



Antimicrobial Activities of Selected Four Less Known Pulses

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Authors' contributions

This work was carried out in collaboration between all the authors. Author RP designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PNP, KJ and BJ managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The antimicrobial activity of hot water extracts of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* seeds were analysed against three different microbial strains with respect to various concentrations (30–90 µg/ml) the zone of inhibition of test concentrations were compared with standard concentration of control (Erythromycin 10 µg/ml and Chloramphenicol 10 µg/ml). Among the two different bacteria used in *Streptococcus pyogenes* the zone of inhibition is higher (19.05±0.29) in 90 µg/ml concentration in aqueous extracts of *E. scandens*, in *Vicia faba* the zone of inhibition is (21.10±0.27 mm) in the case of *V. aconitifolia* the zone of inhibition was observed in 30 µg/ml concentration (20.2±0.21) and in *V. sinensis* at 90 µg/ml concentration the zone of inhibition was (21.4±0.28 mm) observed. *Klebsiella pneumoniae* the extracts of *E. scandens* shows the zone of inhibition is higher (20.01±0.12 mm) in *Vicia faba* the zone of inhibition (20.3±0.14 mm) In the case of *V. aconitifolia* (19.3±0.14) and *V. faba* is (21.4±0.11 mm). The human fungal pathogen like *Candida albicans* the zone of inhibition was observed in hot water extracts of *Entada scandens*, was (20.00±0.19 mm) in 90 µg/ml concentration, in *V. faba* was (20.2±0.11 mm). In case of *V. aconitifolia* the zone of inhibition was (19.3±0.19 mm) and in *V. sinensis* the zone of inhibition is higher in *C. albicans* (19.1±0.11 mm) in 90 µg/ml concentration.

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1. INTRODUCTION

Pulses belong to the family Leguminosae [1]. The family Leguminosae is made up of many species which are cultivated all over the world [2]. The use of pulses range from their forming a staple diet to their being used as condiments, milk, cheese and snacks [3,4]. In common pulses contain abundant nutrients with biological activities the chemical constituents such as flavonoids, phenolic acids, organic acids, amino acids, carbohydrates, and lipids. Moreover, they also used as biological activities, including antioxidant, antimicrobial, anti-inflammatory, antidiabetic, antihypertensive, lipid metabolism accommodation, antihypertensive, and antitumor effects, etc., so its better application of this commonly used food as a medicine [5].

Entada scandens (L) Benth. Belonging to family Mimosaceae it's a perennial, vines, twining this plant has been used by the folkloric medicinal practitioners for long the treatment like skin ulcer (cancer), snake bite, stomach disorders and ureterolithiasis [6]. *E. rheedei* seeds used the treatment of jaundice [7], antibacterial activities and antifungal activities [8]. *E. abyssinica* used for anti-inflammatory activity in acute and chronic models of inflammation [9]. *E. africana* are traditionally used as source of medicines against liver related diseases [10].

Faba bean, *Vicia faba*, is a winter growing pulse, belongs to the family Fabaceae. Antimicrobial activity was observed with the leaves of this bean from sterile distilled water extract [11,12].

Vigna aconitifolia (Jacq.) Marechal is an annual, slender, hairy herb belongs to the family Fabaceae commonly called moth bean, seeds are used medicinally in diets to treat fevers; roots are said to be narcotic. *Vigna sinensis* (L.) Walp. from Fabaceae, the Pea family. It is commonly called Cowpea. It is an annual legume and herbaceous vine. The seeds also have deworming and diuretic properties, and promote stomach health; when powdered and burned they alleviate insect bites. The leaves and seeds are made into compresses to treat blisters. The root also has medicinal properties and is used as a cure for snakebites and as medicine for epilepsy, chest pain, and dysmenorrhea. The plant is also used as animal feed. In Nigeria, the fibers of the peduncles are used to make fishnets and paper [13].

2. MATERIALS AND METHODS

2.1 Source of Seed Materials

The seeds of *Entada scandens* L. (Mimosaceae) were collected from Noolpuzha of Wayanad (Dt), Kerela identified and authenticated by Botanical Survey of India Coimbatore. The other seeds *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* (Fabaceae) were obtained from Tamilnadu Agricultural University, (TNAU) Coimbatore, Tamilnadu.

2.2 Extraction from Seeds

The seeds was air dried and powdered, stored in room temperature. Ten grams of powder was soaked in 20 ml of hot water overnight and then filtered through Whatman filter paper No. 41 along with 2 grams sodium sulphate to remove the sediments and traces of water in the filtrate. Before filing the filter paper along with sodium sulphate is wetted with hot water. The filtrate was then concentrated by bubbling nitrogen gas into the solution and reduces the volume to one ml [14].

2.3 Antimicrobial Activity

The test organisms used were clinical isolates viz., *Streptococcus pyogenes* (Gram positive) *Klebsiella pneumoniae* (Gram negative). The human fungal pathogens like *Candida albicans* are obtained from Department of Microbiology, PSG College of Arts and Science Coimbatore. The bacterial and the fungal cultures were maintained on nutrient agar medium and potato dextrose agar (PDA) medium respectively.

2.4 Growth and Maintenance of Test Organism for Antimicrobial Studies

The bacterial and fungal cultures were maintained on nutrient broth (NB) at 37°C and fungus were maintained on Potato dextrose agar (PDA) at 28°C. The gram positive bacteria *S. pyogenes*, and gram negative bacteria *K. pneumoniae* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm). The fungal inoculums *C. albicans*, were prepared from 5 to 10 day old culture grown

on potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

2.5 Antibacterial Activity [15]

The aqueous extract were tested by the well diffusion method. Different concentration of the extracts (30 -90 μ l/ml) was prepared by reconstituting with aqueous solution. The test microorganisms were seeded into respective medium by spread plate method 10 μ l (10 cells/ml) with the 24 h cultures of bacteria growth in nutrient broth. After solidification the filter paper wells (5 mm in diameter) impregnated with the extract were placed on test organism-seeded plates. Erythromycin (10 μ l) used as standard for antibacterial test. The antibacterial assay plates were incubated at 37°C for 24 hrs. The diameters of the inhibition zones were measured in mm.

2.6 Antifungal Activity [16]

The antifungal activity was tested by well diffusion method. The potato dextrose agar plates were inoculated with each fungal culture (10 days old) by point inoculation. The filter paper wells (5 mm in diameter) impregnated with 100 μ l concentrations of the synthesized silver nanoparticles were placed on test organism-seeded plates. Chloramphenicol (10 μ l) used as positive control. The activity was determined after 72 hrs of incubation at 28°C. The diameters of the inhibition zones were measured in mm.

3. RESULTS AND DISCUSSION

The antimicrobial activity of hot water extracts of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* against three different microbial strains with respect to various concentrations (30 – 90 μ g/ml) were presented in the Tables 1 and 2. The zone of inhibition of test concentrations were compared with standard concentration of control (Erythromycin 10 μ g/ml and Chloramphenicol 10 μ g/ml). Among the two different bacteria used (*Streptococcus pyogenes* and *Klebsiella pneumonia*).

In the case of *E. scandens* against *S. pyogenes* the zone of inhibition was observed (19.05 ± 0.29)

in 90 μ g/ml concentration (Plate 1 B) against the control (16.01 ± 0.10 mm in 10 μ g/ml), followed by 60 μ g/ml concentration (17.23 ± 0.24 mm). The lowest inhibition were observed in 30 μ g/ml concentration (16.55 ± 0.34 mm). In *K. pneumonia* the zone of inhibition is higher (20.01 ± 0.12 mm) in 90 μ g/ml concentration (Plate 1 A and Table 1) against the control (16.65 ± 0.14 mm 10 μ g/ml), followed by 60 μ g/ml concentration (16.01 ± 0.17 mm). The lowest inhibition were observed in 30 μ g/ml concentration (15.33 ± 0.20 mm).

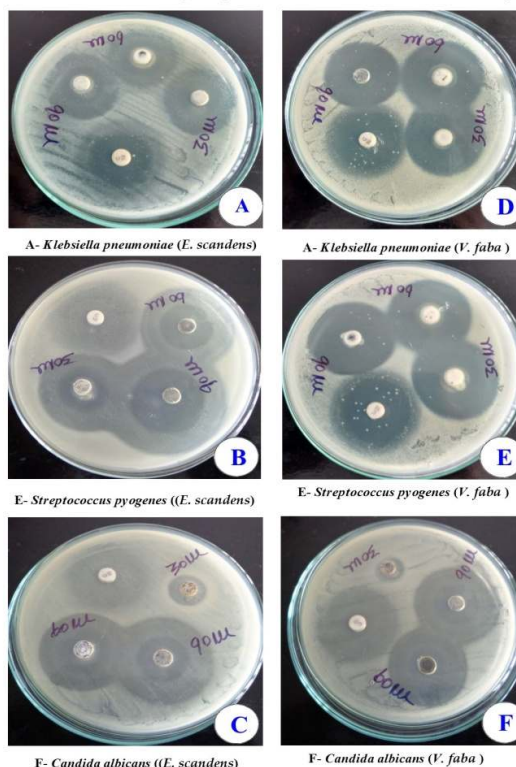


Plate 1. Antimicrobial activity of aqueous extract of *Entada scandens* and *Vicia faba*

In *Vicia faba* the zone of inhibition in *S. pyogenes* is higher in 90 μ g/ml concentration (21.10 ± 0.27 mm) against its control (17.3 ± 0.29 /10 μ g/ml) (Plate 1 E) followed by 60 μ g/ml concentration (19.01 ± 0.8 mm). The lowest inhibition were observed in 30 μ g/ml concentration (17.1 ± 0.29 mm). In *K. pneumonia*, extracts of *V. faba* the zone of inhibition were (20.3 ± 0.14 mm) in 90 μ g/ml concentration (Plate 1 D and Table 1) against the control (16.3 ± 0.31 mm 10 μ g/ml), followed by 60 μ g/ml concentration (19.1 ± 0.2 mm). The lowest inhibition were observed in 30 μ g/ml concentration (16.2 ± 0.18 mm).

Table 1. Antibacterial activity of hot water extract of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis*

S. no	Seed material aqueous extract	Zone of inhibition (mm)							Erythromycin 10 µl
		<i>Streptococcus pyogenes</i>			Standard	<i>Klebsiella pneumoniae</i>			
		30 µl	60 µl	90 µl	Erythromycin 10 µl	30 µl	60 µl	90 µl	
1.	<i>Entada scandens</i>	16.55±0.34	17.23±0.24	19.05±0.29	16.01±0.10	15.33±0.20	16.01±0.17	20.01±0.12	16.65±0.14
2.	<i>Vicia faba</i>	17.1±0.29	19.01±0.8	21.1±0.27	17.3±0.29	16.2±0.18	19.1±0.2	20.3±0.14	16.3±0.31
3	<i>Vigna aconitifolia</i>	20.2±0.21	19.1±0.15	16.2±0.17	17.4±0.16	16.3±0.16	17.4±0.12	19.3±0.14	18.3±0.16
4	<i>Vigna sinensis</i>	19.5±0.16	19.8±0.14	21.2±0.28	19.4±0.17	17.5±0.18	19.2±0.13	21.4±0.11	16.4±0.17

Table 2. Antifungal activity of hot water extract of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis*

S. no	Seed material aqueous extract	Aqueous extract zone of inhibition (mm) <i>Candida albicans</i>			Standard (Chloramphenicol 10 µl)
		30 µl	60 µl	90 µl	
1	<i>Entada scandens</i>	10.40±0.30	18.45±0.25	20.00±0.19	16.40±0.22
2	<i>Vicia faba</i>	8.2±0.30	17.4±0.30	20.2±0.11	17.3±0.31
3	<i>Vigna aconitifolia</i>	9.4±0.16	17.2±0.13	19.3±0.19	16.25±0.29
4	<i>Vigna sinensis</i>	8.11±0.31	15.5±0.16	19.1±0.11	16.3±0.19

In the case of *V. aconitifolia* against *S. pyogenes* the zone of inhibition is higher (20.2 ± 0.21) in 30 $\mu\text{g/ml}$ concentration (Plate 2 B) against the control (17.4 ± 0.16 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (19.1 ± 0.15). The lowest inhibition were observed in 90 $\mu\text{g/ml}$ concentration (16.2 ± 0.17) (Plate 2 B and Table 1). In *K. pneumonia* the zone of inhibition is higher (19.3 ± 0.14) in 90 $\mu\text{g/ml}$ concentration (Plate 2 A and Table 1) against the control (18.3 ± 0.16 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (17.4 ± 0.12 mm). The lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (16.3 ± 0.16 mm).

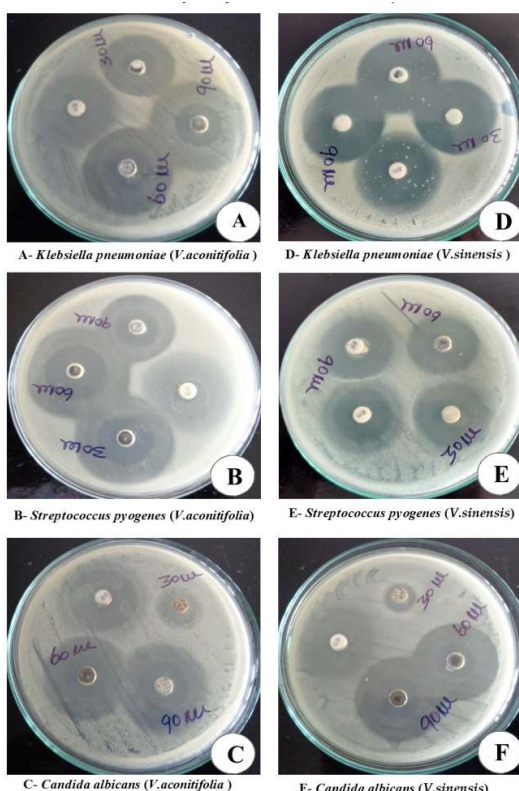


Plate 2. Antimicrobial activity of aqueous extract of *V. aconitifolia* and *V. sinensis*

In *V. sinensis* the zone of inhibition in *S. pyogenes* is higher in 90 $\mu\text{g/ml}$ concentration (21.4 ± 0.28 mm) against its control (19.0 ± 0.24 mm 10 $\mu\text{g/ml}$) (Plate 2 E Table 1) followed by 60 $\mu\text{g/ml}$ concentration (19.8 ± 0.14 mm). The lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (19.5 ± 0.16 mm). In *K. pneumonia*, *V. faba* the zone of inhibition is higher (21.4 ± 0.11 mm) in 90 $\mu\text{g/ml}$ concentration (Plate 2 D and Table 1) against the control (16.4 ± 0.17 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration

(19.2 ± 0.13 mm). The lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (17.5 ± 0.18 mm).

The human fungal pathogen like *Candida albicans* the zone of inhibition was observed in hot water extracts of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* compared with standard drug (Chloramphenicol 10 μl) and presented in Table 2. In case of *E. scandens* the zone of inhibition is higher in *C. albicans* (20.00 ± 0.19 mm) in 90 $\mu\text{g/ml}$ concentration (Plate 1C Table 2) against the control (16.40 ± 0.22 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (18.45 ± 0.25 mm) and lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (10.40 ± 0.30 mm).

In *V. faba* the zone of inhibition was in *C. albicans* (20.2 ± 0.11 mm) in 90 $\mu\text{g/ml}$ concentration (Plate 1F and Table 2) against the control (17.3 ± 0.31 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (17.4 ± 0.30 mm) and lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (8.2 ± 0.30 mm).

In case of *V. aconitifolia* the zone of inhibition is higher in *C. albicans* (19.3 ± 0.19 mm) in 90 $\mu\text{g/ml}$ concentration (Plate 2C, Table 2,) against the control (16.25 ± 0.29 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (17.2 ± 0.13 mm) and lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (9.4 ± 0.16 mm).

In *V. sinensis* the zone of inhibition is higher in *C. albicans* (19.1 ± 0.11 mm) in 90 $\mu\text{g/ml}$ concentration (Plate 2F, Table 2) against the control (16.3 ± 0.19 mm 10 $\mu\text{g/ml}$), followed by 60 $\mu\text{g/ml}$ concentration (15.5 ± 0.16 mm) and lowest inhibition were observed in 30 $\mu\text{g/ml}$ concentration (8.11 ± 0.31 mm).

The antimicrobial activity of hot water extracts of *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* against the *S. pyogenes*, *K. pneumonia* and *C. albicans* the zone of inhibition is higher in 90 $\mu\text{g/ml}$ followed by 60 $\mu\text{g/ml}$ concentration. Increasing the concentration the zone of inhibition also increased.

Similar observation also reported *Entada abyssinica* and *Entada africana* the *in vitro* antibacterial activities eight Gram-negative bacteria, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Providencia stuartii* by [17], similarly *in vitro* antibacterial activity of a phytoconstituent,

entagenic acid isolated from the seed kernel of *Entada pursaetha* the entagenic acid showed the high antibacterial activity against *B. cereus* and *B. subtilis* observed by [18]. The antifungal activities of methanolic fractions from the stem bark of *Entada spiralis* against fungus, *Candida glabrata*, were tested the highest antifungal activity with the inhibition zone diameter of 22 mm [19].

In *Entada leptostachya*, showed the great potential antibacterial activity against *Bacillus cereus*, *Escherichia coli*, *Micrococcus lutea* and *Pseudomonas aeruginosa* [20]. In *E. africana* also has the antibacterial activity of the ethanolic extract of inhibited the growth of *S. typhi* and *B. subtilis* [21].

Similarly in *Entada scandens* also reported in antimicrobial activities against Gram-positive bacteria *Enterococcus* and *Bacillus subtilis*, Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) and the yeast-like pathogenic fungus *Candida albicans* [22]. But in *Entada scandens* contrary to our studies in antimicrobial assay there is no significant inhibition was found against *Escherichia coli*, *Pseudomonas aureus*, *Plesiomonas shigelloides*, *Salmonella typhi*, *S. paratyphi*, *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*, *Proteus vulgaris*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *S. aureus*, *S. epidermidis* and *Streptococcus pyogenes* [23].

In *Vicia villosa* isolated antibacterial compounds from hairy showed potential antibacterial effect the inhibition zones (7.3±0.3 to 11.2±0.4 mm) against *Rhizobium vitis* and *Bacillus subtilis* [12]. In contrary *Vicia faba* the dried seeds of broad beans were investigated the extract did not show antimicrobial activity against all the three gram positive and one gram negative test bacterial pathogens [24].

In broad bean (*Vicia faba* L) such as flowers, leaves, seeds, and seed hulls and were tested against *Bacillus subtilis*, *Serrata marcescens*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsellia pneumonia*, *Shigella* sp and *Micrococcus pyogenes*. Better antimicrobial activity was observed with the leaves of broad bean sterile distilled water extract with a zone of inhibition in between 13-32 mm. Flower and seed hull showed by *Micrococcus pyogenes* a zone of inhibition in between 15-29 and 14-19 mm, respectively leaves, seed hulls flowers of broad

bean with ethanol extract showed by *Escherichia coli* a zone of inhibition in between 25-34, 15-17 and 15 mm, respectively. Flower with ethanol extract had a zone of inhibition in between 15-16 mm by *Bacillus subtilis* and *Micrococcus pyogenes* respectively [11].

Similarly in *V. radiata* the antibacterial activities of extracts from sprouted seeds of *V. radiata* were extracted with three different solvents the methanol extracts showed significant concentration dependent antibacterial activity against almost all the test pathogens [25]. In cowpea *Vigna unguiculata* the ethanol extract showed antifungal activity against *Fusarium proliferatum*. The acetone extract and ethanol extracts inhibited growth of the Gram-positive bacteria, *Staphylococcus aureus* and *Enterococcus faecalis*, at 2.5 mg/ml and *Bacillus cereus*, *B. subtilis* and *Enterobacter cloacae* at 5.0 mg/ml. Ethanol extracts of the same cultivar only showed antibacterial activity against *Enterococcus faecalis* and *E. cloacae* at 5.0 mg/ml [26].

In *Vigna radiata* reported the antibacterial potentials of chloroform and methanol extracts were evaluated against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Salmonella* spp. Chloroform extracts in general exhibited greater antibacterial activity compared to methanol extracts [27]. In *Vigna unguiculata* antibacterial activity in Gram positive bacteria, *Bacillus subtilis* and Gram negative bacteria, *Escherichia coli* the results showed the highest positive antibacterial activity with an inhibition diameter of 22 mm in case of aqueous extract of 300 µl/ml concentration, against the Gram negative bacteria; *Escherichia coli*. The *E. coli* species were found to be more sensitive than that of the *Bacillus subtilis*. The aqueous extract exhibited more antibacterial activity against both the Gram positive and Gram negative organisms than that of the ethanolic extract [28]. The antifungal activity on *V. unguiculata* against two bean fungal pathogens *Colletotricum lindemuthianum* and *C. griseola* [29].

The sprouts of mung beans *Vigna radiata* including fungi remarkable antimicrobial activity against highly infectious *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *S. aureus* and *Salmonella* as well as against human fungal pathogens, *Trichophyton rubrum* and *Trichoderma harzianum* [30].

4. CONCLUSIONS

From the above reports all the four pulses, *Entada scandens*, *Vicia faba*, *Vigna aconitifolia* and *Vigna sinensis* the presence of alkaloids, glycosides, tannins, flavonoids and saponins, might be the reason for the antimicrobial activity. The study showed good results in all the seed extracts. Further studies needs to be isolate the specific compounds from the seeds.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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