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Evaluation of Antibacterial and Anti-Malaria Potential of Wonderful Kola (Buchiolizia coreica) Seed Extract in Male Wister Rats

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Abstract

One of the areas for the search for new antimalarial drugs is from the claimed antimalarial plants from the African flora. Only few of these claims have been authenticated by scientific investigation. Plants form the major part of treatments used by traditional healers in many societies, *Bucholzia Coricea* (B. Coriacea) a perennial plant belonging to the family capparidaceae and genus Bucholzia is popularly known as wonderful kola. Earlier studies carried out on different parts of this plant shows that it has great medicinal potentials thus, the evaluation of antibacterial and anti-malaria potential of wonderful kola *(Bucholzia Coricea)* seed extract in male Wister rats. Material and Methods: Fresh seeds of *B. coricea* were plucked and washed with distilled water, concentrated using rotary evaporator. Standard antibacterial agent (30 µg) tetracycline, antimicrobial susceptibility test discs and Nutrient agar was used. 100g of crude extract taken into a conical flask, the crude extracts were tested by disc diffusion method on nutrient agar medium. selected bacteria were used to evaluate the antibacterial activities of the crude extracts of *Bucholizia Coricea* seed, Escherichia coli (ATCC©25922), *Salmonella typhi*, (ATCC©14028), *Staphylococcus aureus* (ATCC©25923) and *Kliebselia pneumonia*, (ATCC©19155). A

total of seventy-eight Swiss albino mice (18-25 g) of both sexes were used in the study. Eighteen for acute toxicity study while thirty were used for each of the in vivo antiplasmodial test. Results: antibacterial; higher inhibition was observed at 400µg/mL on Klebsiella pneumonia at 15.14 + 0.07mm when compared to standard 21.19+ 0.03mm. while the curative effect of the extract in *P. berghei* infected rats at 40mg/kg show significant parasitaemia density to be 30.98+4.30 and pre(D3) was 2.90+0.11mm** with survival time at post (D7) to be 29.67+0.21mm when compared to CQ as standard drug control with 10mg/kg. Conclusion: This study has however stablished the rationale for the traditional use of this plant in Nigeria and like many others, proved that medicinal plants which have folkloric reputations for anti-bacterial and antimalarial properties, thus, as an agent.

Keywords: Evaluation, Antibacterial, Anti-Malaria, Wonderful Kola, *Buchiolizia Coreica*, Seed, Wister Rats.

INTRODUCTION

There are 300 million acute cases of malaria and its bacterial infections each year globally, resulting in more than a million deaths. Majority of these disease cases and deaths occur in sub-Saharan Africa where the disease is endemic (Greenwood and Mautabingwa, 2002, De Ridder et al., 2008). Malaria is Africa's leading causes of under-five mortality and constitutes 10% of the continent's overall disease burden. The alarming rate at which the parasite particularly *plasmodium falciparum,* has developed resistance to currently used antimalarial drugs makes it imperative to search for newer, more effective therapeutic agents.

One of the areas for the search for new antimalarial drugs is from the claimed antimalarial plants from the African flora (Whitefield, 1995). Only few of these claims have been authenticated by scientific investigation (). Plants form the major part of treatments used by traditional healers in many societies, thus, many plants have acquired reputation for being useful against malaria (5).

Bucholzia Coriacea (B. Coriacea) a perennial plant belonging to the family capparidaceae and genus Bucholzia is popularly known as wonderful kola (Kigigha et al., 2018).

Earlier studies carried out on different parts of this plant shows that it has great medicinal potentials (Adisa et al., 2011; Ibrahim and Fagbohum, 2013).

Buchholzia coriacea is widely known as wonderful kola, a perennial plant which grows as a tree belonging to the family of capparaceae. The seeds are sheltered in a purple aril which is chewed in some Sub-Saharan African countries and known to have a strident spicy taste. It is a medicinal plant folklorically used in the treatment of feverish conditions in human (Ajayi et al., 2021). The presence of alkaloids L-starchydine and L3-hydrostarchydine as the main constituents but not present in the leaves, Lupeol and Bsitosterol were the most active fractions of the methanolic extract of the stem bark. Research output on different parts of the plant has ascribed several medicinal importance such as; anti-helminthic, anti-plasmodic, anti-microbial, hypoglycemia, anti-diarrhea, antispasmodic and analgesic effects to the plant (Nwaehuijor 2015). Buchholzia coriacea seeds in diet hastened gastric ulcer curative rate in acetic acid induced gastric ulcer in rats. The *Buchholzia coriecea* treatment and ischemia-reperfusion gastric ulcer reported better healing rate ascribed to its ability to suppress gastric acid secretion. More notably for this study is the anti-hyperglycemic and the anti-diabetic properties reported for this plant with dearth of knowledge on the role of the intestine in glucose uptake when treated with Buchholzia coriecea (Ezeigbo, 2011)

Analgesic, Anti-Anxiety and Anti-Inflammatory Activity

Analgesics are drugs or agents temporarily used minimize and/or block the sensation of pain. Typically, pain is an unpleasant sensation leading to abnormality in or part of the body. Pain can either physical or psychic/mental depending on its source. Like inflammation, analgesics are used to treat pain caused by physical stimuli, while mental pain is treated with antipsychotic agents (including anti-depressant, anti-anxiety and anti- manic drugs). Plants have demonstrated positive potential for the used as analgesics. Some of the plants such as wonderful kola has demonstrated potential for pain reliving. The ethanol seed extracts of wonderful kola have analgesics and anti-inflammatory activity based on their study in which male rats was used as experimental animal (Nwaehujor et al., 2015).

The plant has analgesic activity, anti-inflammatory potential, leaves extract of wonderful kola plant have anti-inflammatory activity, seed extracts of wonderful kola have anti-inflammatory and analgesic activities. As well as positive potency on carrageenan-induced inflammation in rats. The methanolic seed extract of wonderful kola has anxiolytic (anti-anxiety) and analgesic potentials. The authors further reported that it has stabilizing effect on the motor activity probably due to secondary metabolites. The anti-inflammation may

be associated to the presence of n-Hexadecanoic acid, 9,12-Octadecadienoic acid and 9,12 Octadecadienoyl chloride (Z, Z) (Pundir and Jain, 2010, Ibrahim and Fagbohun 2013)

However, there is currently limited work on the efficacy of Bucholizia Coricea seed extract on pathogen and malaria parasite.

MATERIALS AND METHODS

Collection of Seed Materials

The Seed

Fresh seed of wonderful kola (buchholzia coriacea) were purchased from Wukari market, Wukari Taraba state, Nigeria and were authenticated in the department of biochemistry at federal university Wukari, Taraba, Nigeria.

Preparation of the Seed Materials

Fresh seeds of B. coriacea were plucked and washed with distilled water. The sliced seeds were spread on a clean mat in a well-ventilated room with regular turning to enhance even drying and avoid decaying. The sliced seeds were shade-dried for 10 weeks. The shade dried sliced seeds were pulverized with an electric blender and a known weight (800 g) of the pulverized B. coriacea seeds were macerated in 70% ethanol (700 ml) and allowed to stand for 24 h. The mixture was separated with Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator and water bath. The extract was then weighed and stored in a refrigerator.

Equipment and Materials

Weighing balance, syringes (1ml, 2ml, 5ml, 10ml) Buchholzia coriacea seed extract, distilled water, animal feed (Top feed), cages, formaldehyde, drinking bottles, alloxan, measuring cylinder, cotton wool, methylated spirit, hand gloves, reagent bottles, methanol.

Extract Preparation

100g of crude extract taken into a conical flask, and 100ml of distilled water was added to the sample and mixed to form a solution.

Chemicals

All chemicals used in this investigation were of analytical grade and were obtained from SIGMA. Standard antibacterial agent (30 µg) tetracycline, antimicrobial susceptibility test

discs and Nutrient agar (CM0003) were obtained from Oxoid Ltd, Wade Road, Basingstoke, Hants, RG2 8PW, UK.

Preparation of Test Samples

The crude extracts of Bucholizia Coricea seed extract was used in antibacterial assay, the methanol crude extracts. The crude extracts were tested by disc diffusion method on nutrient agar medium as described by Pundir and Jain (2010). Exactly 3 mg of the crude sample was dissolved homogeneity in 3 mL of methanol giving a stock solution of 1000 µg/mL. Different volumes from the stock solution were taken, amounted to 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL each, and dissolved in 5 mL of methanol to make final concentration respectively.

Preparation of Agar Plates

Preparation of agar plates was performed based on method described by Pundir and Jain (2010). Nutrient agar was prepared according to manufacturer's instruction with 14 g of dried agar dissolved in 500 mL distilled water. The agar solution was heated until boiling followed by sterilization in autoclave at 121°C. The agar solution was then poured into a sterile petri plate and allowed to cool down and forming a gel. The plate was divided into eight sections by making a line marking on the outside surface of the plate. The eight sections were for each test samples namely the 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL samples, tetracycline 30µg (positive control) and methanol (negative control). The plate was sealed using parafilm and keep chilled at 4°C upon bacteria inoculation.

Anti-Bacterial Test

Preparation of Bacteria Broth

Several selected bacteria were used to evaluate the antibacterial activities of the crude extracts of Bucholizia Coricea seed, Escherichia coli (ATCC©25922), Salmonella typhi , (ATCC©14028), Staphylococcus aureus (ATCC©25923) and Kliebselia pneumonia , (ATCC©19155) were obtained from the stock culture provided by Microbiology Laboratory, Moddibo Adama University Yola, the nutrient broth was prepared according to manufacturer's instruction, with 2.6 g of the dried broth dissolved in 200 mL distilled water followed by sterilization in autoclave at 121°C. The bacterial was sub-cultured in a 10 mL of broth, each in universal glass bottle for 16 hours inside an incubator equipped with

shaker at 37°C (Thomas, 1998). After 16 hours incubation, turbidity (optical density/OD) of the bacterial broth was measured by using UV mini spectrophotometer (model 1240 of Shimadzu brand), comparable to that of nutrient broth standard tube for further use (Pedroni et al.,2006). Measurement was performed at wavelength 575 nm and the bacterial broth was ready to be used when its turbidity was between OD 0.6 to 0.9. Nutrient broth was used to adjust the turbidity until the desired value was obtained.

Plate Inoculation

Inoculation of the bacteria was carried out in a biohazard cabinet and the procedure was based on method described by Pundir and Jain (Betty & Sternne, 1998). Approximately 1 mL of the ready bacterial broth were transferred into mini centrifuge tubes. A sterile cotton swap was dipped into the mini centrifuge tube containing bacteria broth and streaked over entire of the agar plate surface, performed in 4 different directions. The agar plate was then left for 5-10 minutes before applying the test samples. The disc used was 6 mm diameter. A volume of 10µL of the test samples of 100µg/mL, 200µg/mL, 300µg/mL, 400µg/mL were each pupated onto the discs and placed onto the agar plate by using sterile forceps and gently pressed to ensure contact. Next to be placed on the agar plate was the disc pupated with methanol as negative control, followed by 30µg of tetracycline as standard antibacterial agent (positive control). The plates were left at room temperature for 10 minutes to allow the diffusion of the test samples and the standards into the agar. Each crude extract was tested in triplicate for each bacterium used. The plate samples were then incubated at 37°C for 24 hours before the inhibition zone around every sample disc being examined. The inhibition zone was measured in diameter to indicate the presence of antibacterial activity for each sample, as compared to the positive control.

Anti-Malaria Test

Acute Toxicity Test

The acute toxicity of the extract was tested to determine the safety of the agent using Lorke's (ENV/MC/CHEM, 1997) method. The study was carried out in two phases. In the first phase, nine mice were randomized into three groups of three mice per cage and were administered orally with graded concentration (10, 100 and 1000 mg/kg-1 of the extract. The mice were observed for signs of toxicity which include paw licking, salivation, stretching of the entire body, weakness, sleep, respiratory distress, coma and death in the

first four hours and subsequently for 4 days. In the second phase, another set of nine mice were also randomized into three groups of three mice per cage and were administered orally with graded concentrations (1600, 2900 and 5000 mg/kg-l of the bark extract also, based on the result of the first phase. The animals were also observed for signs of toxicity and mortality for the first four hours and thereafter for 4 days. The oral LD50 was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Animals Test

A total of seventy-eight Swiss albino mice (18-25 g) of both sexes were used in the study. Eighteen for acute toxicity study while thirty were used for each of the in vivo antiplasmodial test. The animals were obtained from HAUEMM animal facility centre Federal Housing Estate no10 Sanitation Road Gerie Adamawa State. The animals were housed in cages at room temperature and moisture under naturally illuminated environment of 12:12 h dark/ light circle. They were fed on standard diet and had water ad libitum. A standard protocol was drawn up in accordance with the Good Laboratory Practice (GLP) regulation [ENV/MC/CHEM (98)] (NIH, 1985). The principle of laboratory animal care [NIH publication No. 85-23(Hilou et al., 2006) was also followed in this study.

Inoculums

Parasitized erythrocytes were obtained from a donor-infected mouse maintained at animal facility centre, NIPRD. Parasites are maintained by continuous re infestation in rats. The inoculums consisted of plasmodium berghei parasitized erythrocytes. This was prepared by determining both the percentage parasitemia and the erythrocytes count of the donor rats and diluting them with normal saline in proportion indicated by both determinations. Each rat were inoculated intraperitoneally with infected blood suspension (0.2 mL) containing 1x107 plasmodium berghei parasitized red blood cells on day zero. Infected rats with parasitemia of 5- 7% were allocated to five groups of six rats each (Okokon et al., 2005).

In Vivo Antiplasmodial Test

Suppressive test

A total of thirty rats were used for the study using the methods of Akuodor et al., 2010 and Ryle and Peter, 1970. Each rat was inoculated intraperitoneally with standard

inoculums of 1x107 plasmodium berghei infected erythrocytes. The rats were randomly divided into five groups of six per cage and treated with 100, 200 and 400 mg/kg/day of the extract.

Chloroquine diphosphate 10 mg/kg/day was given as positive control and 0.2 mL of normal saline to the negative control group. All administered orally for four consecutive days (D0-D3). On the fifth day (D4), blood was collected from each mouse and thin films made on a slide. The films were fixed with methanol, stained with Giemsa and parasitemia density examined (Nikon YS2-H, Japan) by counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields.

Curative test

Evaluation of curative potential of Bucholizia Coricea seed extract was done by adopting the method of Chandel and Bagai, 2010 and Akuodor et al., 2010 with slight modification. A total of thirty mice were inoculated intraperitoneally with standard inoculums of 1x107 P.berghei berghei infected erythrocytes on the first day. Seventy-two hours later, the mice were divided into five groups of six per cage and treated with 100, 200 and 400 mg/kg/day of the extract. Chloroquine diphosphate 10 mg/kg/day was given to positive control and 0.2 mL of normal saline to the negative control group, all administered orally.

Treatment continued daily until the eight day when thin films were prepared with the blood collected from the tail of each mouse. The films were fixed with methanol, stained with Giemsa and parasitemia density examined by counting the parasitized red blood cells on at least 1000 red blood cells in 10 different fields. The mean survival time for each group was determined by finding the average survival time (days) of the mice (post inoculation) in each group over a period of 30 days (Do-D29) (Betty & Sternne, 2003).

Acute toxicity test

There was no mortality observed in rat after oral administration of the aqueous extract, even at doses as high as5000 mg/kg signifying that the oral LD50 was greater than 5000 mg/kg. Hence, the experimental doses used (100,200 and 400mg/kg p.o.) were within safe margin.

Suppressive effect

The aqueous Bucholizia Coricea seed extract exhibited a dose dependent chemosuppressive effect at the different doses employed. Doses of 100, 200 and 400 mg/kg caused chemosuppression of 84%, 89% and 96.74% respectively. The effect of the extract was significant ($P<0.05$) when compared with the control. The standard drug, chloroquine (10mg/kg/day), caused 96.93% suppression (Table 1).

Curative effect

The aqueous Bucholizia Coricea seed extract caused a dose-dependent decrease in parasitemia in the extract treated group similar to chloroquine treated group unlike the saline treated group in which there was a consistent increase in the blood parasite density. The survival values showed that the plant extract significantly ($P<0.05$) suppressed established infection at the doses employed. Death was observed in the control group on day 9, and by day 12, all mice in the group died. On the other hand, mice in extract treated groups survived beyond 24 days However, some of the mice in the 400 mg/kg/day group survived the 30 days observation period, while chloroquine treated group recorded no death at all (Table 3).

Statistical Presentation and Processing

Every data collected in the course of this work was statistically presented, processed and analyzed to the best of standards.

Method of Data Collection

Collection of data was done at interval of 7 days (1 week) after the administration of the first treatment dose in the albino rates. Parameters were duly collected and analyzed.

Method of Data Analysis

Data collected were subjected to Analysis of Variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) for test of hypothesis for the final results, discussion and conclusion in statement of facts of this research work.

RESULTS

Phytochemical test Phytochemical analysis of the Bucholizia Coricea seed extract revealed the presence of alkaloids, terpenes, sterols, flavonoids, tannins, saponins and carbohydrates

Result presentation and description of Effect of buchiolizia coreica seed extract on Gram positive and Gram-negative bacteria in millimetre (mm) which demonstrated the extract's impact on various selected bacterials.

Table 1: Effect of *buchiolizia coreica* seed extract on Gram positive and Gram-negative bacteria in millimetre (mm)

Conc. (µg/mL)	**Organism**	**Tetracycline (30 µg/mL)**	**Methanol**
100µg/mL	*Escherichia coli*	21.12± 0.03	**12.12 + 0.12**
	Staphylococcus aureus	21.13± 0.05	10.13 ± 0.12*
	Klebsiella pneumonia	21.23± 0.03	10.12 ± 0.13
200µg/mL	*Escherichia coli*	21.21± 0.04	11.11 ± 0.15
	Staphylococcus aureus	21.15± 0.02	10.15 ± 0.17
	Klebsiella pneumonia	21.13± 0.05	**18.10 ± 1.16***
300µg/mL	*Escherichia coli*	21.19± 0.06	**18.13 ± 0.03***
	Staphylococcus aureus	21.15± 0.04	13.11 ± 0.14
	Klebsiella pneumonia	21.17± 0.03	12.21 ± 0.06
400µg/Ml	*Escherichia coli*	21.18± 0.13	16.11 ± 1.12*
	Staphylococcus aureus	21.20± 0.11	10.12 ± 3.11
	Klebsiella pneumonia	21.19± 0.03	**15.14 ± 0.07**

Result is presented as Mean ± SD. N = 3. *= significant activity was observed when compared to the control ($p<0.05$). Concentration of standard is 30 µg/mL of tetracycline, Conc= Concentration.

Impact of buchiolizia coreica seed extract on Antibacterial Activity According to Table 2, significant antibacterial activity was observed at all concentrations compared to the control, as indicated by '*'.

Table 2: Suppressive activity of *Buchiolizia coreica* seed extract in plasmodium berghei-infected rats

Treatment	Dose (mg/kg)	Mean parasitaemia density	%
		D5	
Control	0.2 ml/kg	16.43±0.91	--------
Buchiolizia coreica	100	2.46±0.15	84**
	200	1.78±0.08	89**
	400	0.6±0.10	96.74**
CQ	10	0.49±0.06	96.93**

D5= Day five, CQ=Chloroquine * significantly different from control at P< 0.05.

** High significantly different from control at P<0.01

Table 3: Curative effect of Buchiolizia coreica seed extract in P. berghei-infected rats

Treatment	Dose (mg/kg)	Mean parasitaemia density		Survival time
		(Days)	Pre (D3)	post (D7)-
Control	0.2ml/kg	30.73±1.77	39.62±3.2	10.67±0.49
Buchiolizia coreica	10	29.42±2.22	5.72±0.49**	24.83±0.95
	20	30.34±3.10	4.45±0.37**	27.17±0.75
	40	30.98±4.30	2.90±0.11**	29.67±0.21
CQ	10	29.6 8±3.11	2.32±0.07**	30.0±0.0

D3=Day three, D7=Day seven, CQ=Chloroqiune * significantly different from control at P< 0.05.

** High significant different from control at P<0.01

DISCUSSION

The study evaluated the antibacterial potential of methanol Wonderful Kola seed extract against selected bacterial strains. Significant antibacterial activity was observed at all concentrations tested, indicating the potential of the extract as a natural antimicrobial agent. With Escherichia coli having the highest inhibition at 300µg/mL with inhibition rate of 8.13 + 0.03mm* when compared to the control. Low inhibition rate was observed on Escherichia coli at 100µg/mL with inhibition rate of 12.12 + 0.12 when compared to the control 21.12+ 0.03.

These findings support the traditional use of Wonderful Kola seed extract for treating bacterial infections and underscore its therapeutic potential in combating antibiotic-resistant bacteria.

Although wonderful Kola seed (Buchiolizia coreica) provide a significante antibacterial potential. The activity of the extract on the infected rats with Plasmodium berghei has been used in studying to determine the activity of potential antimalarials in the rats.

However, the primate models provide a better prediction of efficacy in human than the rodent models, the later have also been validated through the identification of several conventional antimalarial, such as chloroquine, mefloquine, halofantrine and more recently artemisinin derivatives (Thomas et al., 1998). Plasmodium berghei has been used in studying the activity of potential antimalarials in mice (Pedroni et al., 2006) and in rats

(English et al., 1996). It produces diseases similar to those of human plasmodium infection (Kumar et al., 2006, Peter et al., 1998).

As this parasite is sensitive to chloroquine, this drug was used as a standard drug in the study. Chloroquine has been used for suppressive and curative antiplasmodial activities. In early and established infection, chloroquine interrupts with the hem polymerization by forming a PF-chloroquine complex. This complex is responsible for the disruption of the parasite's cell membrane function and ultimately leads to auto digestion.

The 4-day suppressive test is a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia is the most reliable parameter. A mean group parasitemia level of less than or equal to 90% of the mock-treated control animals usually indicate that the test agent is active in standard screening studies (Ene et al., 2008).

The results obtained showed that in Plasmodium berghei infected rat treated with aqueous Buchiolizia coreica seed extract, there was significant decrease in parasitemia. The extract exhibited a dose dependent activity. In addition, the result of the chemo suppressive activity suggests that the seed extract of this plant can suppress parasite growth to non-detectable levels in erythrocytes. It is important to know that scientific evaluation of traditional medicine preparations for claimed antimalaria efficacy be carried out even up to the level of finding out the degree of suppression of parasite growth in erythrocytes (Auodor et al., 2010).

The Buchiolizia coreica seed extract of this plant also exerted significant curative effect during established infection. Curative activity of potential antimalarial agents of ethnomedicinal materials should be discernable during testing for antimalarial. The observed antimalarial activity of the plant extract is consistent with the traditional use of the plant as herbal medication against the disease and indicative of its potential as a chemotherapeutic antimalarial agent.

This was confirmed by the mean survival time values which at doses employed were twice or more than that of control group. In untreated rats, the parasite count increased daily until the death of the animal, which was also observed by (Steele et al., 1999).

However, the traditional use of Buchiolizia coreica seed extract could be attributed to the presence of certain phytochemicals that constitute the bioactive principles in the plant. Numerous plants containing a wide variety of phytochemicals as their bioactive principle

have shown antiplasmodial activities (Aishawsh et al., 2007, Matur et al., 2009, Philipson & Wright, 1990). The antiplasmodial activity of this plant extract might be attributed to the presence of alkaloids, flavonoids and terpenes which have been variously implicated in antiplasmodial activities of many plants (Ayoola et al., 2008). These compounds have also been shown to exert antiplasmodial activity by elevating the red blood oxidation and inhibiting the parasite's protein synthesis. This counteracts the oxidative damage induced by the malaria parasite (Arokiyaraj et al., 2008).

CONCLUSION

This study has however stablished the rationale for the traditional use of this plant in Nigeria and like many others, proved that medicinal plants which have folkloric reputations for anti-bacterial and antimalarial properties can be investigated in order to establish their efficacy and to determine their potentials as sources of new anti-bacterial and antimalarial drugs.

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Conflict Of Interests: All authors have none to declare.

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