

Sentul Fruit (*Sandoricum koetjape*) Peel as Anti-Inflammation for Gingivitis after Scaling

by I Nyoman Wirata, A.a. Gede Agung, Ni Wayan Arini, Ni Ketut Nuratni

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10 *Sentul* Fruit (*Sandoricum koetjape*) Peel as Anti- Inflammation for Gingivitis after Scaling

I Nyoman Wirata¹, A.A. Gede Agung², Ni Wayan Arini³, Ni Ketut Nuratni⁴

1,2,3,4 16 Department of Dental Hygiene, Poltekkes Kemenkes Denpasar, Bali

Correspondence: I Nyoman Wirata, Department of Dental Hygiene, Poltekkes Kemenkes Denpasar, Bali.
E-mail: wiratainyoman@yahoo.co.id

Abstract

9 Various herbs are used as analgesic, anti-inflammatory, anti-bacterial, anti-fungal, expectorant, anti-plaque and odorant. *Sentul* is an edible fruit and is also used in traditional medicinal herbs which can treat diarrhea, relieve fever, and as an anthelmintic. *Sentul* bark methanol extract can inhibit the growth of fungus *Candida albicans* by 39.65%. In addition, the ethyl acetate extract of the *Sentul* leaves also has anti-bacterial activity. The aim of this study is to determine the effect of fractionation with different types of solvents on the phytochemical compounds of *Sentul* fruit peel in Bali province. This research is an experimental study in a laboratory with qualitative and quantitative analysis models of chemical compounds. This research was carried out from March to August 2021. The research location was carried out in the laboratory of the Faculty of Food Technology, Udayana University. Sample criteria was old *Sentul* peel, about 30 kilograms. Data was collected based on the results of examinations from the Laboratory of the Faculty of Food Technology, Udayana University which subsequently analyzed qualitatively and descriptively. From several phytochemical compounds, flavonoids, saponins and tannins are aromatic hydroxyl groups that act as antibacterial. Therefore, seen from the highest levels of flavonoids, saponins and tannins, aqua fraction of *Sentul* ethanol extract is the best treatment with flavanoid levels of 11476.16 mg/100g QE, tannins 88.605 mg/g and saponins 6.862 mg/g.

Keywords: *Sentul* Peel, Anti-Inflammation, Gingivitis

1. Introduction

14 17 The results of a national socio-economic survey in 2001 as many as 57.7% of the Indonesian population did self-medication without medical assistance, 31.2% of them used traditional medicinal plants 9.8% chose other traditional medicine methods (Agustiningsih et al., 2010). The advantages of herbal medicines compared to modern medicines are relatively low side effects and one plant has more than one pharmacological effect (Karimi, et al., 2015). Many types of herbs have been used and their use has been developed as analgesic, anti-inflammatory, anti-bacterial, anti-fungal, expectorant, anti-plaque and odor medicine. To increase the benefits of herbal medicines, selective purified extracts by observing effective compounds should be conducted and limit as small as possible the ballast substances involved (Zhang et al., 2018).

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World Health Organization (WHO) reports that 10-15% of the world's population suffers from periodontal, 80% of teenagers suffer from gingivitis, while almost all of the adult population has suffered from gingivitis (Tanjaya & Auerkari, 2011). Gingivitis is an inflammation of the gingiva with swelling, redness, exudate and changes in the normal contour of the gingiva. Gingivitis is caused by the accumulation of bacteria plaque, and plaque that accumulates in the mouth will mineralize to form tartar (Murakami et al., 2018). Tartar is a medium for the growth and proliferation of bacteria that can cause inflammation of the gums.

Several studies have shown that mouthwash can inhibit plaque formation and has been shown to reduce the severity of gingivitis (Hodge, 2016; Moein et al., 2020; Grover et al., 2021). In general, mouthwash has the same way of working, namely destroying bacterial cells, breaking down enzymes in the plaque matrix, inhibiting bacterial aggression or inhibiting the attachment of bacteria to the tooth surface (Prasanth, 2011).

Sentul fruit is known edible and used as traditional medicinal herbs such as its roots can treat diarrhea, the leaves can relieve fever, and the powdered part of the stem can be used as an anthelmintic (Diansari et al., 2018; Aria, et al., 2013). Several researchers have proven the efficacy of the sentul plant as a white discharge medicine as stated in (Warsinah et al., 2011) that the methanol extract of sentul bark can inhibit the growth of fungus *Candida albican* by 39.65%. In addition, the ethyl acetate extract of the leaves of the sentul also has anti-bacterial activity (Toobpeng et al., 2017). The results of the phytochemical screening of sentul fruit peel simplicia powder showed the presence of groups of alkaloids, flavonoids, tannins, saponins, glycosides, anthraquinone glycosides and steroids (Heliawati et al., 2017).

The objective of our study is to observe sentul fruit peel in Bali province in determining its effect of fractionation with different types of solvents on the phytochemical compounds.

2. Method

2.1 Research Design

This research is an experimental study in a laboratory with qualitative and quantitative analysis models for chemical compounds. This research was carried out from March to August 2021, on samples of old sentul fruit peels, about 30 kilograms. The experiment included laboratory examination of the extracts of aquadest, ethanol, the fractions of aquadest, hexane and Ethyl Acetate at the Laboratory of the Faculty of Food Technology, Udayana University. Then the data were analyzed qualitatively and descriptively.

2.2 Experimental Procedure

The peel of sentul fruit was collected and then washed, and dried in an oven to obtain simplicia for about 72 hours. After drying, the sample was powdered using a blender, then extracted/separated biochemical compounds by maceration/soaking method with distilled water and ethanol for 24 hours. The maceration method was chosen in this study because it is an easy method and uses simple tools, which just need to soak the sample in a solvent. After the extraction process, the next was to proceed with liquid-liquid fractionation, using a separating funnel according to the solvent applying hexane, ethyl acetate and aquadest as solvents. The fractionation solvent is based on the level of polarity of the solvent, polar aquadest, non-polar hexane, semi-polar ethyl acetate.

After obtaining the extract, phytochemical screening was carried out to determine the class of active compounds. Phytochemical screening is a simple way to perform qualitative analysis of compound content in plants. In this study, the screening carried out was the alkaloids test, flavonoids test, saponins test, tannins test, and triterpenoid test because these tests already represented several groups of compounds contained in plants. Afterwards, the extract was further tested for the levels of several phytochemical compounds such as phenol, flavanoid, tannins, alkaloids, and saponins levels to know which fractionation had the highest phytochemical content. In addition, antioxidant activity tests were also carried out to determine which type of solvent fractionated extract had the highest antioxidant activity.

3. Results

3.1 Phytochemical Screening

Table 1 shows the results of phytochemical screening tests with the fractionation of aqua, ethyl acetate and hexane from sentul fruit peel extract. Based on the test results, the sentul fruit peel extract contain alkaloids, triterpenoids, tannins, flavonoids and saponins, except for the hexane fraction which is declared negative to contain saponins. This is presumably due to the small content of saponins in the hexane fraction of sentul fruit peel extract so that qualitative compounds were not detected.

Table 1: Phytochemical screening of sentul extract

Sample Code	Variable				
	Saponins	Alkaloids	Triterpenoids	Tannins	Flavonoids
Aqua fraction of sentul aqua extract	positive	positive	positive	positive	positive
Ethyl acetate fraction of sentul aqua extract	positive	positive	positive	positive	positive
Hexane fraction of sentul aqua extract	negative	positive	positive	positive	positive
Aqua fraction of sentul ethanol extract	positive	positive	positive	positive	positive
Ethyl acetate fraction of sentul ethanol extract	positive	positive	positive	positive	positive
Hexane fraction of sentul ethanol extract	negative	positive	positive	positive	positive

3.2 Quantitative Test

Table 2 shows the results of quantitative test of saponins, alkaloids, and tannins while table 3 shows the quantitative results on phenol, flavonoids and antioxidant activity. The average value followed by the same letter in the same column shows a non-significant difference (Duncan 5%). As seen from the tables, the liquid-liquid fractionation of the two extracts using aquadest, ethyl acetate and hexane, the aqua and ethyl acetate fractions tend to contain higher chemical compounds than the hexane fraction. Aquadest is a polar solvent so they can attract polar compounds such as tannins, alkaloids, saponins and phenolics, while ethyl acetate is a semi-polar solvent so that it can still dissolve polar and non-polar components (Abarca-vargas et al., 2016), but hexane is a non-polar solvent so that only few components of the extract are soluble.

Table 2: Quantitative results of saponins, alkaloids, tannins of sentul extract

Code Sample	Alkaloids (mg/g)	Tannins (mg/g)	Saponins (mg/g)
Aqua fraction of sentul aqua extract	16.978	b	85.439
Ethyl acetate fraction of sentul aqua extract	12.110	c	77.807
Hexane fraction of sentul aqua extract	7.120	d	23.465
Aqua fraction of sentul ethanol extract	36.412	a	88.605
Ethyl acetate fraction of sentul ethanol extract	16.673	b	6.512
Hexane fraction of sentul ethanol extract	5.957	d	4.085

Table 3: Quantitative results of phenol, flavonoids and antioxidant activity of sentul extract

Code Sample	Phenol (mg/100g GAE)		Flavonoids (mg/100g QE)		Antioxidant activity (% 100 ppm)	
Aqua fraction sentul aqua extract	7657.96	a	9560.23	c	23.684	a
Ethyl acetate fraction sentul aqua extract	944.99	c	19672.96	a	13.727	b
Hexane fraction aqua extract sentul	626.28	c	13928.07	b	6.117	c
Faction aqua ethanol extract sentul	1444.49	bc	11476.16	c	26.529	a
Fraction of the ethyl acetate extract of ethanol sentul	1772.12	b	17380.43	a	16.429	b
Fraction hexane extract ethanol sentul	1337.18	bc	10436.92	c	8.606	c

3.2.1 Alkaloids

Figure 1 shows the alkaloids content in sentul peel extract. According to the results of the ANOVA test, the fractionation treatment with hexane, ethyl acetate and aqua solvents on the ethanol extract and aquades of sentul fruit peel gave a significant effect (sig<0.005) on the alkaloids content.

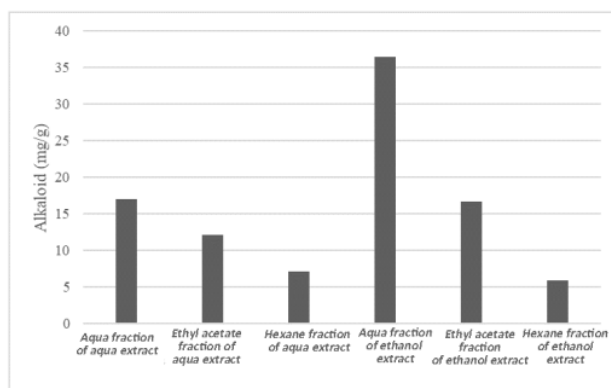


Figure 1: Alkaloids in sentul fruit peel

The highest alkaloids content is obtained in the aqua fraction of the ethanol extract with a concentration of 36.412 mg/g which is significantly different from other treatments. The lowest alkaloids content is obtained in the hexane fraction of ethanol extract with a concentration of 5.957 mg/g which is not significantly different from the treatment with the hexane fraction aqua extract with a concentration of 7.120 mg/g.

3.2.2 Tannins

Figure 2 shows the tannins content in sentul peel extract. Based on the results of ANOVA test, fractionation treatment with solvent hexane, ethyl acetate and ethanol extract and aqua in ethanol and aquadest extract achieves a significant influence (sig <0.005) on levels of tannins.

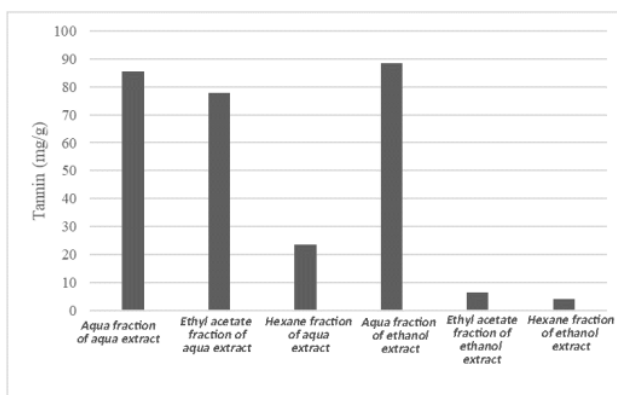


Figure 2. Tannins in sentul fruit peel

The figure indicates the highest tannins content was obtained in the aqua fraction of ethanol extract with a concentration of 88.605 mg/g which is not significantly different from the treatment with the aqua fraction of aqua extract with a concentration of 85.439 mg/g and significantly different from other treatments. The lowest tannins content is obtained in the hexane fraction of ethanol extract with a concentration of 4.085 mg/g which is not significantly different from the treatment of the ethyl acetate fraction of ethanol extract with a concentration of 6.512 mg/g.

3.2.3 Saponins

Figure 3 shows the saponins content in sentul peel extract. Based on the ANOVA test results, fractionation treatment with hexane, ethyl acetate and aqua solvents on ethanol and aquadest extracts of sentul fruit peel obtain a significant effect (sig<0.005) on saponins levels.

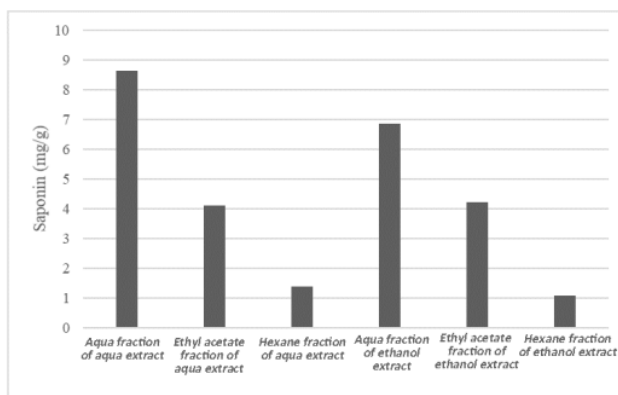


Figure 3: Saponins in sentul fruit peel

The highest levels of saponins is obtained in the aqua fraction of aqua extract with a concentration of 8.639 mg/g which is significantly different from other treatments. The lowest levels of saponins is obtained from the hexane fraction of ethanol extract with a concentration of 1.090 mg/g which was not significantly different from the treatment with the hexane fraction of aqua extract with a concentration of 1.388 mg/g.

3.2.4 Phenol

Figure 4 shows the phenol content in sentul peel extract. According to the results of the ANOVA test, the fractionation treatment with hexane, ethyl acetate and aqua solvents on the ethanol extract and aquades of Sentul fruit peel gave a significant effect ($\text{sig} < 0.005$) on the phenol content.

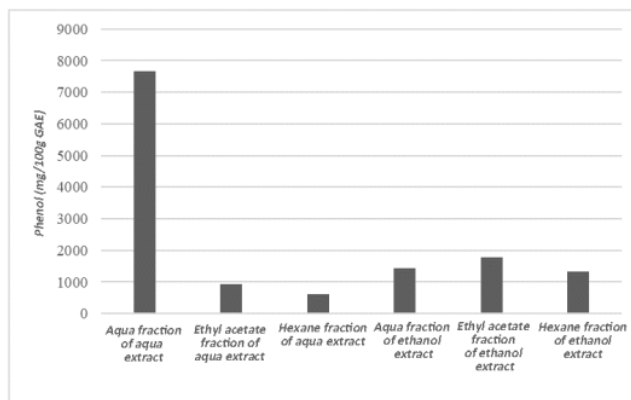


Figure 4: Phenol in sentul fruit peel

The highest phenol content is obtained in the aqua fraction of aqua extract with levels of 7657.96 mg/100g GAE (Galat Acid Equivalent) which is significantly different from other treatments. The lowest phenol content is achieved in the hexane fraction of aqua extract with a concentration of 626.28 mg/100g GAE.

3.2.5 Flavonoids

Figure 5 indicates the flavonoids content in sentul peel extract. Based on the ANOVA test results, fractionation treatment with hexane, ethyl acetate and aqua solvents on ethanol and aquades extracts of sentul fruit peel gave a significant effect ($\text{sig} < 0.005$) on flavonoids levels.

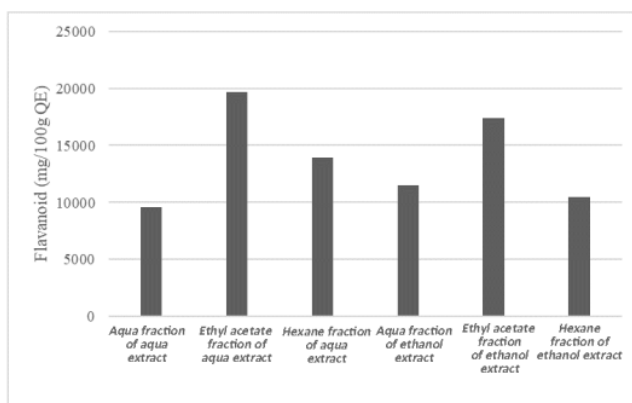


Figure 5: Flavonoids in sentul fruit peel

The highest flavonoids content is obtained in the ethyl acetate fraction of aquades extract of with levels of 19672.96 mg/100g QE (Quersetin Equivalent) which is not significantly different from the treatment of the ethyl acetate fraction of ethanol extract with levels of 17380.43 mg/100g QE and is significantly different from other

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treatments. The lowest flavonoids content is obtained in the hexane fraction of the ethanol extract with a concentration of 10436.92 mg/100g QE.

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3.2.6 Antioxidant activity

Figure 6 is the antioxidant activity of sentul peel extract. Based on the results of the ANOVA test, fractionation treatment with hexane, ethyl acetate and aqua solvents on ethanol and aquades extracts of sentul fruit peel holds a significant effect (sig < 0.005) on the antioxidant activity of 100 ppm extract.

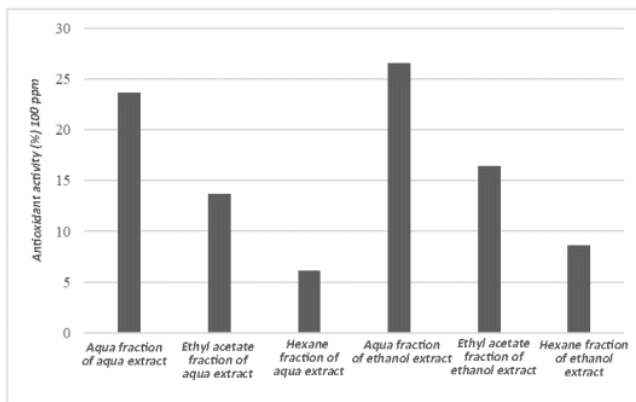


Figure 6: Antioxidant activity in sentul fruit peel

The highest antioxidant activity is from aqua fraction of ethanol extract with antioxidant activity of 26.529%, in which it is not significantly different from the treatment of the aqua fraction of aqua extract with antioxidant activity of 23.684% and significantly different from other treatments. The lowest antioxidant activity is obtained in the hexane fraction of aqua extract of with an antioxidant activity of 6.117%.

3.2.7 IC50 results of sentul peel extract

In addition, the antioxidant activity of the extract was tested at a concentration of 100 ppm against DPPH. (2,2-diphenyl-1-picrylhydrazyl). We also tested IC 50 (Inhibition Concentration 50%) of sentul fruit extract. Table 4 shows the results of sentul extract tested with IC50.

Table 4: Results of IC50 testing on sentul fruit

Treatment	IC50(ppm)	AAI (Antioxidant Activity Index)	Description
Aqua fraction sentul aqua extract	299.96	0.135	Weakest
Ethyl acetate fraction sentul aqua extract	365.63	0.110	Weakest
Hexane fraction aqua extract sentul	753.79	0.053	Weakest
Faction aqua ethanol extract sentul	189.54	0.211	Weak
Fraction of the ethyl acetate extract of ethanol sentul	340.98	0.117	Very weak
Fraction hexane extract ethanol sentul	608.78	0.066	Very Weak

According to table 4, the results of research, Sentul fruit extract has weak to very weak antioxidant activity with the value of Antioxidant Activity Index (AAI) ranging from 0.066 -0.211. The aqua fraction of sentul fruit peel ethanol extract has the highest AAI value of 0.211 (weak).

4. Discussion

Sentul fruit (*Sandoricum koetjape*) is known for its ability as traditional medicine. Our research evaluate the compounds in sentul fruit concerning as its use in gingivitis. On the quantitative test results i.e. table 2, based on the ANOVA²⁴, fractionation treatment with hexane, ethyl acetate and aqua solvents on the ethanol and aquadest extract hold a significant effect (sig<0.005) on the content of saponins, alkaloids, tannins, phenols, flavonoids and antioxidant activity. This shows that the type of solvent affects the content of phytochemical compounds in sentul fruit peel. Based on the test results, the ethanol extract has a higher content of alkaloids, tannins and flavonoids than the aquadest extract. The polarity of ethanol is lower than aquadest so that it can dissolve alkaloids, diglycosides, phenolics, flavonoids, and a small amount of essential oils (Agustiningih et al., 2010; Widyawati et al., 2014).

Alkaloids are generally non-polar compounds, while the pseudoalkaloids and protoalkaloids are soluble in water²³ (Truczynik, 2012). Alkaloids are more commonly found in polar solvents because the class of alkaloids compounds that have the potential as antioxidants are polar compounds that will be extracted in polar solvents (Gan et al., 2017).

Many tannins compounds are found in the use of aquadest as solvent. This is because tannins are more soluble in water solvents. This component is¹² found in the hexane fraction, because hexane is a non-polar solvent, so there are fewer soluble components. The results of our study are in accordance with the research of (Aini & Mardiyansih, 2016) which reported that tannins compounds are found in the aquadest fraction in the ethanol extract of pandan leaves²⁵ according to (Yuliana et al., 2014) tannins are polar compounds with hydroxyl groups, so to extract them, polar solvents such as methanol, ethanol, acetone and water are needed.

Saponins compounds tend to be polar soluble. Saponins are detected in polar and semi-polar solvents (Yusnawan, 2013; Robinson, 1995), but the smallest detected in non-polar solvents even on phytochemical s¹² taining, saponins are not detected in hexane fractionation which is because of the very small amount extracted. The results of this study are in accordance with the research of (Supriyanto et al., 2017) which stated that saponins compounds are found in neem leaf extract in polar solvents.

²⁹ Based on the results of the study, the highest phen⁷olic compounds were obtained in the fractionation of aquadest of sentul fruit peel extract. Phenol compounds have many hydroxyl functional groups or in free conditions (aglycones) will produce high levels of total phenol (Juan Moreno, 2012). The total phenol test is non-specific only on polyphenols. Phenolic components are known as polar antioxidants (Minatel et al., 2017). Aquadest has a polar nature so that it is able to dissolve polyphenol compounds well.

The graph of the flavonoids content of sentul fruit peel extract can be seen in Figure 5. Flavonoids are compounds that can be soluble in polar to non-polar solvents (Ferreira & Pinho, 2012). This statement is comparable to the results of the study where the highest flavonoid content is obtained in the ethyl acetate fractionation of Sentul fruit peel aquadest extract and the lowest is found in the hexane fraction.

Regarding figure 6 about antioxidant activity of sentul fruit peel extract, the highest antioxidant activity is held in the aqua fractionation of ethanol extract since ethanol solvent is semi polar so it is able to extract flavonoids compounds, phenols, tannins and alkaloids which have antioxidant properties.

³¹ Referring to table 4, the difference in IC50 value can be caused by the number of a¹⁹ xidant compounds such as flavonoids, phenols, tannins and alkaloids contained in the extract (Supriyanto et al., 2017). ¹³ higher the concentration of bioactive compounds in the extract, the more antioxidants extracted. Ethanol is a semi-polar solvent so that it has the ability to dissolv¹⁴ polar and non-polar compounds so that a lot of bioactive compounds with antioxidant properties are extracted. Based on the results of the research, the ethanol fraction of sentul fruit peel aqua extract contains high tannins, flavonoids, phenol and alkaloids content.

Conclusion

We have conducted a study regarding the chemical content in sentul fruit (*Sandoricum koetjape*). From several phytochemical compounds, flavonoids, saponins and tannins are aromatic hydroxyl groups that act as antibacterial. Therefore, observing from the highest levels of flavonoids, saponins and tannins, the aqua fraction of sentul ethanol extract is the best treatment with flavonoids content of 11476.16 mg/100g QE, tannins 88.605 mg/g and saponins 6.862 mg/g.

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