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(RESEARCH ARTICLE)

Chemical compositions and antioxidant activity of leaf and stem essential oils of *Bryophyllum pinnatum* (lam.) Kurz

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Abstract

Essential oils of air-dried samples of leaf and stem *Bryophyllum pinnatum* obtained by hydro-distillation in an all glass clevenger-type apparatus gave percentage yield of 0.14 and 0.55 respectively. The essential oils (EOs) were subjected to analysis using gas chromatography (GC), gas chromatography-mass spectrometry (GC-MS). Fifteen (15) compounds representing 97.78% of *B. pinnatum* leaf essential oil and seventeen (17) compounds, accounting for 97.23% *B. pinnatum* stem essential oil were identified. The major compounds present in leaf *B. pinnatum* essential oil were 1-octen-3-ol (19.52%), supraene (16.63%), 1-heneicosanol (12.00%), 2,5-dimethylheptane (8.42%) and (E)-9-eicosene (6.90%) while the main compounds of *B. pinnatum* stem were (E)-5-eicosene (25.71%), oleamide (20.25%), isolongifolol (13.07%) and β-gurjunene (7.71%). Both leaf and stem essential oils contain oleamide (5.63% and 20.25%), (E)-5-eicosene (6.99% and 25.71%), hexahydropseudoionone (2.24% and 2.66%) and phytol (3.73% and 3.09%) respectively. The Inhibitory concentration (IC_{50}) values (in µg/mL) of scavenging activity of the leaf essential oil were 789 and stem essential oil had 829 compared to standards (butylated hydroxyl anisole 40.39 and ascorbic acid 55.22) which indicated a moderate antioxidant activity. We report the chemical compound present in leaf and stem essential oils and their antioxidant properties.

Keywords: *Bryophyllum pinnatum*; Antioxidant; Essential oil; Hydro-distillation; Gas Chromatography-Mass spectrometry

1. Introduction

Bryophyllum pinnatum (Lam.) Kurz., (Crassulaceae) commonly known as the leaf of Life or life plant is a succulent perennial medicinal herb that grows 3 – 5 feet tall, with a fleshy dark green foliage, distinctively scalloped and hemmed in red and pendulous flowers [1]. It usually grows in tropical, sub-tropical and warm temperate climatic zone used in folk medicine in Africa, tropical America, India, China and Australia. The plant is available throughout the Southern part of Nigeria [2]. Some species are cultivated as ornamentals [3][4]. *B. pinnatum* is known as a universal antidote due to its abundant medicinal uses for the treatment of several ailments and it is well known for haemostatic and wound healing properties [3]. The leaf and bark are bitter tonic, astringent to the bowels, analgesic; useful in diarrhoea and vomiting [5]. It is used either externally or internally for all types of pains and inflammations, various bacterial, viral and fungal infections, leishmaniasis, upper respiratory infections, stomach ulcers, flu and fever [6]. The pharmacological activities on different extracts and fractions showed significant neuropharmacological effect [7], antinociceptive, anti-inflammatory, antidiabetic [8][9][10], antimicrobial [11][12][13], anti-ulcer[14] and antitumor activities [15]. Several active compounds have been isolated from this specie. They include: Syringic acid, caffeic acid, 4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, p-hydroxycinnamic acid, p-coumaric acid, ferulic acid, phosphoenol pyruvate,

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protocatechuic acid from aerial parts of the plant [16]. Its leaf contains astragalin, 3,8-dimethoxy-4,5,7-trihydroxyflavone, friedelin, epigallocatechin-3-o-syringate, luteolin, kaempferol [17], the plant is a good source of bufadienolides [18]. The cardienolide and steroidal contents includes β-sitosterol, bryophyllol, bryophynol, bryophyllin A & B, bryotoxin C, bufadienolide-1,3,5-orthoacetate with potent cytotoxicity [19][20][21][15][22][23]. Essential oils obtained from plant samples have been reported to possess unique medicinal effects such as digestive, antimicrobial, antioxidant, cytotoxic, antifungal, hypoglycemic and antispasmodic activities [24][25][26][27]. The essential oil composition of leaf *Bryophyllum pinnatum* had been evaluated [28], but no account of biological and antioxidant activities were conducted. As a result of the medicinal importance of *B. pinnatum*, therefore, this research is aimed to determine the chemical constituents of the leaf and stem essential oils of *B. pinnatum* and the antioxidant activity using 1,1–diphenyl–2–picrylhydrazyl radical (DPPH).

2. Material and methods

2.1. Plant materials

Bryophyllum pinnatum leaf and stem were collected from Forestry Research Institute of Nigeria (FRIN), Ibadan, identified and authenticated at the herbarium unit, Department of Botany, University of Ibadan, Nigeria where voucher samples had been deposited, with voucher number UIH-22780.

2.2. Extraction of essential oils

Sample of *Bryophyllum pinnatum* was collected, which were separated into leaf and stem parts giving 834 g and 211 g respectively. They were crushed separately and hydro-distilled for 3 hours in an all glass Clevenger-type apparatus designed to British Pharmacopeia specifications [29]. Oils were collected under an iced condition with 1 mL of distilled n-hexane, which the analysing GC corrected. The essential oils were obtained and stored at 4 °C before analysis [30].

2.3. Identification and quantification of the essential oil constituents

Leaf and stem *Bryophyllum pinnatum* essential oils were subjected to GC-MS analysis on an Agilent 7809 A Gas Chromatography hyphenated with an Agilent Mass Detector having split/splitless injector interfaced to mass selective detector operating at 70 eV. The ion source temperature was set to 200 °C over a mass spectral range of m/z 50-700 at a scan rate of 1428 amu/sec. The column of the GC used was HP-5MS with a length of 30 m, with an internal diameter of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was programmed as follows: initial temperature 80 °C for 2 min, increased at 10 °C/min to a temperature of 240 °C for 6 minutes. Helium was used as the carrier gas at a flow rate of 1 mL/min. Injection volume, linear velocity and pressure were adjusted at 1.0 µL, 362 cm/s and 56.2 KPa respectively. The oven temperature was set at 60 °C, hold for 1 min to 180 °C for 3 min at 10 °C/min, the final temperature was 280 °C for 2 min at 10 °C/min both the injector and detector temperatures were fixed at 250 °C. Identification of the essential oil components were based on comparison of mass spectral fragmentation patterns (NIST database 14.L/chemstation data system) with the data previously reported in the literature [31][32].

2.4. Antioxidant inhibition assay

The effects of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay of the essential oils were determined according to Onocha *et al* [33]. DPPH (3.95 mg) was dissolved in methanol (100 mL) to give 100 µM of methanolic DPPH. Aliquots of 0.5 mL of methanol solutions of each, containing essential oils in triplicates were added to 2.0 mL of methanolic DPPH. The absorbance at 517 nm was noted after 10 minutes in UV-Spectrophotometer. Five serially diluted concentrations (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL and 62.5 µg/mL) of the essential oil samples were prepared. The same procedure was carried out on butylated hydroxyl anisole (BHA) and ascorbic acid, which are standard antioxidants. The decrease in absorption was measured against that of the control (methanolic DPPH without samples) and the percentage inhibition was also calculated using the formula:

$$\% \, \mathrm{I} = \frac{\mathrm{Ac} - \mathrm{As}}{\mathrm{Ac}} \times 100$$

Where Ac is absorbance of the control, As is absorbance of sample and % I is percentage inhibition. All tests and analyses were done in triplicates and the average was obtained. The scavenging activity was expressed in terms of 50 % Inhibition concentration (IC_{50}), the concentration of the samples required to give a 50 % reduction in the intensity of the signal of the DPPH radical, was evaluated from the graph representing the percentage inhibition against sample concentration using regression analysis on Microsoft Excel.

3. Results and discussion

Essential oils from leaf and stem *Bryophyllum pinnatum* obtained by hydro-distillation gave colourless herbal aroma. The percentage yields obtained were 0.14 and 0.55 for *B. pinnatum* leaf and *B. pinnatum* stem respectively [Table 1]. The oils were analysed using Gas Chromatography [GC], Gas Chromatography-Mass Spectrometry [GC-MS]. The chromatograms were presented in Figs 1 and 2. Fifteen (15) compounds representing 97.23 % of leaf essential oil of *B. pinnatum* and Seventeen (17) compounds making a total of 97.98 % of Stem essential oil of *B. pinnatum* were identified. Results of the 28 identified compounds in both leaf and stem essential oil and comparison of the amount of classes of compounds found in the two essential oils were presented in Table 2. The percentage Inhibition (% I) and inhibitory concentration (IC_{50}) of leaf and stem B. *pinnatum* essential oils compared with reference standard were shown in Table 3. Structures of some terpene/terpenoid from leaf and stem essential oil of *B. pinnatum* were presented in Fig 3 while percentage inhibition of leaf and stem oils at different concentration compared with standards were made known in Fig 4.

Table 1 Essential oils obtained from leaf and stem parts of *Bryophyllum pinnatum*

Plant parts	Weight of sample (g)	Weight of essential oil obtained (g)	% Yield of essential oil obtained	Odour assessment
Leaf	834	1.17	0.14	Leafy aroma
Stem	211	1.16	0.55	Herbal aroma

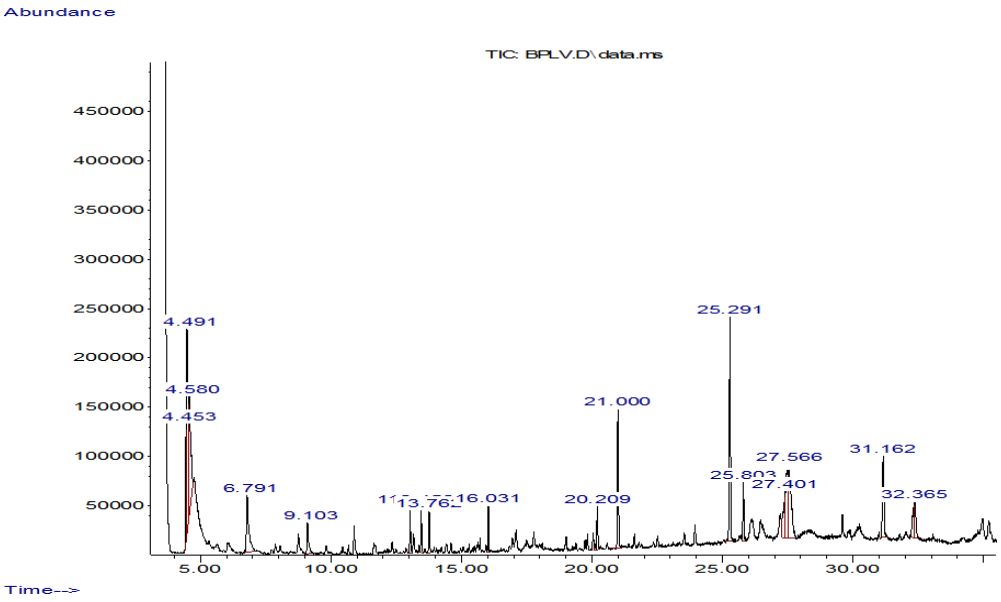

Figure 1 Gas chromatogram of the leaf essential oil of *Bryophyllum pinnatum*

The significant compounds present in *B. pinnatum* leaf essential oils include, 1-octen-3-ol (19.52%), supraene (16.63%), 1-heneicosanol (12.00%), 2,5-dimethylheptane (8.42%), (E)-5-eicosene (6.90%), oleamide (5.63%), tritetracontane (5.09%), phytol (3.09%), trans-fernesol (3.96%) and β-cyclocitral (2.32%) while the remaining compounds appeared in trace amount.

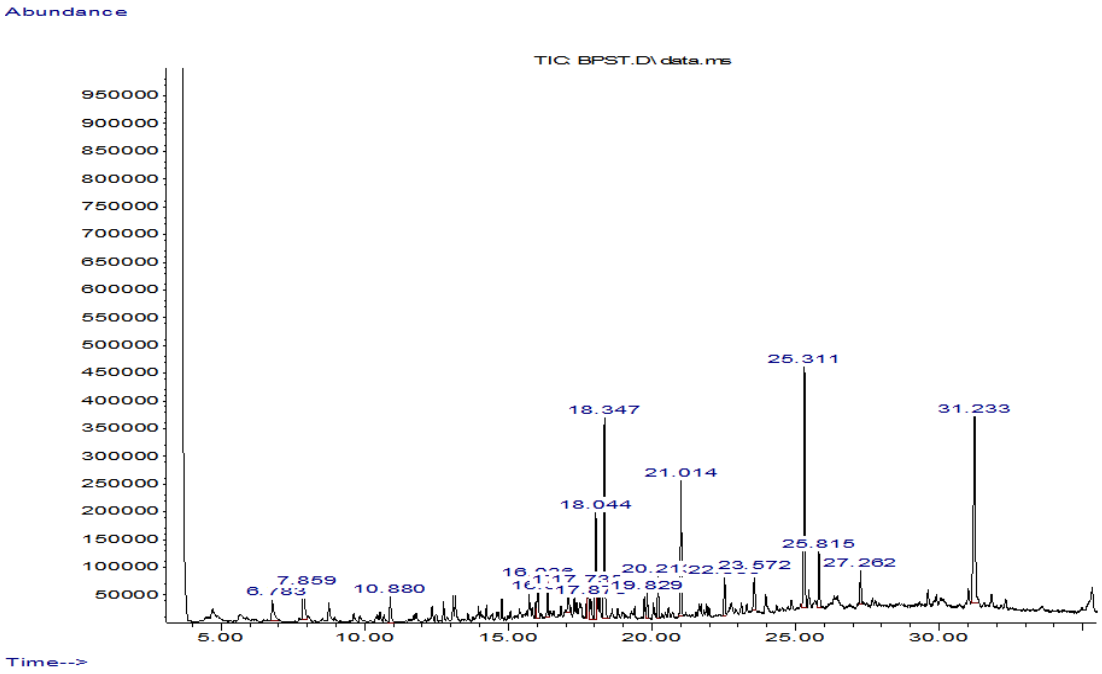

Figure 2 Gas chromatogram of the stem essential oil of *Bryophyllum pinnatum*

The most abundant compounds present in stem essential oil were (E)-5-eicosene (25.71%), oleamide (20.25%), 5-isolongifolol (13.07%) and β-gurjunene (7.71%). The stem oil also features significant quantities of (E)-2-nonenal (3.30%), phytol (3.73%), β-bisabolene (2.95%), hexahydropseudoionone (2.66%), cycloheptane (2.60%) and (Z)-hexadecanamide (2.09%) [Table 2]. However, both leaf and stem essential oils contain oleamide (5.63% and 20.25%), (E)-5-eicosene (6.99% and 25.71%), hexahydropseudoionone (2.24% and 2.66%) and phytol (3.73% and 3.09%) respectively. The leaf oil consists of 32.90% terpene/terpenoid while total terpene/terpenoid present in stem essential oil was 59.42%. The essential oils had a significant amount of sesquiterpenes/sesquiterpenoids such as β-gurjunene, isolongifolol etc and diterpenes which include phytol. This result when compared with an earlier report by Aboaba *et al* [28] showed variation in identified compounds, which could be as a result of differences in plant location. Their report had given a basis for comparison, and we have improved on it.

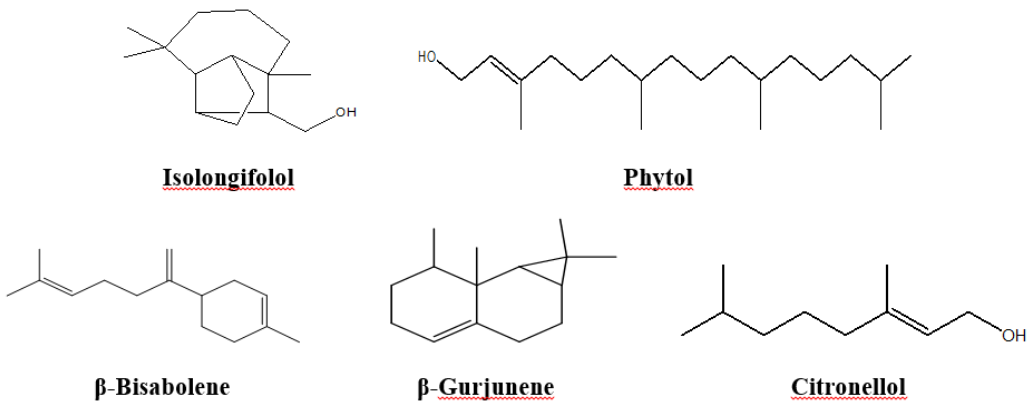

Figure 3 Structures of some terpene/terpenoid from leaf and stem essential oil of *B. pinnatum*

The results for antioxidant activity and scavenging ability on DPPH radicals of the two essential oils [Table 3 and Figure 3] revealed moderate activity of the oils compared to the standards as shown by the percentage inhibition (% I) and inhibitory concentration (IC_{50}) of the result obtained. The effectiveness of the essential oils and the reference standards were in descending order, thus; BHA > Ascorbic acid > Bpleaf > Bpstem. The ethno-medicinal activity of the two essential oils may be dependent on the compounds present in these oils.

Table 2 Chemical compositions of leaf and stem essential oils of *Bryophyllum pinnatum*

S/N	Retention time (min)	Compounds	Molecular formula	Molecular weight (g/mol)	% Area	
					leaf	Stem
1	4.45	1-octen-3-ol	$C_8H_{16}O$	128.22	19.52	-
2	4.58	2,5-dimethylheptane	C_9H_{20}	128.26	8.42	-
3	6.78	cycloheptane	C_7H_{14}	98.19	-	2.60
4	6.79	nonanal	$C_9H_{18}O$	142.24	6.18	-
5	7.86	(E)-2-nonenal	$C_9H_{16}O$	140.23	-	3.30
6	9.10	β-cyclocitral	$C_{10}H_{16}O$	152.23	2.32	-
7	10.88	(E,E) 2,4-decadienol	$C_{10}H_{16}O$	152.23	-	1.95
8	13.46	2-ethenyl-1,3,3-trimethyl-cyclohexene	$C_{11}H_{18}$	150.26	2.01	-
9	13.76	trans-β-ionone	$C_{13}H_{20}O$	192.3	1.84	-
10	16.03	1,1-(1,2-dimethyl-1,2-ethanediyl)-bis-cyclohexane	$C_{13}H_{30}$	222.41	1.86	-
11	16.04	β-Bisabolene	$C_{15}H_{24}$	204.35	-	2.95
12	16.37	2,7-dimethyl-3,6-bis(methylene)-1,7-Octadiene	$C_{12}H_{18}$	162.27	-	1.62
13	17.08	1-Tetradecanol	$C_{14}H_{30}O$	214.39	-	2.10
14	17.74	8,9-dehydrocycloisolongifolene	$C_{15}H_{22}$	202.34	-	1.68
15	17.87	Benzenepropanamine	$C_{10}H_{15}N$	149.23	-	1.64
16	18.04	β-Gurjunene	$C_{15}H_{24}$	204.35	-	7.71
17	18.35	Isolongifolol	$C_{15}H_{26}O$	222.37	-	13.07
18	19.83	Isopropyl myristate	$C_{17}H_{34}O$	270.45	-	1.55
19	20.21	Hexahydropseudoionone	$C_{13}H_{26}O$	198.34	2.24	2.66
20	21.01	(E) 5-Eicosene	$C_{20}H_{40}$	280.54	6.99	25.71
21	23.57	Citronellol	$C_{10}H_{20}O$	156.27	-	2.62
22	25.29	1-Heneicosanol	$C_{21}H_{44}O$	312.58	12.00	-
23	25.82	Phytol	$C_{20}H_{40}O$	296.33	3.09	3.73
24	27.26	(Z) Hexadecanamide	$C_{16}H_{33}NO$	281.48	-	2.09
25	27.40	trans-Fernesol	$C_{15}H_{26}O$	222.37	3.96	-
26	27.49	Supraene	$C_{30}H_{50}$	410.72	16.63	-
27	31.16	Oleamide	$C_{18}H_{35}NO$	281.48	5.63	20.25
28	32.34	Tritetracontane	$C_{43}H_{88}$	605.18	5.09	-
% identified					97.78	97.23
Class of terpenes/terpenoids						
Monoterpenoid					2.32	4.57
Sesquiterpene					-	12.34
Sesquiterpenoid					3.96	13.07
Diterpene					6.99	25.71
Diterpenoid					3.09	3.73
Triterpene					16.63	-
Total terpene/terpenoid					32.90	59.42
Non-terpene derivatives					64.88	37.81

Samples having phenolic moieties exhibit good antioxidant activity [34]. Hence, the moderate antioxidant activity of the essential oil could be due to the presence of phenolic/hydroxyl group constituents such as phytol, citronellol and 5-isolongifolol. Phytol is used as a precursor for the manufacture of vitamin E and K, in which Vitamin E is a good antioxidant drug. The compound, 1-Octen-3-ol is a good insect (mosquitoes) repellent that works by blocking the insects' octenol odorant receptors [35]. Therefore, the presence of 1-Octen-3-ol in leaf essential oils of *B. pinnatum* as the most abundance confirmed that the plant could have insecticidal property [36].

Table 3 % Inhibition and IC_{50} of leaf and stem B. *pinnatum* essential oils compared with reference standard

Sample	1.0 mg/mL	500 µg/mL	250 µg/mL	125 µg/mL	62.5 µg/mL	IC_{50} µg/mL
BHA	96.23	96.05	95.06	94.40	94.65	40.39
Asc. acid	96.96	97.37	96.38	86.91	85.84	55.22
Bpleaf	50.12	43.37	43.37	40.16	36.38	789
Bpstem	51.36	43.62	41.15	40.33	39.67	829

BHA = Butylated hydroxyl anisole, Asc. acid = Ascorbic acid, Bpleaf = *Bryophyllum pinnatum* leaf, Bpstem = *Bryophyllum pinnatum* stem

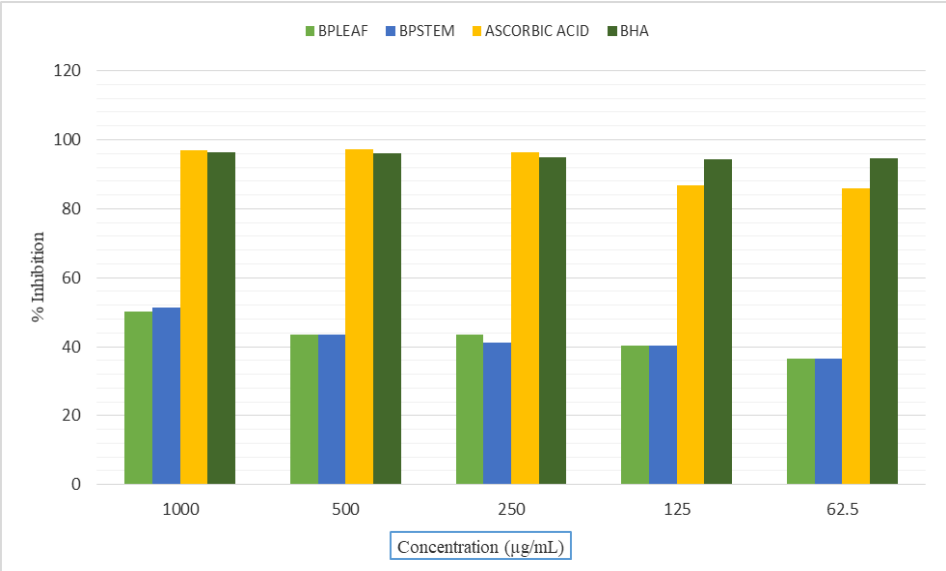

Figure 4 % Inhibition of the leaf and stem essential oils at different concentrations compared with reference standard

4. Conclusion

The chemical constituents of leaf and stem essential oil of *B. pinnatum* are been reported, with moderate antioxidant activity. This could be traced to the presence of vital compounds such as phytol, isolongifolol, β-gurjunene, β-bisabolene, citronellol, which are important terpene/terpenoid compounds. The compounds present in these essential oils could account for their usage in ethno-medicine.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

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