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Research Article

Evaluation of Antimicrobial Properties of *Manilkara zapota* (L) Royen. Fruit

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Abstract

The current study was investigated the antimicrobial properties of *Manilkara zapota* (L) Royen grown at two different sites in Tamil Nadu state of India (Murasancode of Kanyakumari district (site I) and Kudankulam of Tirunelveli District (site II). Bioassays for antimicrobial activities were carried using aqueous, petroleum ether, ethyl acetate, chloroform and ethanol extracts of fruits from plants grown at the selected sites were used for find out the antimicrobial activities through bioassays against few pathogenic bacteria and fungi. Gram positive bacteria such as *Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis*, gram negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Proteus vulgaris* and fungal strains like *Candida albicans, Aspergillus flavus* and *Aspergillus niger* were utilized for the current study. The fruit fractions from plant of the two sites showed variation in degrees of antimicrobial activity against the microorganism investigated. The ethanolic fruit extract of *Manilkara zapota* plants of site II showed significant antibacterial activity in cultures of *Bacillus subtilis* showing growth inhibition zone of 30 mm compared to all tested bacterial strains. Antifungal activity of ethanolic fruit extracts from plants of site II exhibited higher activity in cultures of *Aspergillus flavus* producing growth inhibition zone of 20 mm. The study shows that more antimicrobial activity is shown by ethanolic extract of fruits of plants from site II, the reason for which is discussed.

Keywords: *Manilkara sapota, Antibacterial activity, Antifungal activity, Disc diffusion*

INTRODUCTION

Manilkara zapota, commonly known as sapodilla belonging to the family of Sapotaceae. The plant is evergreen glabrous tree with a smooth juice.

Sapota is a climacteric natural product, for the most part used for its sweet and delectable natural products. It contains a rich assortment of phenolic compounds including tannins, flavonoids, flavanols, hydroxy benzoic acid, phenolic acid etc.

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According to a 100 g of eatable organic product contains (dampness 73.7 g, carb 21.49 g, protein 0.7 g, fat 1.1 g, calcium 28 mg, phosphorus 27 mg, iron 2 mg and ascorbic acid). It is an abundant source of dietary fiber that acts as natural laxative adding bulk to the stool, promotes bowel movement and support to safeguard the mucosal lining of the colon from cancer-causing toxins. This organic product is a significant source of polyphenol compound tannin which act as a causing agent of contraction, antimicrobial anti-inflammatory, antiviral, anti-arthritic and the ability of management and treatment the infections by parasites are used to treat swollen and inflamed veins and GI tract issues. The seeds are aperients, diuretic, tonic and febrifuge (Bose et al., 2001). Sapota tremendously plentiful in nutrients A, C backings to help sound vision, battles irresistible specialist, supports in susceptibility and keep you sickness free. Besides, it additionally contains vital measures of other fundamental supplements like potassium, copper, iron and nutrients like folate, B1 and B5 which are fundamental for keeping up with ordinary metabolic cycles in the body and upgrades generally prosperity (Osman et al., 2011).

MATERIALS AND METHODS

Collection of plant material

Fresh leaves and fruits of *Manilkara Zapota* L, were purchased in the month of December, 2016 from Murasancode of Kanyakumari district and *Manilkara zapota* plants growing at Kudunkulam of Tirunelveli district. The species and taxonomy of the plant was identified and registered by Professor R. Medo Merina, Department of Botany, Women's Christian College, Nagercoil (Konuku et al., 2017).

Preparation of plant powder

The collected fresh leaves and fruits were washed thoroughly and air dried in shade until dried completely. The drying process was continued until the moisture content was decreased. After drying, the plant material was macerated using mixer grinder. Then the powder was stored in air tight containers and kept in refrigerator for future use.

Preparation of plant extracts

The dried and powdered leaves and fruits of *Manilkara Zapota* were extracted with 10 grams of plant powder and 250 ml of ethyl acetate, chloroform methanol and ethanol separated through a soxhlet extractor and the temperature was adjust to the boiling point of the solvent. The extracts were separated through whatman (No.1) filter paper and then concentrate at 40°C by using rotary evaporator. The resultant product was kept in a freezer for further experiments (Abreu et al., 2012).

Determination of antimicrobial assay

Six bacterial strains (*Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, E.coli, K.pneumoniae*, *P.vulgaris*) and three fungal strains (*C.albicans, A.flavus and A.niger*) were collected from the Institute of Microbiology division of Sree Chitra Thirunal Institute of Medical Sciences and Technology Thiruvananthapuram, Kerala. The crude extracts of *Manilkara zapota* fruits were mixed with ethanol extract for the antibacterial activity test. Disc diffusion method was approached for find out the antibacterial effect. The fruits extracts of *Manilkara zapota* were investigated for their *in vitro* antimicrobial properties against selected both gram positive and gram negative bacteria (Baky et al., 2016).

Paper disc diffusion assay

The required medium was made by mixing of both distilled water and muller Hinton agar medium (33.9 g). Then the prepaid medium was autoclave for 15 min (pH 7.3). After autoclaving the media was allow to solidify at room temperature under an UV light. For disc diffusion assay, 50 mg of each extract was mixed with 1 ml of representative solvents and from that 5 mg/ml was applied to sterile paper discs (6 mm). Then the discs were kept on an agar plates, incubated with 19 hours culture using the test pathogens (10^6 bacterial cells/ml) in nutrient broth. In this method we consider a disc as a positive control, here an antibiotic disc such as ampicillin was used and the corresponding solvent loaded disc taken as negative control. Then following incubation for one day at room temperature. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition in centimeters. The lack of zone of inhibition means the particular compound is inactive against the pathogen. If the zone is less than 7 mm indicates the activity of the compound, the zone may be from 8 mm-10 mm, the activity of compound is intermediate and sensitive if more than 11 mm.

Antifungal assay by disc diffusion method

Antibiotic susceptibility tests were identified by agar disc diffusion (Kirby-Bauer) method, Fungi strains *C.albicans, A.niger, A.flavus* were spreaded in separate SDA agar plates and the different extracts were applied into the sterile discs Then each discs were placed on the SDA medium plates and following incubation at 22°C for two whole days. After the incubation period, the zone of inhibition were checked and measured with transparent ruler in millimeters. The results were statistically analysed using ANOVA and also done to find out the effect of plant extracts from different sites on the pathogens (Ayaz et al., 2019).

RESULTS

Antimicrobial activity of *M. zapota* dried fruit extracts against different pathogenic microbes showed variation in their activity. The results revealed that extracts has potential to suppress the microbial growth. Positive control used for our study was amikacin which showed good activity and inhibition was higher in all the strains. Ethanolic extract of *M. zapota* fruits from plants of site II (Koodunkulam of Tirunelveli) exhibited higher activity in which their zone of inhibition against *B. subtilis* (30 mm) *P. vulgaris* (22 mm), *S. mutans* (19 mm), *E. coli* (19 mm) *S. aureus* (17 mm) and *K. pneumonia* (17 mm). Samples from site I (Murasancode of Kanyakumari) revealed its better activity against *B. subtilis* (21 mm), *K. pneumonia* (18 mm), *Proteus vulgaris* (18 mm), *Streptococcus mutans* (17 mm), *E. coli* (17 mm) and *S. aureus* (16 mm) (Figure 1). Results exhibiting maximum zone of growth inhibition for bacterial and fungal cultures (Bakkali et al., 2008).

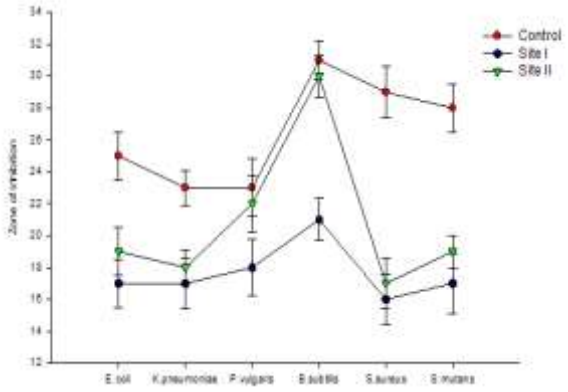

Figure 1. Anti-bacterial activity of *M. zapota* ethanolic extracts.

M. zapota fruits from plants of site I and II extracted using ethyl acetate showed distinct zone of inhibition (Blair et al., 2015). Samples from site I exhibited growth inhibition against *E. coli* and *S. aureus* (11 mm), *S. mutans* and *B. subtilis* (12 mm) *P. vulgaris* (13 mm), *K. pneumonia* (15 mm). Samples from site II revealed inhibition zone against *B. subtilis (*15 mm*)* and *S. mutans* (13 mm) *K. pneumonia* (12 mm) *S. aureus* and *P. vulgaris* (11 mm), *E. coli* (9 mm) (Figure 2).

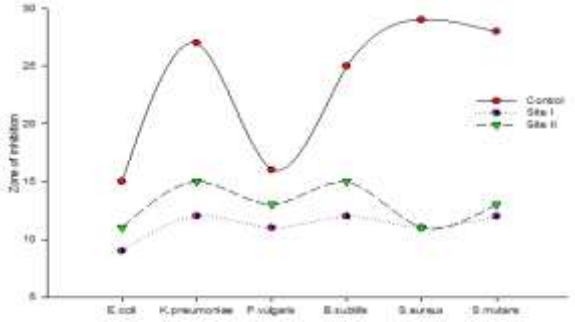

Figure 2. Anti-bacterial activity of *M. zapota* ethyl acetate. extracts.

Petroleum ether fruit extract from plants of site I showed growth inhibition in *K. pneumonia* (14 mm) and *S. aureus (*12 mm), *P. vulgaris* (12 mm), *S. mutans* (11 mm). Least growth inhibition activity was shown in *B. subtilis* (8 mm). Samples from plants of site II exhibited more growth inhibition against *P. vulgaris* (16 mm), *S. mutans* (12 mm), *K. pneumonia* (11 mm), *S. aureus* (11 mm) and *B. subtilis* (10 mm). The samples from two sites exhibited same zone of inhibition against *E. coli* (13 mm) (Figure 3).

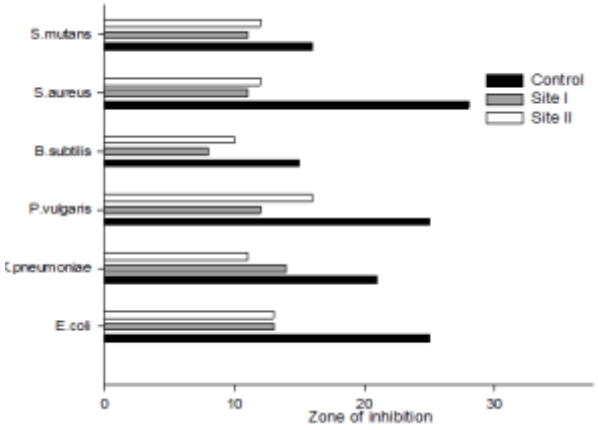

Figure 3. Anti-bacterial activity of *M. zapota* petroleum ether extracts.

Chloroform extracted *M. zapota* fruits from plants of site II showed good activity against tested microbial strains when compared to extract of fruits form plants of site I. Samples from plants site II showed higher zone of inhibition against *E. coli* (16 mm), *S. mutans* (14 mm), *B. subtilis* (13 mm), *K. pneumonia* and *P. vulgaris* (10 mm), *S. aureus* (9 mm). Samples from site I showed its competency against strains by producing inhibition zones in cultures of *E. coli* (13 mm), *P. vulgaris* (13 mm), *S. mutans* (11 mm), *B. subtilis* (10 mm), *K. pneumoniae* (9 mm) and *S. aureus* (6 mm). Aqueous extract of samples from plants of site I and II showed minimal activity against tested microbial strains. Samples from site I showed lower activity than site II, which was noted against *S. mutans* and K. *pneumonia* (12 mm)*, P. vulgaris and S. aureus* (8 mm). No activity from site I was exhibited against *B. subtilis* which is clearly displayed by their zone of inhibition. Extract from plants of site II revealed good growth inhibition activity of *K. pneumonia* (13 mm), *S. aureus* (10 mm), *P. vulgaris* (9 mm) and *B. subtilis* (8 mm).The samples from two sites exhibited same zone of inhibition in *E. coli* (10 mm). Statistical analysis of the data of antibacterial activity was analysed to support the results of the present investigation. The *P value* for the ethanol and petroleum ether extract was highly significant at 0.005% level with *P value* 0.0056, while petroleum ether showed a calculated *P value* of 0.0002. The P value for chloroform, ethyl acetate and aqueous extract was significant at 0.0015% (0.0001, 0.0014 and 0.0001) respectively (Bukola et al., 2008).

Antifungal activity

Antifungal activity of *M. zapota* fruit extracts was also analysed to find the ethanolic extracts of *M. sapota* fruits of plants from site II showed higher activity by suppressing the growth of *C. albicans* (16 mm), *A. niger* (18 mm) and *A. flavus* (20 mm) in cultures (Casal et al., 2005). Whereas fruit extract from site I plants was less competent to control the growth of fungal strains cultures of *A. niger* (14 mm), *C. albicans* (15 mm), *A. flavus* (18 mm) (Figure 4).

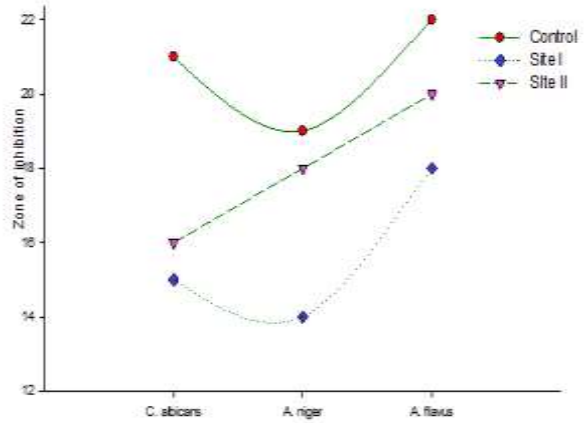

Figure 4. Anti-fungal activity of *M. zapota* ethanol extracts.

The dried fruits of *M. zapota* extracted through ethyl acetate from site I and II showed similar growth inhibition zone in cultures of *A. flavus* (9 mm), while extract from plants of site I showed minimum inhibition against *C. albicans* (7 mm) and a maximum growth inhibition zone of 11 mm in *A. niger* cultures. Strain *A. niger* (12 mm), *C. albicans* growth was suppressed by nystatin (20 mm) followed by extract from site II (10 mm) (Figure 5).

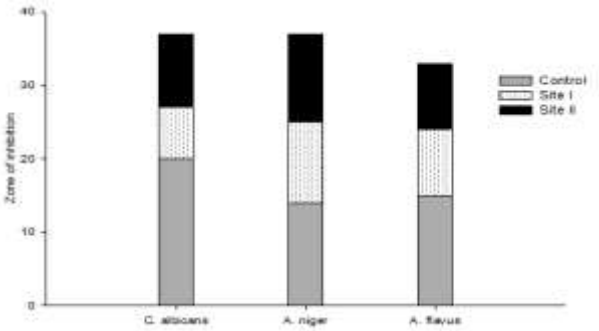

Figure 5. Anti-fungal activity of *M. zapota* ethyl acetate extracts.

Petroleum ether extracted *M. zapota* fruits from site II showed good activity against tested fungal strains than site I. The extract has capability to compete or suppress the growth of *C. albicans* (11 mm), *A. niger* (10 mm) and *A. flavus* (12 mm). The extract from site I has no activity against *A. flavus* by exhibiting no zone of inhibition. *A. niger* (9 mm), *C. albicans* (8 mm).

Chloroform extracted *M. zapota* fruits from plants of site II showed good antifungal activity against all the fungal strains such as *A. niger* 13 mm, *C. albicans* 11 mm and *A. flavus* 10 mm (Sumitra et al., 2010).

This shows the extract has its potential to inhibit the growth of fungal culture than site I (*A. niger* 12 mm, *C. albicans* 10 mm and *A. flavus* 7 mm) (Kothari et al., 2010). The positive control nystatin showed good inhibitory action against *C. albicans* (20 mm), *A. niger* (16 mm), *A. flavus* (15 mm) (Figure 6).

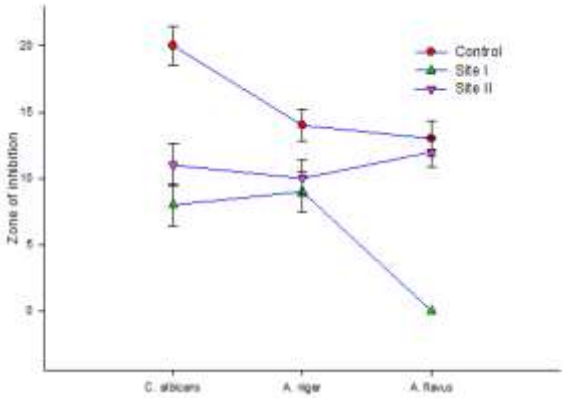

Figure 6. Anti-fungal activity of *M. zapota* petroleum ether extracts.

M. zapota fruits from plants of site I and II extracted in water shown minimal activity only against few strains and there was no growth inhibition zone in few plates. This fruit extract from site II plants showed no inhibition zones against *A. niger* and *A. flavus.* This implies the extract was inactive has no capability to inhibit the growth of fungal culture (Maiyo et al., 2010).

Statistical analysis of the data of antifungal activity was analysed supported the results in the present investigation. The P value for the petroleum ether and ethyl acetate extract was high significant at 0.05% (0.0412 and 0.0226) respectively. The P value for chloroform and ethanol extract was significant at 0.01% level with P value (0.0178 and 0.0166) respectively.

DISCUSSION

The usage of botanicals have been elevated now a days due to wide range of applications against diverse groups such as pharmaceutical, medicine, agricultural, cosmetic and food industries of synthetic drugs. The alarming increase in infection has urged researchers to find potential compounds against antibiotic-resistant microbes (Mandalari et al., 2007). According to Blair et al. resistance to drugs by pathogenic organism are acquired through signal transmission among bacterial species.

According to Ullah et al., bacterial species drug resistance can also be developed through extracellular drug efflux mediated by efflux pumps, degradation of drugs by enzymes and modification of target.

M. zapota fruit is the major source of polyphenol compound such as tannin which possess the ability for the contraction of skin cells and other body tissues, act as an agent for reduces inflammation or swelling, act against viral, parasites and bacterial infection, that are well-known to treat hemorrhoids and GI tract related issues. The flower of *M. zapota* used to treat diarrhea, dysentery and breathing issues. Earlier works reveals that herbal based extract exhibits higher antimicrobial activity when compared with synthetic antimicrobial agents.

The ethanolic extracted *M. zapota* fruits from trees of site I and II when exposed to *E. coli, K. pneumonia, P. vulgaris, B. subtilis, S. aureus* and *S. mutans* cultures showed good growth inhibitory activity. Fruit extracts from plants of site II when incorporated in cultures medium of *B. subtilis*, the growth of which was inhibited to the maximum, when compared to all other strains. Inhibition zone was maximum in *B. subtilis* (30 mm) followed by *P. vulgaris, S. mutans, K. pneumonia* and *S. aureus* cultures when grown in fruit extract of plants from site II.

The availability of phyto-constituents helps to have good antimicrobial activity. In bacterial cells, the phytocompounds in the pant cause cell content coagulation and also inactivate their DNA. The results implied shows that the ethanolic fruit extract contain phytoconstituents which can combat pathogenic microorganisms, which is supported statistically by showing significant difference in their activity having $F_{2,15}$=7.45, P<0.005). Earlier research report also reveals the availability of terpenoids, flavonoids and glycosides in sapota plant extracts which helps to exhibit antimicrobial properties. The chloroform extract of fruits from *M. zapota* plants of site II showed better activity against *E. coli* cultures when compared to all other strains.

The activity of ethyl acetate extract of *M. zapota* fruits from plants of site I was maximum in *K. pneumoniae* cultures, while the same solvent extract of fruits of plants from site II inhibited the growth of *B. subtilis* cultures to the maximum. However, as compared to the control, all of the results in relation to the growth inhibitory action of fruit extract from sapota plants from the selected sites were lower (Amikacin). Petroleum ether extracted fruits from plants of site II exhibited better activity when compared to same solvent extract of fruits from trees from site I. The aqueous extract of *M. zapota* fruits from plants of the selected sites showed lower activity against strains when compared with other extracts. Even though antibiotics in the present markets are potential, still development of new drugs are needed to suppress drug resistant microbial pathogens, as most of the synthetic antimicrobial drugs can result in carcinogenic effects and acute toxicity. In this investigation gram positive bacterial strains were more affected.

The findings of the current study are in agreement with Nair and Chanda.

Antifungal activity

The ethanolic extracted *M. zapota* fruits from plants of site I displayed good antifungal activity than fruit extract of plants from site II against all the fungal strains screened. The activity was maximum in the cultures of *A*. *flavus* (20 mm) followed by *A. niger* and *C. albicans,* which implies the extract has its potential to control the growth of fungus. This may due to the presence of secondary metabolites like terpenoids which causes closure of the cell wall of microbes by waning the tissue, while Zablotowicz et al., is of the opinion that some proteins and various enzymes were leaked from the cells because of the activity of saponins.

In the present investigation results obtained in the antifungal assay were significantly different among all the tested concentrations with $F_{2,6}$=8.75, P<0.005. Chloroform fruit extract of plants from site II showed greater activity when compared with fruit extract of plants from site I against *A. niger, C. albicans* and *A. flavus.* Ethyl acetate extracts of fruit from plants of site I inferred its activity against *A. niger* as maximum of (12 mm) with $F_{2,6}$ value of 7.60 with P<0.02. Petroleum ether extract of fruits of plants from site I displayed lower growth inhibitory activity in *C. albicans, A. niger* cultures and there was no activity observed in *A. flavus* cultures which implies that the extract does not has potential to compete the fungal growth.

The fruit extract from plants of site II exhibited higher antifungal activity in all the strain culture tested which was displayed by their zone of growth inhibition. The results were significant with $F_{2,6}$=5.68 at P<0.005. Similar to the present work, petroleum ether extract of *M. zapota* fruits against different fungal strains such as *Mucor hiemalis*, *Fusarium eumartii* and *Candida albicans* showed good activity. The aqueous extracted *M. zapota* fruits from plants of site I and II exhibited less antifungal activity in the fungal cultures analysed.

This work proves that plant based compound isolation and their activity against microorganisms can help to overcome drug resistant pathogen and control various infectious diseases. Mulyaningsih et al., suggests that the plant based compounds; essential oil can be useful to combat against epidemic multi drug resistant pathogenic microorganisms. Earlier researchers suggest that the crude plant extracts studied against pathogens or their activity will paves the way for the discovery of novel bioactive compounds and that opens the door for the development of dynamic bioactive compounds.

Studies on antimicrobial actives of *Sapotaceae* families revealed their activity against many pathogenic microbes which was seen through earlier findings. *Manilkara huberi* against *Candida albicans, Candida glabrata* and *Candida parapsilosis*.

The phytochemicals such as tannins and flavonoids have the same mechanism like create a supply of stable free radical and develop a composite with nucleophilic amino acids, these amino acids have the capacity to block the activity of protein and loss of function, their possible antimicrobial activity is large as they possibly target microbial cell, cell wall polypeptides and membrane bound enzymes as suggested by Stern et al.

Thus the observations of present study says that these underutilized can be further used to identified the bioactive natural substances that would provide and make possible pharmacological studies leading to synthesis of a more potent drug with low toxicity. The phytocompounds which are present in the extracts might disruption of the cell membrane of the pathogen, which cause cellular components leakage, coagulation, interfaces with membrane protein and this retards enzyme synthesis, nutrient absorption and ultimately leads to death of microbes. Similar pathway were explained when bacteria are treated with essential oil. Thus plant based natural compounds and extracts has its potential to control pathogenic micro-organisms.

CONCLUSION

Fruits of *Manilkara zapota* were commonly consumed, however in the present days this fruit is not given much importance. Hence it is the need of the hour to create awareness among the population about this medicinal property of the plant. So that highly sensitive microbial strains can be combated with the use this fruit. The outcomes of this study offer a systematic basis for separation and purification of bioactive principles from the fruits of *Manilkara zapota.* Our next studies are focus on the identification of active phytocompounds and establish their effect against microbes, are in progress.

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