Antioxidant activities of leaf extracts of *Euphorbia fusiformis* Buch. -Ham. ex D. Don (Euphorbiaceae)

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ABSTRACT

Introduction: Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to causes damage to the cellular biomolecules, resulting degenerative diseases. Antioxidants are prevent the oxidative damage caused by free radicals. Medicinal plants are having significant free radical scavenging potential due to the presence of various phyto-constituents. The present study was selected *Euphorbia fusiformis* Buch. -Ham. ex D. Don is a rare rhizomatous and medicinal herb belongs to Euphorbiaceae family. The purpose of current study to assess the antioxidant potential of various solvents (methanol, acetone, ethyl acetate, chloroform and hexane) leaf extracts of *E. fusiformis*. **Methods:** The antioxidant assays like, DPPH radical, super oxide radical, nitric oxide radical, hydrogen peroxide radical scavenging activity and ferrous ion chelating potential were carried out in this study. **Results:** The results revealed that all extracts of *E. fusiformis* expressed varying degree of antioxidant property on different tested methods. Remarkable antioxidant activity was observed in acetone extract on the DPPH radical scavenging activity with the lowest IC_{50} value of 15 µg/ml followed by hydrogen peroxide scavenging activity (IC_{50} values of 42 µg/ml). The highest percentage of inhibition of nitric oxide radical, superoxide radical and hydrogen peroxide radical scavenging activity was observed in methanol extract with low IC_{50} values. Acetone extract of E. fusiformis showed good chelating potential on ferrous ions in ferrous ion chelation assay, with least IC_{50} value. **Conclusion:** The results of present investigation suggest that *E. fusiformis* leaf extracts may be used as a natural antioxidant agent.

Key words: DPPH, $O_2^{\bullet-}$, *Euphorbia fusiformis*, H_2O_2 assays, Leaf extracts, NO.

INTRODUCTION

The continuous use of synthetic antioxidants (butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)) was reported to have some health risks (suspect to carcinogens) and induce toxicity level in the body system.[1,2] Hence, an alternate strategy has been urgently required to overcome these troubles, plant based antioxidants are preferred by healers. Extracts from plant materials like herbs, vegetables, fruits, cereals and plant materials which were rich in phenolics, are reported to have high antioxidant property and routinely used in food and pharmaceutical industry, because they hold back oxidative degradation of lipids.[3] Droge[4] clearly stated that free radicals can develop several age related diseases by rising oxidative damages and it could be eliminated in body health problems against chronic diseases and its progression by natural antioxidants.[5,6]

The present study has chosen *Euphorbia fusiformis* Buch. -Ham. ex D. Don (Euphorbiaceae), is a rare herbaceous plant found in the Eastern Ghats of India, especially in dry deciduous forests.[7] The ethnobotanical knowledge of the plant shows the dried root powder and fresh rhizome have

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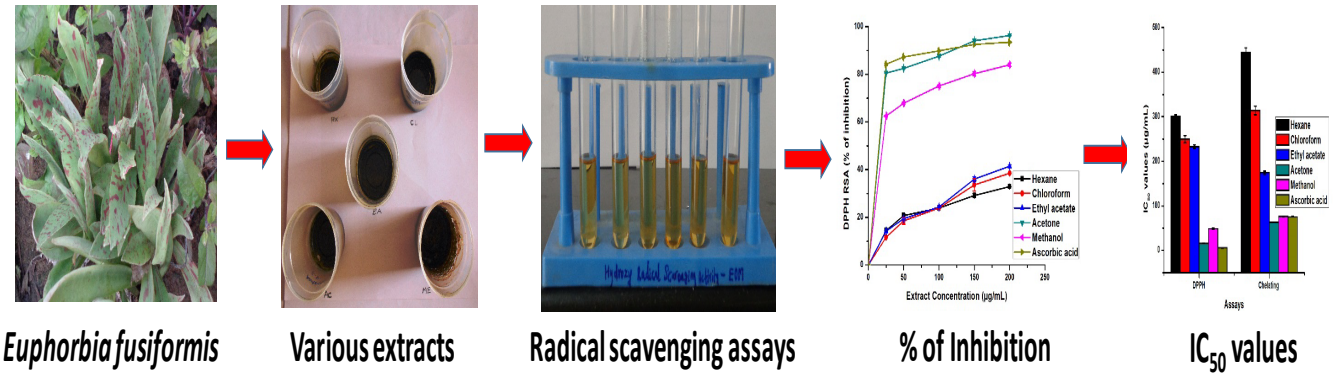

Graphical Abstract

been found to increase secretion of mother's milk. The dried rhizome extract is also administrated orally for relief from joint pain and rheumatism and it is more effective against diarrhea. The fresh milky latex is externally applied to heal chronic wounds and cracks and to cure skin diseases. Crushed leaf poultice is applied on forehead to getting relief from acute headache.[8] The earlier pharmacological studies of plant have been reported as effective anti-inflammatory, antimicrobial, hepatoprotective properties.[9-11] Recent findings reported that the antioxidant activity of isolated compounds from *E. fusiformis* rhizomes.[12] Keeping this view in mind, the present study was carried out the antioxidant potentials of different solvent extracts from the leaves of *E. fusiformis*, a rare medicinal herb.

MATERIALS AND METHODS

Collection of Plant Materials

The fresh and healthy leaves of *E. fusiformis* was collected from Chitteri hills, Eastern Ghats of Tamil Nadu. The identification of botanical nomenclature of the plant was done by Dr. D. Natarajan, Asst. Professor, Department of Biotechnology, Periyar University, Salem. The herbarium specimen is maintained in the research laboratory for further reference. The collected plant materials were shade-dried for 15 days under laboratory condition.

Solvent extraction

The air-dried, powdered leaf was extracted in cold percolation method successively with hexane, chloroform, ethyl acetate, acetone and methanol. Each time before extracting with the next solvent, the plant material was dried in hot air oven at 40°C. Finally, the material was filtered using Whatman No. 1 filter paper allowed to dry a fine paste developed was weighed and stored at 4°C for future use.

Extract preparation

About 1 mg of plant extract was weighed and suspended in 1ml of respective solvents (1 mg/ml concentration). They were used for analyzing all antioxidant assays.

Antioxidant assays

Determination of DPPH radical scavenging activity (DPPH RAS)

The DPPH radical scavenging assay was carried out according to the method Chew *et al.* with some modifications.[13] 1 ml of extract with various dilutions (25-200 µg/ml) was added to 2 ml of 1 mM DPPH in methanol and incubated for 30 minutes in the dark. The color intensity was measured at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. The DPPH scavenging ability was calculated by the following formula

$$\text{DPPH RSA (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

Ferrous ion chelating activity

The ferrous ion chelating activity was measured by the method Glucin *et al.*[14] 100 µl of 2 mM $FeSO_4$ and 300 µl of 5 mM ferrozine were mixed with different concentration (25-200 µg/ml) dilutions of extracts. The reaction mixture was incubated for 10 min and the intensity of color measured at 562 nm. The ferrous ion chelating ability of the extracts was calculated by the following formula

$$\text{Nitric oxide RSA (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \text{X 100}$$

Nitric Oxide Radical Scavenging Activity

The nitric oxide scavenging activity of different solvent plant extracts on free nitric oxide radical was estimated by the method Srinivasan *et al*.[15] Different aliquots (25–200 mg) of extracts were used in the study. 10 mM sodium nitroprusside prepared in phosphate buffered saline, was mixed with test extracts and kept for 2 hours and 30 min at room temperature. 0.5 ml of griess reagent (containing 1% sulphanilamide, 2% boric acid and 0.1% N-(1-naphthyl) ethylene diamine dihydrochloride) was added to the test samples followed by the incubation. The chromophore developed at the reaction was absorbed at 546 nm in a UV–Visible spectrophotometer. BHT with the same reaction mixture without plant extracts were employed as a positive control respectively. Radical scavenging activity was expressed as an inhibition percentage of free radical by the sample and was calculated using the formula.

(Nitric oxide RSA (%)=) with (FICA (%)=)

Superoxide Radical ($O_2^{\bullet-}$) Scavenging Activity

Plant materials having tendency of inhibiting the formazon formed during the superoxide radical scavenging generated in riboflavin–light–NBT system.[16] 3 ml reaction mixture containing sodium phosphate buffer (50 mM) (pH 6), riboflavin (20 mg) and EDTA (12 mM) and NBT (0.1 mg). The reaction was initiated by illuminating the reaction mixture with different concentrations of sample extracts for 90 seconds. Immediately, the absorbance was measured at 590 nm in a spectrophotometer. The percentage inhibition of superoxide anion generation was calculated using the formula.

$$\text{Superoxide RSA (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

Hydrogen Peroxide Scavenging Activity

The ability of extracts to scavenge hydrogen peroxide was calculated by the method described by Ruch *et al*.[17] A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). Extracts (20-200 mg/ml) were added to a hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide was read spectrophotometrically at 230 nm after 10 min against the blank solution (phosphate buffer without hydrogen peroxide). For each concentration, a separate blank sample was used for background subtraction. The percentage inhibition activity was calculated using the formula.

$$\text{Hydrogen peroxide RSA (\%)} = \frac{\text{Absorbance of control - Absorbance of sample}}{\text{Absorbance of control}} \text{X100}$$

Statistical analysis

All assays were done in triplicates. Data were presented as mean ± standard deviation (SD) of three determinations. The inhibitory concentration (IC_{50}) was calculated by non-linear regression analysis.

RESULTS

DPPH radical scavenging activity

The DPPH radical scavenging potential of different solvent

Table 1: DPPH radical scavenging activity of different solvent extracts of *E. fusiformis*

Extract Concentration (µg/ml)	DPPH radical scavenging activity					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Ascorbic acid
25	14.43 ± 0.04	11.63 ± 0.09	14.21± 0.36	80.53 ± 0.04	62.49 ± 0.27	84.19 ± 0.40
50	20.76 ± 0.49	18.53 ± 1.38	19.42 ± 0.67	82.49 ± 0.13	67.88 ± 0.40	87.26 ± 0.18
100	23.92 ± 0.09	23.83 ± 0.09	24.32 ± 0.04	87.62 ± 0.36	75.06 ± 0.53	89.80 ± 0.22
150	29.18 ± 0.80	33.63 ± 2.67	36.04 ± 0.98	94.08 ± 0.31	80.27 ± 0.85	92.52 ± 0.00
200	32.83 ± 0.09	38.49 ± 0.13	41.43 ± 0.58	96.30 ± 0.13	84.01 ± 0.67	93.45 ± 0.22
IC_{50}	301.55 ± 2.82	249.74 ± 7.86	232.96 ± 4.39	15.43 ± 0.33	48.99 ± 1.06	05.51 ± 0.28

Table 2: Ferrous Ion chelating activity of different solvent extracts of *E. fusiformis*

Extract Concentration (µg/ml)	Ferrous Ion chelating activity					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	Ascorbic acid
25	02.16 ± 0.17	05.66 ± 0.45	12.69 ± 1.10	52.31 ± 0.13	45.48 ± 2.33	45.52 ± 2.12
50	07.98 ± 1.02	12.81 ± 0.89	17.33 ± 0.70	64.30 ± 0.45	59.44 ± 1.32	55.54 ± 0.21
100	12.81 ± 1.57	17.06 ± 1.36	35.89 ± 3.48	70.60 ± 0.17	65.68 ± 0.59	64.11 ± 0.51
150	17.56 ± 0.38	24.78 ± 1.27	47.16 ± 2.06	76.75 ± 1.48	73.80 ± 0.23	75.80 ± 1.17
200	21.98 ± 0.55	32.01 ± 0.23	51.15 ± 0.95	81.97 ± 0.55	76.77 ± 1.04	83.16 ± 0.21
IC_{50}	444.22 ± 10.58	314.58 ± 9.50	174.68 ± 3.45	62.86 ± 0.40	75.86 ± 0.68	75.52 ± 0.51

Table 3: Nitric Oxide Radical Scavenging Activity of different solvent extracts of *E. fusiformis*

Extract Concentration (µg/ml)	Nitric Oxide Radical Scavenging Activity					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	BHT
25	67.16 ± 1.0	69.12 ± 1.0	68.25 ± 0.6	62.82 ± 1.4	73.48 ± 7.7	80.25 ± 4.1
50	67.56 ± 1.7	69.14 ± 1.0	68.51 ± 0.5	68.04 ± 1.5	76.65 ± 8.9	86.57 ± 4.4
100	68.0 ± 2.0	69.15 ± 1.0	68.84 ± 0.5	68.06 ± 1.6	86.48 ± 8.7	88.50 ± 4.3
150	68.35 ± 2.0	69.26 ± 1.0	70.05 ± 0.7	69.04 ± 1.3	96.08 ± 9.8	91.49 ± 4.7
200	68.67 ± 1.8	69.36 ± 0.9	70.46 ± 0.8	69.68 ± 2.7	97.40 ± 10.9	92.49 ± 4.9
IC_{50}	568.2	663.3	726.74	524.36	70.30	152.84

Table 4: Superoxide Radical ($O_2^{\bullet-}$) Scavenging Activity of different solvent extracts of *E. fusiformis*

Extract Concentration (µg/ml)	Superoxide Radical ($O_2^{\bullet-}$) Scavenging Activity					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	BHT
25	96.18 ± 1.2	97.19 ± 0.9	96.32 ± 0.9	93.71 ± 1.7	90.70 ± 4.8	78.60 ± 1.4
50	97.19 ± 1.3	97.92 ± 0.9	97.66 ± 0.8	94.38 ± 1.1	91.64 ± 2.5	80.61 ± 1.4
100	98.12 ± 1.2	98.39 ± 0.9	98.46 ± 0.9	95.38 ± 0.9	93.31 ± 1.7	86.63 ± 4.1
150	98.52 ± 1.2	98.99 ± 0.8	99.06 ± 1.3	96.12 ± 1.1	94.65 ± 1.5	89.30 ± 5.0
200	99.33 ± 1.2	99.33 ± 0.8	99.46 ± 1.2	96.79 ± 1.2	96.32 ± 2.2	93.31 ± 6.0
IC_{50}	1035.68	18424.12	1255.29	1099.36	808.44	278.87

Table 5: Hydrogen peroxide assay of different solvent extracts of *E. fusiformis*

Extract Concentration (µg/ml)	Hydrogen peroxide assay					
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol	BHT
25	15.49 ± 3.6	28.33 ± 1.0	27.14 ± 1.9	27.21 ± 1.8	34.38 ± 1.2	93.74 ± 1.0
50	20.33 ± 4.3	29.61 ± 0.8	31.16 ± 1.8	37.47 ± 1.6	38.30 ± 1.2	95.19 ± 1.0
100	27.64 ± 4.2	30.10 ± 0.8	57.74 ± 1.8	49.85 ± 1.6	49.52 ± 1.2	96.77 ± 1.5
150	41.13 ± 3.4	63.54 ± 1.5	63.21 ± 1.8	59.59 ± 1.5	73.93 ± 1.6	98.35 ± 1.9
200	46.62 ± 1.3	64.13 ± 1.8	65.41 ± 1.8	62.84 ± 1.4	95.39 ± 2.5	99.27 ± 2.2
IC_{50}	46.85	36.90	36.11	42.17	24.70	27.46

extracts of plant materials are given in Table 1. A stable free radical (DPPH) was routinely used to analyze the antioxidant potential of plant materials. Importantly, IC_{50} of different extracts was also calculated to determine the amount of extract needed to quench 50% of free radicals. Least concentration which showed maximum antioxidant potential activity was considered as IC_{50} values of the tested plant materials. Out of which the acetone extracts showed least IC_{50} value of 15.43 µg/ml, followed by the methanol extract, showed comparable antioxidant potential than the other tested solvents. The IC_{50} values of standard ascorbic acid were noticed as 05.51 µg/ml. However, the values were compared with the results of our present study and noticed potential radical scavenging capacity in acetone and methanol extracts.

Ferrous ion chelation property

The ability of ferrous ion chelating by the different extract was increased in a dose dependent manner (Table 2). Different solvent extracts of test plant showed significant ferrous ion chelating ability, while the potential effect (about 81.97 %) was observed in the acetone extract (at a higher concentration of 200 mg/ml). Whereas, the methanol and other solvent extract showed relatively comparable chelation potential. At the same time ascorbic acid carried higher chelating ability (83.16%) was comparable with that of acetone extract of the test plant.

Nitric Oxide Radical Scavenging Activity

The scavenging percentage of nitric oxide was noticed increasing in increasing concentration of the plant extracts. It was recorded higher in the methanolic extract (with an optimum IC_{50} value 70.30 µg/ml) and lower in ethyl acetate extract (726.24 µg/ml). Simultaneously, the acetone and hexane extracts exhibited significant radical scavenging activity in increasing concentrations. As a natural antioxidant provider, this plant can be clearly compared with the standard BHT. The positive control BHT show least IC_{50} value i.e., 152.84 µg/ml (Table 3).

Superoxide Radical ($O_2^{\bullet-}$) Scavenging Activity

The scavenging ability of plant materials exhibited that the inhibition of formazan was recorded and presented in Table 4. The highest percentage of inhibition was noted in the methanol extract (showed potential RSA in

least IC_{50} value 808.44 μg/ml). The other solvent radical scavenging was noticed very promising in hexane and acetone (IC_{50} 1035.68 and 1099.36 μg/ml) respectively. The total scavenging activity of the extracts was compared with standard BHT, which showed a promising inhibition at 278.87 μg/ml. Increasing concentration of the extract developed best radical scavenging activity and the IC_{50} values are given in Table 5.

Hydrogen peroxide assay

The solvent extracts of plant materials showed an efficient scavenging activity in the significant concentration dependent manner and are presented in Table 5. The increased concentration quenches best radical tendency. Among the tested solvent extracts methanolic extract showed promising inhibition at the concentration of 24.70 μg/ml, which was comparatively higher than the standard BHT (27.46 μg/ml) and the least efficiency was estimated in hexane extract (46.85 μg/ml). Ethyl acetate (36.11 μg/ml) and chloroform (36.90 μg/ml) extracts also revealed a prominent scavenging activity. The results can be compared with the standard BHT and seems to be an effective scavenger. IC_{50} values of the different concentration pertaining to the significant antioxidant potential were also recorded and tabulated.

DISCUSSION

Oxygen is essential in living systems, the formation of free radicals in metabolic functions provokes uncontrolled conditions and cause several degenerative diseases like cancer, diabetes mellitus, liver cirrhosis, nephrotoxicity etc.[18] ROS species like super oxide anions, hydroxyl radicals and nitric oxide inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation. Antioxidants may offer resistance against the oxidative stress by scavenging the free radicals and inhibiting the lipid peroxidation.[19] Antioxidants of plant materials were actively against the free radicals and convert them to less reactive or non-harmful metabolic product.[20]

DPPH is a stable free radical which inhibition was calculated to show the potential antioxidant compound from plant materials. It gives more accurate and repeatable results for determination of antioxidant potential.[21] The radical scavenging potential of test plant extract possessed significant activity in acetone followed by methanol extract, which were capable of reducing DPPH radical into hydrogen ion, thus the intensity of the color change in the reaction depends upon the number of electrons transferred during the reaction.[22] Contribution of electron sharing was the tendency, rendered from the natural/ plant phenolic compounds.[23] Hence, it could be assumed that the higher antioxidant potential of methanol and acetone extracts was due to the electron sharing by the phenolic compounds present in them.

Besides the radical sharing tendency, the free radical reducing capacity of a compound may serve as a significant indicator of its potent antioxidant activity.[24] The antioxidant capacity of the plant based compounds have been attributed to various mechanisms like binding of transition metal ions, decomposition of peroxide/superoxide's, prevention of chain initiation, and prevention of continued proton abstraction.[25] Reducing the power of the metabolic and acetone extracts of the test plant increased with increasing concentration of the extract, indicated that phenolic or related compounds in them donated electrons and could also react with free radicals to convert them into more stable products and to terminate radical chain reactions.

The total antioxidant potential tested by phosphomolybdenum assay successfully registered the Vitamin E present in the test plant extract. It's directly related to the antioxidant properties of the natural compounds. It measures the total potential independently. So the results were expressed in standard ascorbic acid equivalents. So that the results of the test plant can be correlated with its free radical scavenging activity. The results of the present study showed that the methanol and acetone extracts rendered higher amount of total antioxidant capacity.Wong and Yen[26] reported the methanolic extracts of mung bean sprouts and radish sprouts only exhibited a chelating ability of 60% and 40%, respectively, at a concentration of 3 mg/ml.

Superoxide radicals are well known for their harmfulness to cellular components as they were a precursor of more ROS.[27] The percentage inhibition obtained in this study clearly demonstrate the efficiency of the extracts in scavenging the ROS. Methanol extract followed by the acetone has superior radical scavenging potential. In the mitochondrial electron transport chain the superoxide anion act as a free radical which involved in the reduction of oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form a superoxide anion. It plays an important role in the formation of hydroxyl radical or singlet oxygen in living systems.[28] The results of our present study were comparable with the results of other reports on superoxide radical scavenging activity on *Monochoria vaginalis*[29] *Pothos scandens*[21] *Clitoria ternatea*,[30] who reported similar findings.

Nitric oxide or reactive oxygen species formed during their reaction or with superoxide such as NO_2, N_2O_4, and NO_2^- are very reactive. Recently considerable interest has arisen with the plant products to overcome the nitric oxide radical with their quenching ability. These compounds are responsible for altering structural and functional behavior of cellular components. Nitric oxide has also implicated in inflammation, cancer and other pathological conditions.[31] In our studies it was found that the methanol extract had a higher scavenging activity (86.16%) followed by the ethyl acetate and acetone extracts (69.42 & 67.59%). Similar kind of observations was recorded.[32] (*Brassica junea*) and they found remarkable nitric scavenging activity. The present results were correlated with the earlier findings[29] (*M. vagnalis*)[21] (*P. scandens*); Siddhuraju and Manian[33] (*Macrotyloma uniflorum*) has shown noticeable activity against NO_3^- radicals.

Hydroxyl radicals can be converted into more reactive species in H_2O_2 and they cause more unfavorable effects to humans. Hence, the results of the present study reporting methanol extract followed by ethyl acetate and acetone extracts having significant radical scavenging potential. They can act as a good scavenger of such harmful radicals. This study was further compared to other related medicinal plants i.e. *Allium sativum*,[34] *Ananas cosmosus*,[35] and *P. scandens*[21] who reported potential antioxidant activity. Hence forth, the test plant materials possesses higher amount of antioxidant potential in methanol and acetone extracts.

CONCLUSION

The present study concluded that *E. fusiformis* leaf materials possess strong antioxidant activity. Further detailed exploration of phytochemical constituents and related chemical studies would enable this medicinal plant may be useful in pharmaceutical and neutraceutical industries for development of antioxidant drugs/products in future.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

Highlights of the paper

- *Euphorbia fusiformis* leaf materials possess strong antioxidant activity.
- *E. fusiformis* expressed varying degree of antioxidant property on different tested radicals.
- Remarkable antioxidant activity was observed in acetone extract on the DPPH radical scavenging activity with lowest IC_{50} value of value of 15 μg/ml followed by hydrogen peroxide scavenging activity (IC_{50} values of 48 μg/ml).

About Authors

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