Research Article

Physicochemical Analysis, *in vitro* Antioxidant and Antimicrobial Potential of Leaves of *Bridelia montana* (Roxb.) Willd

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ABSTRACT

Aim: The aim of this particular study is to analyse the anti-oxidant and the anti-microbial potential of the leaves of an ethnomedicinal plant, *Bridelia montana* (Roxb.) Willd. which belongs to the family Phyllanthaceae. **Materials and Methods:** The physicochemical parameters of the powdered plant samples was analyzed by the methods suggested in the The Indian Pharmacopoeia (1996). The phytochemical constituents of the leaf extracts were studied, the anti-oxidant potential and the anti-microbial activity of the ethanol leaf extract was analysed using DPPH free radical scavenging method and agar well-diffusion method respectively. **Results:** The physicochemical analysis revealed that the sulphated ash was found to be the highest among all the other parameters and the moisture content was relatively less. The qualitative phytochemical analysis showed the presence of flavonoids, tannins, phenols, glycosides and alkaloids in their leaf extracts. *In vitro* antioxidant analysis showed that the ethanol leaf extract had good free radical scavenging activity when compared with standard Ascorbic acid. The *in vitro* antimicrobial analysis showed good zone of inhibition against most of the tested microorganisms in the concentration dependent manner. **Conclusion:** The present study showed that the ethanol extract of *B. montana* leaves has significant amount of phytochemicals, antioxidant and antimicrobial activity. Many biologically active secondary metabolites were present in the leaf extract which could be responsible for its therapeutic potential.

Keywords: Organoleptic, Physicochemical, Antioxidant, Antimicrobial, *Bridelia montana*, Phytochemicals, Ethnomedicine.

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INTRODUCTION

Healing with natural sources especially medicinal plants are practiced from the time as old as humankind itself. Connection between human beings and the hunt for drugs from nature dates from the far past, among them there are ample evidences from numerous sources like preserved documents, written documents, monuments and also through plant medicines.[1] Plants have provided human beings with all their needs in terms of food, shelter, clothing, medicines, flavours and fragrances. Plants have formed the basis of traditional medicine systems among which are Ayurveda, Siddha, Unani, Chinese amongst others.[2] Herbal formulations now-a-days offer many options that can alter the progress of the medicines. Now-a-days, medicinal plants has formed the basis of the preparation and marketing of drugs and their scientific and commercial significance has been increasing. These products that are derived from plants and are meticulously standardized to ensure their efficacy and safety for a specific application.[3]

"Among around 258,650 higher plant species reported from the world; more than 10% are used to cure ailing communities. Beside many known drugs (e.g. tubocurarine, reserpine, aspirin and morphine etc) are discovered based on traditional knowledge".[4] Plants are the rich resources of medicines and most

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of the modern medicines own their origin from plant. Ethnobotanically, all over the world, plants are the chief sources of medicines till date, which are mostly used by traditional, folklore, complementary and alternative medicinal forms. Although the use of plants as medicine is as old as mankind itself throughout the history, their scientific role and mechanism of action are being researched only recently.[5]

Secondary metabolites (SM) are the compounds that are present in the plants and are not necessary for a cell or an organism to live, but play a vital role in the interaction of the cells with their environment. These compounds are often involved in plant's protection against the biotic and abiotic stresses. Secondary metabolites are highly inducible in response to stresses. A few secondary metabolites are used as drugs, flavours, fragrances, insecticides, and dyes and thus have a great economic value.[6]

Approximately 60 species of *Bridelia*, (Phyllanthaceae) are found throughout the tropical and subtropical regions of the world, mainly in Africa and Asia. Several species of *Bridelia* are used in popular medicines as anti-diabetic, anti-anemic, anti-inflammatory, anti-bacterial, anti-convulsant, anti-diarrhoeal, anti-helmintic, anti-malarial, anti-nociceptive, anti-viral, anti-microbial, hypoglycemic and for abdominal pain, gynaecological, sexual and cardiovascular diseases.[7] *Bridelia montana* is an ethno-medicinally important plant that is being used for treating diarrhoea, dysentery and cold in children, boils, blisters and cuts by the Irula tribal people in the southern region of India,[8-10] this study explores it's pharmacological values by various methods.

MATERIALS AND METHODS

Plant Collection

The plant samples of *Bridelia montana* was collected from Thoovaipathy, a tribal hamlet situated in Anaikatti village, Coimbatore, Tamil Nadu, India. The plant was authenticated at Botanical Survey of India (Southern Wing), Coimbatore. The leaves were then separated from the plant for further processing.

Preparation of Sample

The fresh leaves of the plants were first washed with water to remove adhering dirt and then cut into small pieces, sun dried for 4 days. After complete drying, the entire portions were pulverized into a coarse powder with the help of a grinding machine and were stored in an airtight container and kept in a cool, dark and dry place for further use.[11]

Organoleptic Evaluation

Organoleptic characters like colour, odour, taste, surface characteristics, texture and some specific characteristics of the powdered plant material were evaluated as the first step towards establishment of identity and degree of purity of the drug.[12]

Physico-chemical Analysis

The physicochemical characters like total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content in the powdered plant samples was analyzed by following the methods suggested in the The Indian Pharmacopoeia (1996).[13]

Extraction

The powdered leaf samples were weighed and 20g of the sample was extracted using hot extraction method suggested by López-Bascón, M. A. (2020) with soxhlet apparatus.[14] Different solvents were used to extract the phytochemicals present in the sample viz, Hexane, Ethanol and Water in decreasing polarity.

Qualitative Phytochemical Analysis

Qualitative phytochemical screening was done to analyze the phytochemicals present in the leaf extracts. Hexane, ethanol and aqueous leaf extracts were screened by the following methods.[15]

Test for Alkaloids

To 1 ml of the plant extract, 2 ml of Mayer's reagent was added. Development of creamy precipitate was noted which indicated the presence of alkaloids in the extracts.

Test for Flavonoids

1 ml of extract was added with the few drops of 2% Sodium hydroxide solution which was resulted in the formation of intense yellow color. On addition of dilute acid, it became colorless which indicated the presence of flavonoids.

Test for Glycosides

3 drops of Molisch's reagent was added to 2 ml of extract and mixed well, to this few drops of conc. H_2SO_4 was added carefully. Formation of reddish-purple ring at the junction of 2 layers indicated the presence of glycosides.

Test for Phenols

2 ml of extract was treated with the few drops of Ferric chloride solution. Formation of a bluish-black colour indicated the presence of phenols.

Test for Tannins

1 ml of extract was added with 3 drops of lead sub-acetate solution, formation of creamy gelatinous precipitate indicated the presence of tannins.

Test for steroids

3 ml of extract was mixed with 2 ml of chloroform and conc. H_2SO_4 was added side wise. A red colour produced in the lower chloroform layer and yellow-green fluorescence in H_2SO_4 layer indicated the presence of steroids.

Test for Terpenoids

2 ml of extract was dissolved in 2 ml chloroform and evaporated to dryness. To this, 2 ml of conc. H_2SO_4 was added and heated for 2 min. Formation of greyish colour indicated the presence of terpenoids.

Quantitative Phytochemical Analysis

Estimation of Total Phenol Content

The total phenolic content was estimated using the modified Folin-Ciocalteu photometric method. The appropriate amount of filtered extracts were oxidized with Folin-Ciocalteu's reagent, after 5 min the reaction mixture was neutralized with saturated Sodium carbonate. The solution was then immediately diluted to the volume of 50 ml with distilled water. The absorbance was measured at 750 nm after 90 min of incubation at room temperature against the blank, gallic acid. The total phenolic content is expressed as g gallic acid equivalents (GAE) per 100 g of dry weight (dw).[16]

Estimation of Total Flavonoid Content

Total flavonoid content was determined using Al-owaisi *et al.* (2014). The sample was mixed with 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 mol/L potassium acetate and 2.8 ml of distilled water was mixed. The mixture was incubated for 30 mins at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at 415 nm with UV-Vis spectrophotometer against blank. The amount of total flavonoid was calculated from linear regression equation derived from the quercetin calibration curve. Total flavonoid content of the sample was expressed as (mg of QE/gm) of dried extract.[17]

Estimation of Total Tannin Content

Estimation of total tannin content in leaf and stem was determined by Folin-Denis method with minor modifications. To 0.1 ml of sample extract, 1 ml of distilled water was added and then mixed with 0.5 ml of Folin-Denis reagent. The reaction mixture was alkalinized by the addition of 1 ml of 15% Sodium carbonate solution and kept in dark for 30 min at room temperature. The absorbance of the solution was read at 700 nm using spectrophotometer, and the concentration of tannin in the extract was determined using pure tannic acid as standard (1 mg/ml). The results of tannins are expressed in terms of Tannic acid mg/g of extract.[18]

In vitro Antioxidant Analysis

The ethanol extract was selected for further analysis since the concentration of phytochemicals were high when compared with the others. The antioxidant profile of the ethanol leaf extract of *Bridelia montana* was determined by 2,2 Diphenyl -1-picryl hydrazyl (DPPH) assay to estimate free radicals and scavenging activity in *in vitro* condition. Different concentrations (20, 40, 60, 80 and 100 µl) of the leaf extract was prepared and 150 µl DPPH solution was added to each test tubes. The reaction mixture was well mixed and incubated at room temperature for 30 min. Ascorbic acid was used as standard, the control was prepared by adding 2 ml of DPPH solution and 1 ml of methanol and the absorbance was taken at 516 nm in UV spectrophotometer (Shimadzu, Japan) after 15 min using methanol as a blank. The percentage of radical scavenging activity was calculate using the formula i.e.

$$\% \text{ RSA} = \left[\frac{\text{AC} - \text{AS}}{\text{AC}}\right] \times 100$$

Where, AC is the absorbance of the control and AS is the absorbance of the samples.

In vitro Antimicrobial Analysis

The antimicrobial analysis was done using Agar well diffusion assay[19] with different concentrations of the ethanol extract (10, 20 and 30 µl),[20] different microbes were selected for the study. Gram positive bacteria *Staphylococcus aureus* (MT126466), *Bascillus cereus* (MF671985), gram negative bacteria *Escharichia coli* (MN700632), *Salmonella typhii* (KY787190) and fungi *Aspergillus niger* (MK720633), *Aspergillus flavus* (MH174075). The microorganisms were sourced from the Center for Bioscience and Nanoscience Research (CBNR). Amoxyclav (5µg) was used as positive control for bacteria, Fluconazole (5µg) was used as control for fungi. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone.

RESULTS

Organoleptic Evaluation

The organoleptic parameters like touch, color, taste and odor were analyzed. The leaf powder was coarse to

touch, the color was dark green, the taste was pungent or ashy and the odor was characteristic. The results of the organoleptic evaluation is tabulated in Table 1.

Physico-chemical Analysis

The physicochemical analysis of the powdered leaf samples of *B. montana* revealed that the total ash, acid insoluble ash, water soluble ash, sulphated ash and moisture content were 8.13±0.29, 4.1±0.26, 1.63±0.13, 16.66±0.38, 3±1.15% w/w respectively. The sulphated ash was found to be the highest among all the other parameters. The moisture content was relative less which is very important for a drug to be stored for a longer period of time (Table 2).

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis showed the presence of tannins, phenols and flavonoids in the hexane extract. The ethanol extract showed the presence of maximum phytochemicals viz. alkaloids, flavonoids, glycosides, phenols and tannins. Flavonoids, glycosides, tannins and phenols were present in aqueous extract. Terpenoids and steroids were absent in all the three extracts (Table 3).

Quantitative Phytochemical Analysis

The total phenolic content was high in ethanol leaf extract with 78.83±0.15 mg GAE/g of the extract followed by aqueous and hexane extracts with 77.25±0.07 and 45.87±0.11 mg GAE/g of the extract respectively. The total flavonoid content was present highly in ethanol extract with 62.68±1.18 mg QE/g extract, least amount was present in aqueous extract (30.39±0.99 mg QE/g extract). The total tannin content was high in ethanol leaf extract with 98.03±0.05 mg TAE/g extract and least in hexane extract with 45.14±0.09 mg TAE/g extract. (Table 4, Figure 1).

Table 1: Organoleptic characters of dry powder of leaf powder of *B. montana*.

Sl. No.	Parameter	Leaf
1	Touch	Coarse
2	Color	Dark green
3	Taste	Pungent/ashy
4	Odor	Characteristic

Table 2: Physicochemical analysis of dry powder of leaf powder of *B. montana*.

Sl. No.	Characters	Leaf
1	Total ash	8.13±0.29
2	Acid insoluble ash	4.1±0.26
3	Water soluble ash	1.63±0.13
4	Sulphated ash	16.66±0.38
5	Loss on drying/ moisture content	3±1.15

Values are means of three independent analyses of the extract ± standard error n=3.

Table 3: Qualitative phytochemical analysis of *Bridelia montana* leaf extracts.

Sl. No.	Phytochemicals	Hexane	Ethanol	Water
1	Alkaloids	-	+	-
2	Flavonoids	+	+	+
3	Glycosides	-	+	+
4	Terpenoids	-	-	-
5	Phenols	+	++	+
6	Tannins	+	++	++
7	Steroids	-	-	-

+ Present, ++ Highly present, - Absent.

Table 4: Quantitative phytochemical analysis of *Bridelia montana* leaf extracts.

Sl. No.	Phytochemicals	Hexane	Ethanol	Water
1	Phenols (mg GAE/g extract)	45.87±0.11	78.83±0.15	77.25±0.07
2	Flavonoids (mg QE/g extract)	34.37±1.31	62.68±1.18	30.39±0.99
3	Tannins (mg TAE/g extract)	45.14±0.09	98.03±0.05	93.31±0.20

Values expressed as Mean±SE of triplicate determination.

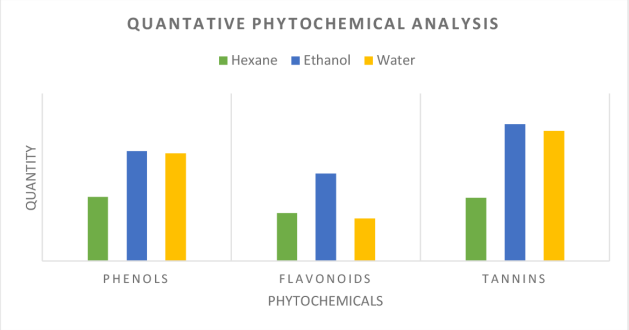

Figure 1: Quantitative phytochemical analysis of *Bridelia montana* leaf extracts.

In vitro Antioxidant Analysis

DPPH free Radical Scavenging Activity

The ethanol plant extract showed a concentration-dependent increase in the DPPH free radical scavenging activity. The highest concentration of the plant extract showed good antioxidant activity when compared with the standard Ascorbic acid. The DPPH scavenging potential of the extract and the std. Ascorbic acid were

Table 5: Antioxidant capacity of ethanol leaf extract of *Bridelia montana*.

Sl. No.	Concentration (µg/ml)	% RSA	
		Extract	Ascorbic acid
1	20	46.94±0.05	58.6±0.32
2	40	51.13±0.11	65.02±0.1
3	60	54.99±0.1	68.79±0.3
4	80	57.5±0.18	74.21±0.22
5	100	61.7±0.08	82.02±0.18
	IC_{50}	20.2±0.13	10.7±0.29

Values expressed as Mean±SE of triplicate determination.

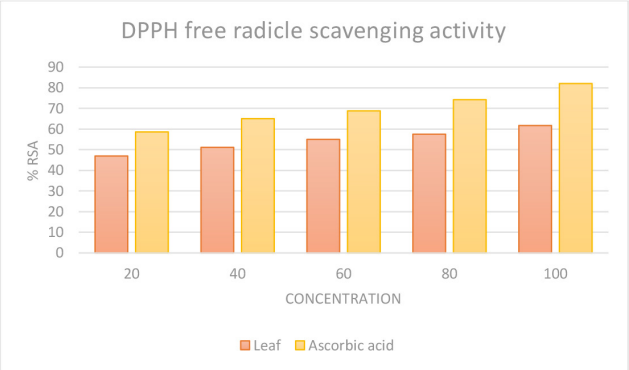

Figure 2: DPPH free radicle scavenging activity of ethanol leaf extract of *Bridelia montana*.

Table 6: *In vitro* antimicrobial activity of ethanol leaf extracts of *B. montana* against different bacterial and fungal species.

Sl. No.	Sample	Type of pathogen	Name of pathogen	Negative control	Positive control	Leaf extract (µl)		
						10	20	30
1	Ethanol leaf extract	Gram positive bacteria	*S. aureus*	NIL	19±1.73	6.67±0.67	15.3±1.33	24.33±1.2
			B. cereus	NIL	2.67±1.76	8±1.15	16.6±0.88	37.67±1.2
		Gram negative bacteria	*E. coli*	NIL	20.3±1.45	11.3±1.76	7±0.57	15±1.73
			S. typhii	NIL	19.67±0.8	4±0.57	11±0.57	18±1.15
		Fungi	*A. niger*	NIL	1.33±1.33	3.33±1.76	5.67±0.67	9±0.57
			A. flavus	NIL	7.33±0.66	6.66±0.67	6.33±0.88	8±1.15

Values expressed as Mean±SE of triplicate determination.

61.7±0.08 and 82.02±0.18% at 100µg/ml concentration respectively. The IC_{50} value of the std. Ascorbic acid was found to be 10.7±0.29% whereas the extract was 20.2±0.13% (Table 5, Figure 2).

In vitro Antimicrobial Activity

Agar well diffusion assay

The results of antimicrobial analysis was concentration dependent. The ethanol leaf extract showed very high antibacterial activity against gram positive bacteria *Bacillus cereus* with the zone of inhibition of 37.67±1.2mm and lowest activity was observed against gram negative bacteria *Escherichia coli* with 15±1.73mm. Highest antifungal activity was observed against *Aspergillus niger* with the zone of inhibition of 9±0.57mm and lowest activity against *Aspergillus flavus* with 3.33±1.76mm (Table 6, Figure 3).

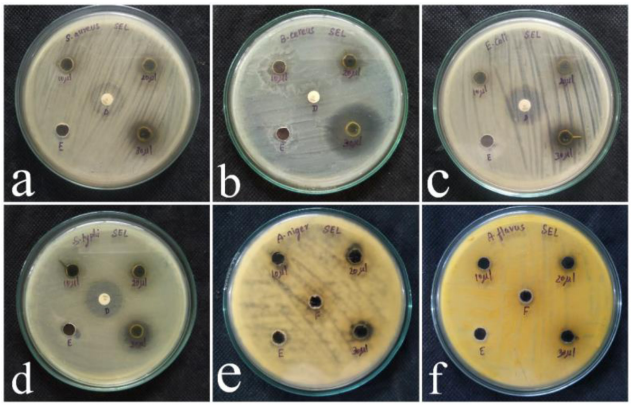

Figure 3: Antimicrobial analysis of ethanol leaf extracts of *Bridelia montana* against (a) *Staphylococus aureus* (b) *Bacillus cereus* (c) *Escherichia coli* (d) *Salmonella typhii* (e) *Aspergillus niger* and (f) *Aspergillus flavus*.

DISCUSSION

Medicinal plants are one of the important substances, the study of their traditional uses through the verification of pharmacological effects is the need of the hour and they can also be natural composite sources that can act as new anti-infectious agents.[21]

Analyzing the physicochemical characters of the sample is very important to prevent adulteration in the plant drugs. Ash values determines the quality and purity of crude drug and indicates presence of various impurities like carbonate, oxalate and silicate. The water-soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash indicates the presence of silica and indicate contamination with

earthy material. Moisture content of drugs should be at minimal level to decrease the growth of micro-organisms like bacteria, yeast or fungi during storage.[22] Similar study on physicochemical properties of *H. ferrugenia, H. grahamii,* and *H. nigra* revealed that the percentage of total ash was 6.09±0.290 for *H. ferrugenia*, 6.433±0.351 for *H. grahamii* and 6.75±0.317 for *H. nigra* respectively. Acid insoluble ash was 0.466±0.061, 0.513±0.015 and 1.47±0.226 the tested plants respectively. The moisture content was 6.73±0.489, 5.566±0.126 and 6.79±0.290 respectively.[23]

The leaf extracts of *Bridelia montana* were tested for the presence of alkaloids, tannins, steroids, terpenoids, phenols, flavonoids and glycosides, the results showed the presence of alkaloids, flavonoids, glycosides, tannins and phenols which is found to have anti-inflammatory, anti-oxidant, anti-microbial, anti-pyretic, anti-septic, hepatoprotective and anti-diabetic properties.[24] The preliminary phytochemical analysis by Vinatha *et al.* (2017) on ethanol leaf extract of *Bridelia montana* showed the presence of different phytocompounds like alkaloid, tannin, glycoside, resins and phenolic compounds.[25] The quantitative phytochemical analysis showed the significant amount of tannins, phenols and flavonoids. Flavonoids has anti-oxidative, anti-inflammatory, anti-mutagenic and anti-carcinogenic properties[26] which were also reported in *Vitex negundo,*[27] *Zingiber officinale,*[28] *Datura metel*[29] and *Emblica officinalis.*[30] The phenols are one of the important phytochemicals with various biological activities were also found to be present in *Ageratum conyzoides,*[31] *Acacia concina,*[32] *Berberis lyceum*[33] and *Rumex hastatus.*[34] Tannins on the other hand are anticarcinogenic, antimutagenic and antioxidative[35] and are also reported in *Sida acuta* and *Sida rhombifolia.*[36]

DPPH assay has been widely used all over the world to evaluate the free radical scavenging efficacy of various substances, plant compounds and other antioxidant substances. During the DPPH free radical scavenging assay, the antioxidants reduces the stable radical DPPH to the yellow-coloured diphenyl-picrylhydrazine. DPPH reduces in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction that takes place during this process.[37] The ethanol leaf extract showed the highest antioxidant activity in 100 µg/ml (61.7%), the % RSA increased with increase in concentration of plant extract. Pearling samples of *Avena sativa* had the highest antioxidant activity (73.4-77.6%), followed by flour (52.6-60.8%), aspirations (56.5%), and trichomes (47.4%).[38] The ethanol leaf extract of *B. montana* at the concentration of 30 µl showed good zone of inhibition against most of the tested bacteria and fungi like *B. cereus, S. typhii, A. niger,* etc. Similar studies on the aqueous extracts of sage and thyme showed good results against most of the tested micro-organisms. Phenolic extract of sage and thyme showed antibacterial activity against *S. aureus* and *Enterococcus sp.* Ethanol extract of parsley on the other hand, showed good activity against *E. coli.* While, it didn't show effect on the tested Gram-positive bacteria. On the other hand, commercial oils of sage, thyme and parsley displayed no antimicrobial activity against *E. coli, Proteus mirabilis* and *Salmonella typhi.*[39]

CONCLUSION

The leaves of *Bridelia montana* is being used in ethnomedicine for many years, to treat various disease like diarrhea and dysentery. The physicochemical analysis showed that the sulphated ash was found to be the highest among all the other parameters and the moisture content was relatively less. The phytochemical analysis showed the presence of alkaloids, flavonoids, glycosides, tannins and phenols. The *in vitro* antioxidant analysis showed that the ethanol leaf extract showed good antioxidant property when compared with the standard Ascorbic acid. *In vitro* antimicrobial analysis showed good zone of inhibition against the tested microbes when compared with the standards. From the above analysis it has been proved that the less explored ethnomedicinal plant *Bridelia montana* has some valuable medicinal properties.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

H_2SO_4: Sulphuric acid; **mm:** Millimetres; **nm:** Nanometre; **mg:** Milligram; **g:** Gram; µg**:** Micrograms; **ml:** Millilitres; µl**:** Microliters; **GAE:** Gallic Acid Equivalents; **QE:** Quercetin Equivalents; **TAE:** Tannic Acid Equivalents; **RSA:** Radical Scavenging Activity.

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