 1 contains 2% agarwood leaf extract
F2 = Cream formula 2 contains 4% agarwood leaf extract
F3 = Cream formula 3 contains 8% agarwood leaf extract

The homogeneity test is an observation of the mixing of the ingredients of a preparation to ensure that the active ingredients and the ingredients that make up the preparation are evenly distributed. A preparation is said to be homogeneous when there are no coarse grains and the color of the preparation is produced evenly (Nur *et al.*, 2022). From the observation of the homogeneity test carried out, it is known that there is no separation between the cream base and the agarwood leaf extract. This shows that the base can support the extract and form a homogeneous cream. By the requirements of the Ministry of Health of the Republic of Indonesia (1985) if the fine preparations are evenly distributed, it can be concluded that the F1, F2, and F3 preparations are homogeneous.

3. pH Test

The pH test is a physicochemical parameter related to the effectiveness and stability of the active substance in the preparation to ensure comfort and safety when applying the preparation. The pH value of the preparation should be in the pH range of the skin, the pH of the preparation that is too acidic can irritate, and the pH of the preparation that is too alkaline can cause dry and flaky skin. The results of the pH test of ethanol extract emulgel preparations of agarwood leaves (*Aquilaria malaccensis* Lam.) can be seen in Table 6.

Tabel 6. pH Test Results

Formula	pH
F0	7.92
F1	7.19
F2	7.11
F3	6.71

The results of the observation were obtained with a consecutive pH of 7.19 (F1); 7.11 (F2) and 6.71 (F3). This shows that the higher the concentration of the extract, the pH of the preparation will decrease, which indicates that agarwood leaf extract has acidic properties that can lower the pH of the preparation. The pH range of topical preparations is 4-8 because with this pH range, the cream will not cause skin irritation when applied. The results of the pH test on F1, F2 and F3 have a pH indicating that the agarwood leaf extract cream preparation has met the requirements of an acceptable preparation for use on the skin.

4. Adhesion Test

The adhesion test aims to determine the ability of the preparation to adhere to the skin. Sunscreen preparations are expected to stick to the skin for a long time so that they can protect the skin from ultraviolet rays for a longer period. The longer the preparation stays on the skin, the more active ingredients can be released.

Tabel 7. Adhesion Test Results

Formula	Time (Seconds)	Specifications
F0	4.1	Not Less than 4 seconds
F1	4.6	Not Less than 4 seconds
F2	4.9	Not Less than 4 seconds
F3	5.4	Not Less than 4 seconds

Testing is carried out using a manually assembled tool following the adhesion test standards of topical preparations according to FDA and BPOM. During the test, an additional load is added that describes the pressure that will be applied when the preparation is applied to the skin. Measurement of the adhesion of the preparation is carried out using a stopwatch, until the glass of the object is detached from other glass. From the results of the adhesion test, the characteristics of the adhesion time (seconds) in several formulas are obtained, as listed in Table .7

The adhesion test produced in the formulation of this study meets the adhesion requirements of topical preparations under the fourth edition of the Pharmacopoeia of not less than 4 seconds. The test results showed that the adhesion of the preparation increased along with the addition of the concentration of the extract to the preparation which showed that the more extract, the thicker the preparation was produced and the longer the preparation was able to stick to the skin (Eka, *et al.*, 2019). The active compounds in plant extracts contain naturally occurring adhesive components such as polysaccharides, tannins, or polyphenol compounds. Aarbor leaf ethanol extract (*Aquilaria malaccensis* Lam.) contains polyphenol compounds with a chemical structure that has many hydroxyl groups (-OH) so that it can interact with the ingredients that make up the preparation through hydrogen bonds and polar interactions. These

interactions increase the cohesion or attraction between molecules in the preparation, thereby increasing the adhesion of topical preparations. The more extracts are added to the preparation, the more active substances are contained in the preparation, and the greater the interaction that occurs between the active substances and the ingredients that make up the preparation so that the adhesion of the preparation increases (Eka, *et al.*, *2019*).

5. Spread Power Test

The diffusion test aims to determine the ability of a topical preparation to spread when applied to the skin. The ability of the preparation to disperse is an important characteristic in topical formulation because it affects the transfer of the active substance of the preparation in the target area. Testing is carried out by the spreadability test standards of topical preparations according to the FDA and BPOM. From the results of the spread power test, the characteristics of the diameter of the spread power are obtained in several formulas, as listed in Table 8.

Table 8. Dispersion Test Results

Formula	Spread power (cm)	Specification (cm)
F0	6.4 cm	5-7
F1	6.3 cm	5-7
F2	6.0 cm	5-7
F3	5.7 cm	5-7

The test was carried out using a 10x10 cm object glass to make it easier to observe. During the test, an additional load is added that describes the pressure that will be applied when the preparation is applied to the skin. The measurement of the dispersion power is carried out after the addition of the last load, i.e. the total load weight of 150 g, until the distribution of the preparation on the glass of the object is constant. The results of the dispersibility test of the preparation of agarwood leaf ethanol extract cream (*Aquilaria malaccensis* Lam.) can be seen in Table 8.

The spreading power of the cream is shown by the diameter of the cream spreading against the load added at regular intervals. The cream spread power test is carried out to determine the amount of spread of the cream when applied to the skin. Experimental data showed that the higher the concentration of the extract, the more active substances contained in the preparation and the greater the interaction that occurred between the compound and the constituent materials of the preparation so that the preparation became thicker and the dispersion of the preparation decreased (Nur *et al.*, 2022). According to the fourth edition of the Pharmacopoeia (1995), the dispersion power of semi-solid preparations that are very comfortable in use is between 5-7 cm. The test results show that overall of the 3 formulas meet the requirements of good spreadability.

6. In Vitro SPF (*Sun Protection Factor)* Test

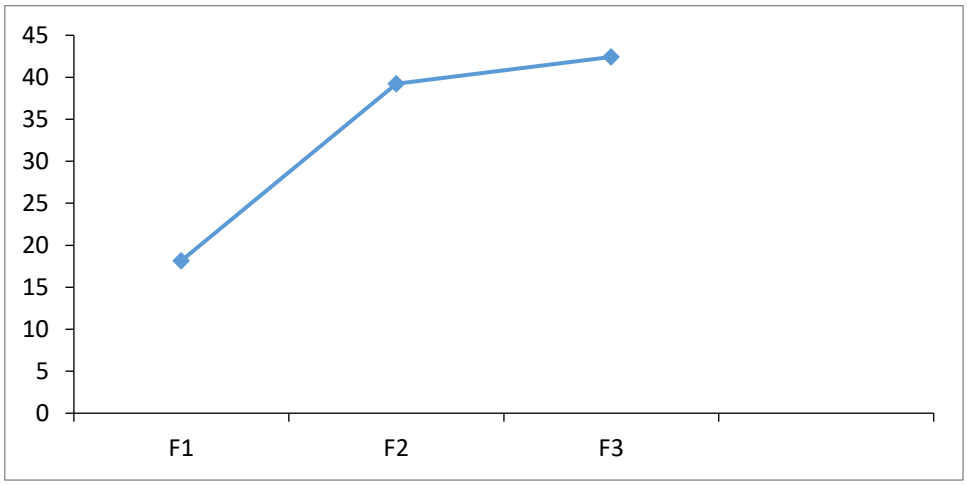
One way to determine the effectiveness of sunscreen products is by measuring SPF in vitro. The effectiveness test of sunscreen emulgel ethanol extract of agarwood leaves (*Aquilaria malaccensis* Lam.) was carried out using a UV-Vis spectrophotometer at a wavelength of 290 – 320 nm and then the SPF value was calculated from the sample absorbance data. The absorbance data from measurements using a UV-Vis spectrophotometer will be converted into the formula for calculating the SPF value. The

results of the calculation of the SPF value of ethanol extract emulgel preparation of agarwood leaf (*Aquilaria malaccensis Lam*.) can be seen in Table 9.

Table 9. F1 cream SPF Test Results

Formula	SPF Test Results
F1	18.16
F2	39.22
F3	42.42

From the results of SPF test, the following graph is obtained:



Picture 1. SPF Test Results

Information:
F1 = Cream formula 1 contains 2% agarwood leaf extract
F2 = Cream formula 2 contains 4% agarwood leaf extract
F3 = Cream formula 3 contains 8% agarwood leaf extract

The results of the SPF test showed that the cream preparation had SPF values of 18.16, 39.22; and 42.42 respectively. From this data, the level of ultraviolet index ability can be grouped based on the SPF value according to WHO 2003. Based on the results obtained, it can be seen that agarwood leaf extract cream with a concentration of 2%, 4%, and 8% obtained SPF values of 18.16 respectively; 39,22; and 42.42 are in the range of ≥11, including having maximum level of protection capability. In the results of this study, the SPF value in agarwood leaf extract cream has maximum sunscreen ability, and the higher the addition of the amount of agarwood leaf extract in the cream, the higher the SPF value.

Agarwood leaf ethanol extract (*Aquilaria malaccensis* Lam.) contains various active compounds that can act as antioxidants such as flavonoids, triterpenoids, and other phenolic compounds. These compounds are one of the secondary metabolites that provide the most antioxidant activity in plants, including agarwood leaves (*Aquilaria malaccensis* Lam.) have very strong antioxidant activity (Halim *et al*, 2022).

Antioxidant activity is often associated with a substance's ability to protect itself from exposure to UV radiation. Free radicals from the interaction of UV rays with the skin can damage skin structures that cause skin irritation, redness, aging, and skin cancer. Antioxidants play a role in neutralizing these free radicals through various

chemical reactions such as hydrogen transfer or electron transfer. Secondary metabolites in the form of polyphenols in the ethanol extract of agarwood leaves (*Aquilaria malaccensis* Lam.) capture free radicals produced by UV radiation and then neutralize them by releasing electrons or hydrogen atoms. The higher the concentration of the extract in the preparation, the higher the concentration of active substances that can act as antioxidants so that the protection against UV radiation is also better, which is shown by the increase in the SPF value of the preparation (Halim *et al.*,2022).

3 Conclusion

Agarwood leaf ethanol extract cream preparations (*Aquilaria malaccensis* Lam.) have SPF activity as evidenced by in vitro measurement of SPF values using UV-Vis spectrophotometers, where the SPF test results for F1, F2, and F3 respectively are 18.16; 39.22; and 42.42 which are categorized as maximum protection. Variation in the concentration of agarwood extract (*Aquilaria malaccensis* Lam.) Affecting the physical properties of sunscreen cream preparations, the higher the concentration of agarwood leaf extract in the cream preparation, the denser the texture of the cream will be and the spreading power will decrease. The results showed that Formula 3 (F2) with an 8% agarwood extract concentration had the highest maximum protection with an SPF value of 42.42.

4 Author contribution

All authors declare no conflict of interest and agree with the content of the manuscript.

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