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Physicochemical Properties and Chemical Constituents of Oil from Joan Seed (*Trachyspermum ammi L)*

S. Morshed[1], M. K. Alam[2], A. Begum[3], S. M. S. Shahriar[1], K. N. Sharmin[3] and S. Akther[2]

[1]Dept. of Applied Chemistry and Chemical Technology, [2]Dept. of Food Processing and Engineering, [3]Dept. of Applied Food Science and Nutrition;
Chittagong Veterinary and Animal Sciences University, Chittagong-4202, Bangladesh

Abstract

The paper deals with the investigation of the fatty oil of carom seed (*Trachyspermum ammi*) for its physicochemical characteristics and fatty acid composition. Oil from carom seed collected from local market was extracted with n-hexane in a glass Soxhlet apparatus. Extracted oil was dark green, burning taste, spicy in odour and soluble in n-hexane, chloroform, pet. ether and benzene. Its chemical characteristic such as acid value (6.69), Iodine value (79.39), Peroxide value (457.11), Saponification value (184.32), Unsaponified matter (9.11), Free fatty acid value as petroselinic acid (48.1) were determined by conventional method. The composition of fatty oil was determined by Gas Liquid Chromatography (GLC). From GLC analysis the main constituents of fatty oil such as Petroselinic acid (89.35%), Oleic acid (5.86%), Linoleic acid (4.79%) were determined. For the better evaluation of these spices, it is necessary to know their complete chemical composition.

Keyword: *Trachysprermum ammi* (L), Fatty oil, Physicochemical properties, Chemical constituents

Introduction

Trachyspermum ammi, commonly referred as Bishop's weed, carom seed (English name) and joan in Bengali language, is an erect annual herb with straight stem originated in Persia, India and Bangladesh (Wadikar *et al.,* 2012). *Trachyspermum ammi* (L) Sprague is a Greek work Trachy = rough and spermum = seeded, whereas ammi is name of plant in Latin. Syn. Carum copticum, commonly known as Joan belonging to family Apiaceae or Umbelliferae (Dwivedi *et al.*, 2012). The plant has a similarity to parsley. Because of their seed-like appearance, the fruit pods are sometimes called seed. They are egg-shaped and greyish. According to Ayurveda, the joan seeds are hot, pungent, stomachic, appetizer, aphrodisiac, carminative, laxative and diuretic. Joan is traditionally used as remedy for gastric disturbances and as a digestive aid. The thymol and carvacrol derivatives and other minor components from joan are responsible for their functional properties (Anilakumar *et al*., 2009 and Pathak *et al*., 2010). Some disagreement about the presence of its constituents was observed. Therefore the present work was designed to carry out a total investigation of Joan seed's fatty oil for the purpose to get information for industrial as well as medicinal applications of fatty oil and characterization and identification of the constituents of fatty oil isolated from the seed by GLC.

Materials & methods

1. Raw materials: Joan **(***Trachysprermum ammi*) seeds were procured from the local market.

2. Chemicals and reagents: Solvents and chemicals of analytical or reagent grade were used in all experiments unless otherwise stated. Chloroform, acetic acid, acetic anhydride, ether, bromine, ethanol, Pet.ether (b.p. 60-80°C), (sigma E.merck or BDH) were used after distillation. BF_3-CH_3OH complex and standard methyl esters of fatty acid (LIPID STANDARD) were obtained from E.MERCK and SIGMA, respectively.

3. Gas Liquid Chromatography: Gas liquid chromatography was conducted with a varian gas chromatograph fitted with a flame ionization detector and glass column (2800×0.2 cm i.d.). A 3% OV-225 on gas chrom Q (100/120 mesh) column was used at 200°C isotherm with a nitrogen flow of 25 ml per minute.

4.Investigation of joan seed (*Trachyspermum ammi*) fatty oil

4.1. Extraction of the fatty oil from joan seed

The fatty oil of sun dried joan seed was extracted with n-hexane (bp 68.2°C) in a glass Soxhlet apparatus.

4.2. Analysis of the essential oil

4.2.1. Determination of physical properties of joan seed fatty oil

4.2.1.1. Solubility of fatty oil in different solvent

A small amount of fatty oil was taken into a test tube and 3 ml of n-hexane was added to it. Then the clear solution was obtained. Then a small amount of fatty oil was taken into another test tube and 4 ml of benzene solvent was added to it. The solubility of the fatty oil was performed with chloroform and pet. ether (b.p 60-80°C) and clear solution was obtained in each solvent.

4.2.2. Determination of chemical properties of joan seed fatty oil

4.2.2.1. Determination of acid value and free fatty acid

Accurately 0.25 g of the fat was taken in a flask. Fifty ml of 95% ethanol was added into the flask, and the mixture was neutralized with 0.1 N aqueous alkali using 0.5 ml of the 1% phenolphthalein indicator. The neutralized ethanol was poured in the flask and mixed the contents of the flask. Then the solution was boiled as hot as possible titration was carried out with 0.1 N aqueous alkali solutions. The solution was shaked vigorously during the titration. The first appearance of the red coloration that did not fade within 10 sec. was considered the end point and the volume of the alkali required were recorded (British Standard Methods of the Analysis of Oils and Fats, 1958).

Acid value = $\frac{56.1 \times A \times N}{W}$

Free fatty acid (as petroselinic acid) = $\frac{2.82T}{W}$

Where, A = Volume of the alkali required.

N = Normality of the NaOH solution.

W = Weight of the sample taken in g.

4.2.2.2. Determination of saponification value

Accurately weighed 0.26 g of sample was taken into a round bottomed flask. Twenty five ml of alcoholic KOH solution was added into the flask through a pipette taking definite time of draining. Then the flask was boiled continuously for 1 h under a reflux condenser and swirling the contents of the flask at frequent intervals. The excess alkali was determined while the solution was stilled hot by titration with the 0.5 N HCl solution using 0.5 ml of the chosen indicator. Blank determination was carried out with the same KOH solution at the same time under the same condition (British Standard Methods of the Analysis of Oils and Fats, 1958; Jolly S.C., 1963 and Scott W.W, 1939).

$$\text{Saponification value} = \frac{56.1 \times N \times (A-B)}{W}$$

Where, N = Normality of KOH solution.

A = Volume of HCl used (in ml) for the sample.

B = Volume of HCl used (in ml) for blank titration.

W = Weight of the sample taken in g.

The equivalent wt. = The molecular wt. of KOH = 56.1

4.2.2.3. Determination of unsaponified matter

An accurately weighed 0.32 g of the fatty oil or fat was taken into a 250 ml round bottom flask and 25 ml of alcoholic KOH was added to it. The flask was attached to a refluxing condenser and heated on a boiling water bath for an hour. The contents of flask were occasionally stirred to mix the solution properly and to ensure complete saponification.

After completion of saponification, the flask was removed from the bath, the condenser was detached and the content of the flask was transferred to a 250 ml separating funnel. The solution was washed with 50 ml of distilled water. Then the flask was rinsed with 50 ml of pet. ether and this ether was poured cautiously into the separating funnel. The funnel was covered and shaken vigorously while the contents were still slightly warm. After shaking for about 30 seconds the funnel was then suspended and left stationary till there appeared two distinct layers in the liquid mixture. The upper ethereal layer was collected in a round bottom flask by transferring the lower aqueous layer of the solution into another flask. The aqueous layer of the soap solution was extracted twice more with pet. ether in the similar way. The ether extract were then combined 30 ml of distilled water and then 20 ml of 0.5 N aqueous KOH solutions.

After one or other of these preliminary treatments, the ethereal solution was washed twice with 20 ml of water. It was shaken vigorously on each occasion. Then successively washed with 20 ml of 0.5 N aqueous KOH solutions, 20 ml of distilled water and again with 20 ml of 0.5 N aqueous KOH solution and at least twice more with 20 ml of distilled water. Washing was continued with water until the wash water no longer turned pink on addition of phenolphthalein indicator.

The ethereal solution was transferred to a sample flask and ether was evaporated from the solution. Then the flask being almost entirely immersed, hold obliquely and rotated in a boiling water bath. When the flask becomes dried, the contents to constant weight, at the temperature not exceeding 80°C.

The extract was dissolved in 10 ml of freshly boiled and neutralized 95% ethanol and titrated with the 0.1 N alcoholic NaOH solutions, using phenolphthalein indicator (British Standard Methods of the Analysis of Oils and Fats, 1958 and Hilditch T.P., 1949).

Unsaponified Matter, Percent by Weight = $\frac{100 \times W1}{W}$

Where, W_1 = Weight in g of residue.

W = Weight in g of sample taken.

4.2.2.4. Determination of peroxide value

Exactly 0.26 g of sample was placed in a 250 ml glass stoppered conical flask. A 7.5 ml of a mixture of 7.5 ml of glacial acetic acid and 5ml of chloroform was added and shaken until the sample was dissolved. Then 0.25 ml of saturated potassium iodide solution was also added to it. The solution had been shaken for at least 1 minute.

About 15 ml distilled water was added to the solution and it was titrated carefully with 0.05 N Sodium thiosulphate solutions until the brown color was faded to pale yellow. Five ml of starch solution was then added and the titration was completed with continuous shaking when the blue color just disappeared at the end point (British Pharmacopaeia: 1998).

$$\text{Peroxide Value} = \frac{2.6 \times strength\ of\ Na2S2O3 \times 1000}{Weight\ of\ sample\ in\ g}$$

4.2.2.5. Determination of iodine value

Exactly 0.28 g of fatty oil was taken in a well-stoppered bottle. The oil was dissolved 10 ml of chloroform. 25 ml of Hanus solution was added to the stoppered bottle from a burette and the solution was allowed to stand for half an hour in dark place with occasional shaking. At the end of this period, 10 ml of KI (15%) solution was added and shaked thoroughly and 100 ml of distilled water was also added by washing and free iodine on stopper.

The solution was titrated with standard 0.1 N $Na_2S_2O_3$ solutions, was added gradually with constant shaking until yellow color of the solution was almost disappeared. A few drops of starch solution were added and the titration was continued until the color was initially disappeared. The blank determination was also carried out observing the same condition omitting the oil (Sidney, Williams, 1984).

$$\text{Iodine Value} = \frac{(S-B) \times N \times 12.69}{G}$$

Where, S = Volume of $Na_2S_2O_3$ required for the sample.

B = Volume of $Na_2S_2O_3$ required for the blank titration.

N = Strength of the $Na_2S_2O_3$ solution.

G = Wt. of the fatty oil taken in g.

4.2.3. GLC Analysis of fatty oil

The fatty oil was analyzed as the methyl ester of their fatty acid contents by GLC. During esterification of fatty oil, glycerides and phospholipids were saponified and fatty acids were liberated and esterified in presence of sodium methoxide.

4.2.3.1. Preparation of methyl esters of reference fatty acids

Reference fatty acids (100 mg of each) were taken in a pear-shaped flask. A condenser was attached with it and refluxed for 10 minutes. Then 5 ml BF_3-CH_3OH complex was added from a bulb pipette through condenser and continued boiling for 2 minutes. Five ml of hexane was added through condenser and boiled for another 1 minute. Then hot mixture was poured into a small separating funnel with 10 ml of hexane. Five ml of distilled water was added to it. The funnel was shaken vigorously and the layers were allowed to separate. The aqueous methanolic layer was drained off and discarded. The hexane layer was dried by treating with anhydrous sodium sulphate, filtered into a quick fit flask, concentrated to a small volume by using vacuum evaporator and transferred to a vial. Finally the hexane containing mixture of methyl ester of acids were concentrated to a few drops by blowing nitrogen gas and stored in a refrigerator before analysis by GLC (AOAC, 1984).

4.2.3.2. Preparation of methyl esters of sample fatty acids fraction

Exactly 0.11 g of the joan seed fatty acid fraction was taken in a pear-shaped flask. Four ml of 0.5 N methanolic NaOH solution and some boiling chips were added to the sample. A condenser was attached with it and refluxed for 10 minutes. Then 5 ml BF_3-CH_3OH complex was added from a bulb pipette through condenser and continued boiling for 2 minutes. Five ml of hexane was added through condenser and boiled for another 1 minute. Then hot mixture was poured into a small separating funnel with 10 ml of hexane. Five ml of distilled water was added to it. The funnel was shaken vigorously and the layers were allowed to separate. The aqueous methanolic layer was drained off and discarded. The hexane layer was dried by treating with anhydrous sodium sulphate, filtered into a quick fit flask, concentrated to a small volume by using vacuum evaporator and transferred to a vial. Finally the hexane containing mixture of methyl ester of acids were concentrated to a few drops by blowing nitrogen gas and stored in a refrigerator before analysis by GLC (AOAC, 1984).

4.2.3.3. Determination of fatty acid composition of the fatty oil by gas chromatography

Methyl ester of fatty acids was diluted to 7% with n-hexane solvent. An inert carrier gas (i.e. nitrogen) was introduced, from a large gas cylinder through the port, the column and the detector. The flow rate of the carrier gas was adjusted to insure reproducible retention times and to minimize detector drift. The sample was injected with the help of a micro syringe through a heated injection part. The injected sample was vaporized and carried into the column. The long tube of the column was tightly packed with solid particles. The solid support was uniformly covered with a thin film of a high boiling liquid (the stationary phase). The sample components were partitioned between the mobile phase and stationary liquid phase and it was separated into its individual components.

The carrier gas and the sample components were then emerged from the column and passed through the detector. The amount of each component was measured on the basis of its concentration by this device and generated a signal which was registered electrically. This signal passed to a recorder. The methyl esters of the standard reference fatty acids were analyzed by TLC using column packed with silar 10°C. The reference sample and joan fatty oil sample chromatogram are shown in figure: 2 and figure: 3 respectively (Richard J. Block; Emmett L. Durrum; Gunter Zweig: *Paper Chromatography and Paper Electrophoresis*, 2nd Ed. Revised and Enlarged).

4.2.3.4. Identification

The methyl ester of fatty acids of Joan seed were identified by comparing the retention time of authentic samples viz resin acid, palmitic acid, petroselenic acid, oleic acid, linolic acid. Furthermore the peaks on the chart were identified with the help of respective chain lengths of different fatty acids. The percentage composition was expressed as weight percent. The fatty acid composition is computed by dividing the corrected peak areas of each peak by sum of the corrected peak areas and multiplying with 100.

Results and Discussion

The oil was extracted from the joan seed by solvent extraction method using n-hexane as an extracting solvent in a Soxhlet apparatus. The yield of the oil was found to be 1.43%. The physical characteristics such as color, appearance, taste and solubility of the joan fatty oil were determined by conventional methods. The purified fatty oil was dark green in color. It had burning taste. It appeared as a homogeneous and liquid at room temperature (25°C). The joan fatty oil is completely soluble in n- hexane, chloroform, pet. ether and benzene (Table 1).

The chemical tests were performed to evaluate the nature of the Joan fatty oil. The values were expressed in terms of some customary constants. Acid value of this Joan fatty oil was 6.69. This value indicates the proportion of free fatty acid in this particular oil. Saponification value of this Joan fatty oil was found to be 184.33. The iodine value of this oil was found to be 79.39. The unsaponified matter was found to be 9.11% in this fatty oil sample. The peroxide value of joan fatty oil was found to be 457.11%.

Some characteristics of Joan can be compared with some edible and non-edible fats and oils available in Bangladesh. In the comparison some parameters like iodine value, saponification value and acid value were used. The iodine value showed the presence of moderate unsaturation which was in agreement with physical state of the oil that means the oil is non-drying in nature. It is known that the oils having iodine value less than 95, generally known as non-drying oil. The other hand Joan seed fatty oil has lower acid value. Lower acid value indicates its ability to be considered as edible oil. The fatty acids composition of joan seed fatty oil can also be compared with some edible and non-edible fats or oils. The fatty acid composition of joan seed oil was determined by Gas Liquid Chromatography (GLC). GLC analysis of the fatty oil revealed that the chromatogram has three peaks assigned to the above acids. From the chromatogram, retention time and peak area of Petroselinic acid, Oleic acid, Linoleic acids were studied and the relative percentage of fatty acid in joan fatty oil calculated. The mole percentages of the individual acids were found Petroselinic acid (89.35%), Oleic acid (5.86%), Linoleic acid (4.79%). Menon *et al*. was determined the fatty acid composition of joan fatty oil by ester fractionation and found Palmitic acid (2.6%), Petroselinic acid (41.8%), Oleic acid (34.8%) and Lenoleic acid (20.7%) by weight (Menon *et al.*, 1953). The mole percentages of joan fatty acid of Menon *et al*. findings differs from our findings may be the area, nature of joan seed.

Table: 1. Physical characteristic of joan seed fatty oil

Sl. No.	Characteristics	Result
O1	Percentage of fatty oil	1.43%
02	Taste	Burning taste
03	Odor	Spicy
04	Color	Dark green
05	Solubility in n-hexane	Soluble
06	Solubility in chloroform	Soluble
07	Solubility in pet. ether	Soluble
08	Solubility in benzene	Soluble

Table: 2. Chemical characteristic of joan seed fatty oil

Sl. No.	Characteristics	Result (%)
01	Acid value	6.69
02	Iodine value	79.39
03	Peroxide value	457.11
04	Saponification value	184.32
05	Unsaponified matter	9.11
06	Free fatty acid value as petroselinic acid	48.1

Table: 3. GLC experimental results of fatty acid composition: (Joan Seed)

Sl. No.	Retention time	Peak area	Relative percentage	Remarks
01	3.29	2484	5.86	Oleic acid
02	16.28	37895	89.35	Petroselinic acid
03	22.48	2031	4.79	Linoleic acid

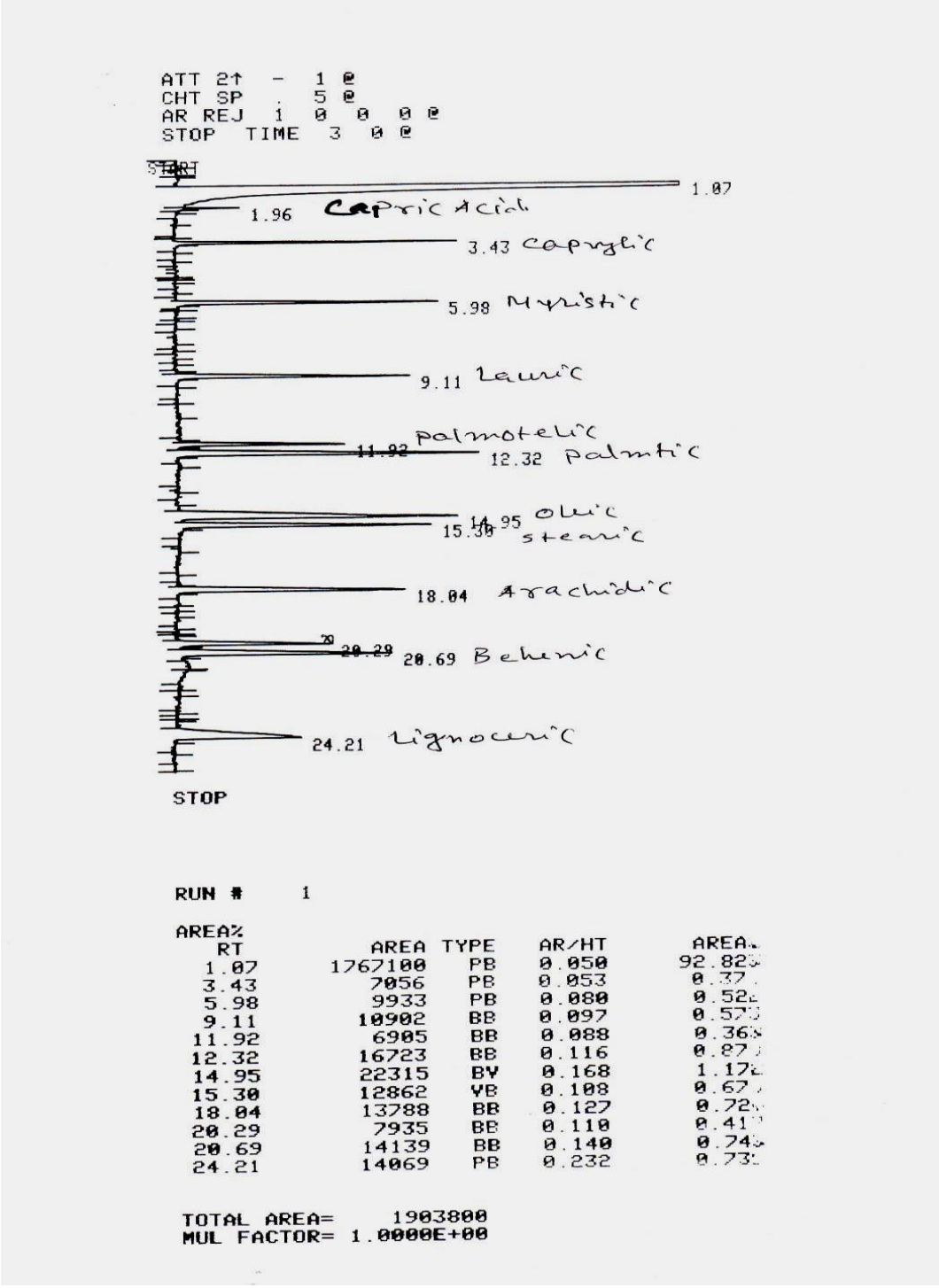
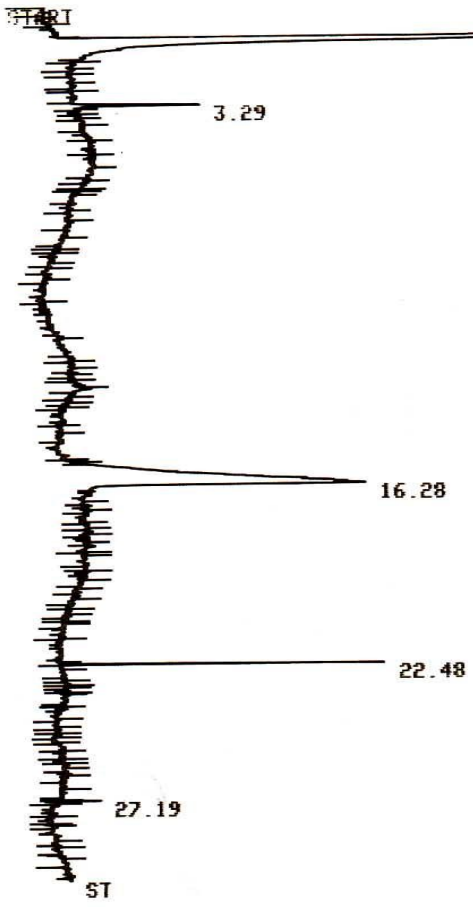


Fig: 1. GLC Chromatogram of reference sample.



RUN # 3

AREA%

RT	AREA	TYPE	AR/HT	AREA%
1.06	401130	BB	0.053	90.43
3.29	2484	BB	0.051	0.569
16.28	37895	PV	0.355	8.54
22.48	2031	D BB	0.016	0.45

TOTAL AREA= 443540
MUL FACTOR= 1.0000E+00

Fig: 2. GLC Chromatogram of the fatty oil of *joan seed*.

Conclusion

The present work therefore, aims to complete chemical investigation of joan seed with particular emphasis on determination of fatty oil. The present investigation has the following steps:

a. Extraction of fatty oil from residue after steam distillation.

b. Characteristic of physical and chemical properties of fatty oil.

c. Investigation of chemical constituents of joan fatty oil.

In the light of above findings obtained from the investigation it is revealed that the joan seed which is now being in cultivated in country, owing to its fatty oil and other valuable nutrients could be used for large scale production of these products. After meeting the internal demand of these products, the remaining amount can earn a considerable amount of foreign exchange through export.

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