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IN VITRO ANTIOXIDANT AND PHYTOCHEMICAL SCREENING OF METHANOLIC EXTRACT OF AGARICUS BISPOROUS (BUTTON MUSHROOM)

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ABSTRACT

Objective: The present study was carried out to evaluate the qualitative and In vitro antioxidant activities of methanolic extract of Agaricus bisporous (button mushroom) Materials And Methods: Agaricus bisporous was extracted with three solvents like methanol,ethanol and chloroform. Three extracts of Agaricus bisporous were tested for different phytoconstituents and the In vitro antioxidant activity of the methanolic extract was studied by using 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, Reducing power activity, hydrogen peroxide scavenging activity, Superoxide scavenging activity and nitric oxide scavenging activity. Results: The yield of phytochemicals is in the order of methanol extract > ethanol extract >chloroform extract were obtained. Finally, methanolic extract was selected for further investigation. Highest free radical scavenging activity of DPPH assay, reducing power scavenging activity, superoxide radical scavenging activity, hydrogen peroxide scavenging activity and nitric oxide radical scavenging activity, was found to be 84.41±5.90 µg/ml, 0.78±0.05µg/ml, 82.30±5.76µg/ml, 72.50±5.07µg/ml, 84.23±5.89µg/ml, at highest concentration of methanol extract of 80 µg/ml respectively in this study. Conclusion: The present study concluded the in vitro antioxidant activity of Agaricus bisporous which might be due to the presence of the phenolic and flavonoid compounds.

KEYWORDS: Agaricus bisporous, Mushroom, Phytochemical, In vitro antioxidant, scavenging activity.

1. INTRODUCTION

Oxidation is essential to many living organisms for production of energy for biological processes. The reactive oxygen species (ROS) and reactive nitrogen species (RNS) are produced due to the oxidation of cell and leads to cell death and tissue damage. Free radicals are responsible for aging and causing various human diseases such as atherosclerosis, diabetes, cancer, hypertension, alzheimer's disease, parkinsonism and cirrhosis.[1]

Antioxidants dealing with an important role in the prevention and treatment of a variety of diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves.[2]

The antioxidants in the human diet are of great interest as possible protective agents to help human body to reduce oxidative damage. To prevent lipid oxidation food industries have long using synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) as preservatives in food products which are restricted due to their carcinogenic effects and has led to increased interest in antioxidant substances from natural resources.[3]

Mushrooms are widely consumed and have been valued as an edible and medical resource. Many studies have found that some species of mushrooms are having therapeutic properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno stimulatory effects (Barros et al., 2007; Oyetayo, 2009.[4]

They accumulate a variety of secondary metabolites, including phenolic compounds, polyketides, terpenes and steroids (Turkoglu et al., 2007.[5]

Agaricus bisporous (A. bisporous) is commonly known as button or table mushroom. The button mushroom, Agaricus bisporus, is one of the world's most economically important vegetable crops.[6] Mushroom growers inoculate compost beds with spawn, which is grain colonized with a specific commercial strain. Mushroom spawn is generally rye grain or millet that has been heat sterilized and inoculated with mycelium from an axenic culture of a particular commercial strain.[7]

Strain stability is of great importance to both spawn producers and mushroom growers. Mushroom strains are usually propagated vegetatively on nutritionally rich substrates, and abnormal growth and poor yield have been recorded occasionally of particular concern to the spawn industry is the phenomenon of sectoring of the mycelium colonizing the grain. When Agaricus bisporus produces sectors on grain or on compost, the sectors usually appear as fluffy patches of rapidly growing mycelium or as thick, rubbery areas of matted growth.[8]

These distinctive morphologies can be observed on agar plates, grain, compost, and the casing material during fruiting. When sectored mycelia complete the growth cycle, it has been observed that they give poor yields and produce poor-quality mushrooms Severe sectors may not produce any mushrooms at all. Although the appearance of sectors has been observed for many years in the mushroom industry, the biological mechanisms that are associated with this growth form are not well understood. The fact that sectoring may appear during the production of spawn or during the colonization of compost on mushroom farms suggests that commercial mushroom strains may possess an inherent ability to spontaneously produce sectors.Thus,this study was to determine the phytochemical compounds and in vitro antioxidant activity, in methanolic extract of Agaricus bisporous (white button) mushroom.[9]

2. MATERIALS AND METHODS

2.1. Chemicals

All chemicals were procured from Ponmani Scientific Chemicals Suppliers, Tiruchirappalli, Tamilnadu, India and were of analytical grade.

2.2. Sample

In this study, white button mushroom (Agaricus bisporus) was purchased from a local market in Tiruchirappalli, Tamilnadu, India.

2.3. Samples preparation

Every sample comprises complete mushroom fruiting bodies (cap, gills, tubes and stipe) of different sizes. The fruiting bodies of mushroom were cleaned and washed to remove any residual compost by using distilled water. These samples were cut into pieces and stored at -80°C. Then, they were lyophilized using freeze-dryer to remove the moisture content. After freeze-drying, the dried samples were ground into fine powder by using micro-grinder and kept in plastic bag prior to analysis. Samples were ground and kept in plastic bag. The samples were kept in airtight container at -20°C prior to analysis.

2.4. Sample extraction

Extraction was performed according to the method of Yang et al. (2002) with some modifications. 250 grams of powdered sample was extracted with 500 ml of methanol,ethanol and chloroform.The mixtures were placed in a conical flask (wrapped with an aluminium foil) and agitated at 200 rpm with orbital shaker for 1 hour at 25°C. The extract was then separated from the residue by filtration through what man No. 1 filter paper.

The remaining residue was extracted twice, and then the two extracts were combined. The residual solvent of methanol extracts was removed under reduced pressure at 40°C using a rotary evaporator to dryness. For aqueous extraction, the aqueous extract was lyophilized using a freeze dryer. Both of the dried extract was used directly for all analysis.[10]

2.5. EVALUATION OF IN VITRO ANTIOXIDANT ACTIVITY

Methanol, ethanol and chloroform extract of Agaricus bisporous were tested for in vitro antioxidant activity using standard procedures.

a. Determination of DPPH scavenging activity

Different aliquots of 0.2 to 1 ml of sample extract solutions were taken in different test tubes. To these entire tubes methanol was added and made up to 1ml. To this 4m1 of methanolic DPPH was added and shaken well.

The mixture was allowed to stand at room temperature for 20 min. The control contains only methanol and DPPH. The readings were noted at 517 nm against methanolic blank.

The change in absorbance of the samples was measured. Free radical scavenging activity was expressed as the inhibition percentage calculated by using the formula Percentage of antioxidant activity = [A - B/A] x 100.Where, ‘A’ is absorbance of control ‘B’ is absorbance of sample.[11]

b. Determination of Reducing power activity

1 ml of Agaricus bisporous extract was mixed with phosphate buffer (2.5 ml 0.2 M, pH 6.6) and potassium ferric cyanide (2.5 ml). The mixture was incubated at 50˚c for 20 minutes. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min.

The upper layer of solution (2.5ml) was mixed with distilled water (2.5ml) and Ferric cloride (0.5ml, 0.1%) and absorbance measured at 700 nm. Increased absorbance of the reaction mixture indicates stronger reducing power. The activity was compared with ascorbic acid standard.

Percentage inhibition scavenging activity was calculated by (A control x A test/A control) x100.Where A control is the absorbance of the control. A test is the absorbance in the presence of the sample.[12]

c. Determination of superoxide scavenging activity

Superoxide anions were generated in samples that contained in 3.0ml, 0.02ml of the Agaricus bisporous (20mg), 0.2ml of EDTA, 0.1ml of NBT, 0.05ml of riboflavin and 2.64ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the plant extracts.

All the tube where the initial optical density was measured at 560 nm in a spectro photometer. The tubes were illuminated using a fluorescent lamp for 30 minutes. The absorbance was measured again at 560 nm. The difference in absorbance before and after illumination was indicative of superoxide anion scavenging activity.[13]

d. Determination of hydrogen peroxide scavenging activity

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the extracts in distilled water was added to 0.6 ml of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as standard. Percentage inhibition of H_2O_2 radical scavenging activity was calculated by (A control x A test/ A control) x 100.Where A control is the absorbance of the control. A test is the absorbance in the presence of the sample.[14]

e. Determination of Nitric oxide scavenging activity

The reaction was initiated by adding 2.0ml of sodium nitroprusside, 0.5ml of PBS, 0.5ml of Agaricus bisporous (50mg) and incubated at 25°C for 30 minutes. Griess reagent (0.5ml) was added and incubated for another 30 minutes. Control tubes were prepared without the extracts. The absorbance was read at 546 nm against the reagent blank, in a spectrophotometer.[15]

Statistical analysis

The determinations were conducted in triplicate and results were expressed as Mean ± SD. Statistical analysis were done by analysis of variance using ANOVA test followed by student' t- test with P <0.05 considered as significance.

3. RESULTS

The qualitative phytochemical screening in methanolic, ethanolic and chloroform extract of Agaricus bisporous was given presented in Table.1

Table 1: Preliminary phytochemical analysis of Agaricus bisporous.

S. No	Name of the Test	Phytochemical Constituents	Chloroform	Ethanol	Methanol
1	Alkaloid	Mayer's test Dragondraff test Wagner Test	+ + +	+ + +	+ + +
2	Carbohydrate	Molish Test Fehling Test Benedict Test	- - -	- - -	+ + +
3	Steroidal Glycosides	Libermann's test Salkowaski test	- -	- -	- -
4	Saponin	H_2So_4 test	-	-	+
5	Tannin	Lead Acetate	-	+	+
6	Flavonoid	Ammonia test	+	-	+
7	Coumarin	Sodium chloride test	-	-	-
8	Phenol	Folinceo test	+	+	+
9	Anthocyanin	H_2So_4 test	-	-	-
10	Terpenoid	Chloroform test	-	-	+
11	Protein	Folinceo test	+	+	+

(+): Present (-): Absent

Different phytochemical like alkaloids, glycosides ,saponins, tannins, terpenoids, reducing sugars, phenolic compounds, flavanoids, protein and carbohydrates were identified and the results were given in Table.1. Our results indicates the presence of phytochemicals in the order of methanol extract >ethanol extract > chloroform extract. The above data showed the more yield of phytochemicals in methanol extract and hence the methanolic extract was selected for the further studies for in vitro antioxidant studies.

3.1. DPPH radical scavenging activity

Table 2 and figure 1 shows the DPPH scavenging effect increased with the increasing concentrations of Agaricus- bisporous powder extract as compared to standard ascorbic acid and highest DPPH scavenging activity of Agaricus bisporous was observed as 84.41%

inhibition at 80 µg/ml concentration which indicates the DPPH scavenging effective of Agaricus bisporous as compared to ascorbic acid.

Table 2- % of DPPH Radical scavenging activity of Agaricus bisporous **extract at different concentrations**

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**	**IC_{50} (µg/ml)**
Mushroom extract	22.21±1.55	48.14±3.36	63.26±4.42	84.41±5.90	45.56
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	35.03

Values were expressed as Mean ± SD for triplicates

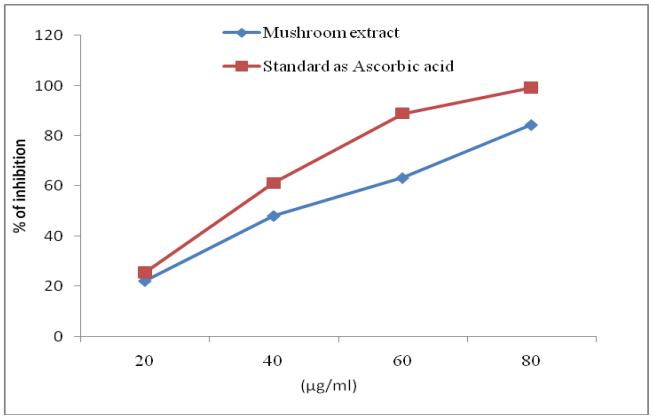

Fig.1 DPPH radical scavenging activity of Agaricus bisporous.

Values were expressed as Mean ± SD for triplicates

3.2. Reducing power activity

Table 3 and figure 2 shows the Reducing power effect increased with the increasing concentrations of Agaricus bisporous powder extract as compared to standard ascorbic acid and highest Reducing power activity of Agaricus bisporous was observed as 0.78 % inhibition at 80 µg /ml concentration which indicates the Reducing power effective of Agaricus bisporous as compared to ascorbic acid.

Table3. % Reducing power assay of Agaricus bisporous **extract at different concentrations**

Parameters	**20 (µg/ml)**	**40 (µg/ml)**	**60 (µg/ml)**	**80 (µg/ml)**
Mushroom extract	0.22±0.01	0.43±0.03	0.66±0.04	0.78±0.05
Standard (Ascorbic acid)	0.41± 0.03	0.71 ± 0.05	0.89± 0.07	0.98 ± 0.08

Values were expressed as Mean ± SD (Optical density) for triplicates

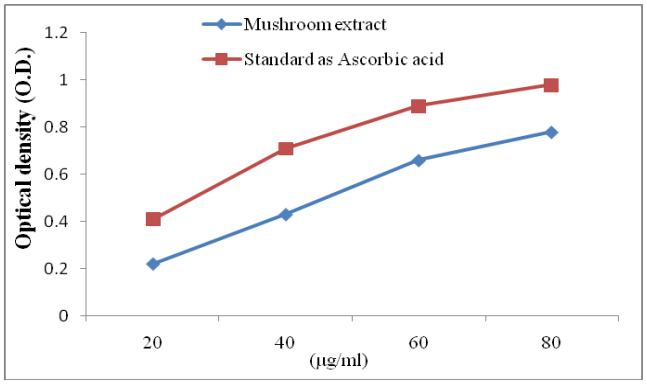

Fig 2. Reducing power assay of Agaricus bisporous

Values were expressed as Mean ± SD for triplicates

3.3. Superoxide scavenging activity

Table 4 and figure 3 showed the superoxide scavenging effect increased with the increasing concentrations of Agaricus bisporous powder extract as compared to standard ascorbic acid and highest superoxide scavenging effect of Agaricus bisporous was observed as 82.30.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of Agaricus bisporous as compared to ascorbic acid.

Table 4- % of Superoxide scavenging activity of Agaricus bisporous extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Mushroom extract	23.07±1.61	41.53±2.90	63.83±4.46	82.30±5.76	47.36
Standard (Ascorbic acid)	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.62

Values were expressed as Mean ± SD for triplicates

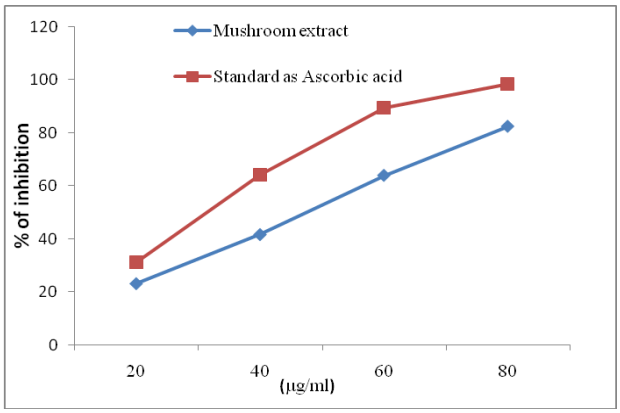

Fig . 3- Super oxide scavenging activity of Agaricus bisporous

Values were expressed as Mean ± SD for triplicates

3.4. Hydrogen peroxide scavenging activity

Table 5 and figure 4 showed the hydrogen peroxide scavenging effect increased with the increasing concentrations of Agaricus bisporous powder extract as compared to standard ascorbic acid and highest hydrogen peroxide scavenging effect of Agaricus bisporous was observed as 72.50.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of Agaricus bisporous as compared to ascorbic acid

Table 5- % of hydrogen peroxide scavenging activity of Agaricus bisporous extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Mushroom extract	20.31±1.31	35.00±2.45	48.12±3.36	72.50±5.07	57.12
Standard (Ascorbic acid)	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	42.41

Values were expressed as Mean ± SD for triplicates

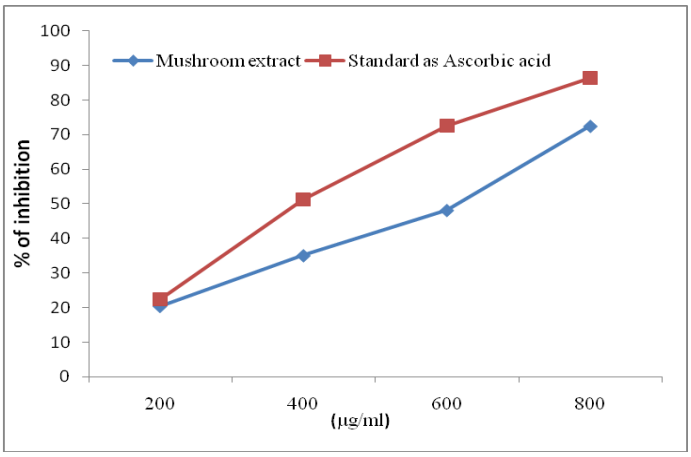

Fig .4.hydrogen peroxide scavenging activity of Agaricus bisporous

Values were expressed as Mean ± SD for triplicate

3.5. Nitric oxide scavenging activity

Table 6 and figure 5 showed the Nitric oxide scavenging effect increased with the increasing concentrations of Agaricus bisporous powder extract as compared to standard ascorbic acid and highest Nitric oxide scavenging effect of Agaricus bisporous was observed as 84.23.% inhibition at 80 µg /ml concentration which indicates the superoxide scavenging effective of Agaricus bisporous.

Table 6- % of Nitric oxide scavenging activity of Agaricus bisporous extract at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Mushroom extract	20.22±1.41	43.16±3.02	68.44±4.79	84.23±5.89	46.88
Standard (Ascorbic acid)	35.23 ± 2.81	65.21 ± 5.28	78.51± 6.28	98.65 ± 7.89	30.65

Values were expressed as Mean ± SD for triplicates

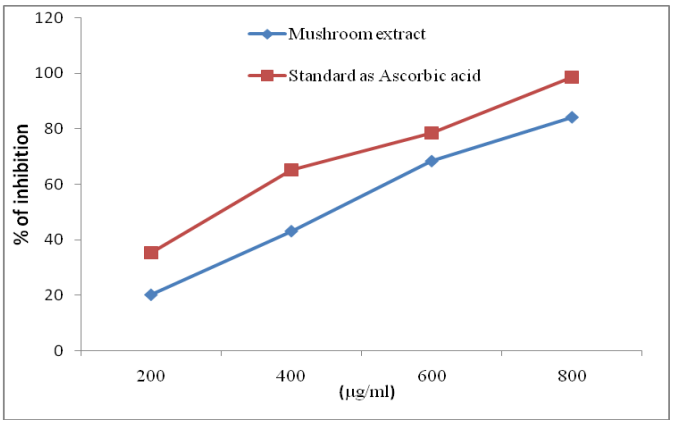

Fig .5.Nitric oxide scavenging activity of Agaricus bisporous

Values were expressed as Mean ± SD for triplicates

DISCUSSION

The presence of secondary metabolites like tannins, saponin, carbohydrates, glycosides, alkaloids, flavonoids, terpenoids, steroids, polyphenols and anthraquinones of Agaricus bisporous suggests that the plant might be of medicinal importance.[16] Due to the presence of flavonoids and phenol suggests that the plant might have an antioxidant, anti-allergic, anti-inflammatory antimicrobial, anticancer activity.[17] The presence of tannins shows that the plant is astringent as documented and suggests that it might have antiviral and antibacterial activities and can and in wound healing and burns.[18] Saponins and glycoside are also very important classes of secondary metabolites as some are cardio active and used in treatment of heart conditions.[19]

The phenolic and flavonoids are widely distributed secondary metabolites in plants having antioxidant activity and have wide range of biological activities as anti-apoptosis, anti-aging, anti-carcinogen, anti inflammation, atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[20] This study also proved that many dietary polyphenolic and

flavanoid constituents derived from Agaricus bisporous are more effective antioxidants than ascorbic acid and thus might contribute significantly to the protective effects in vivo.[21]

The present study was also revealed the in vitro antioxidant properties of Agaricus bisporous which might be due to the presence of phenolic and flavonoid compounds in methanolic extract.[22] DPPH radicals react with suitable reducing agents and then electrons become paired-off and the solution loses colour steriochiometrically with the number of electrons taken up . Such reactivity has been widely used to test the ability of compounds of Agaricus bisporous extracts to act as free radical scavengers .Though the extracts showed good DPPH scavenging activity but it was less effective than standard Ascorbic acid. This activity is due to presence of phenolic and flavonoid components in the plant extracts.[23]

The Reducing antioxidant power assay, a yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. The presence of radicals causes the conversion of the ferric cyanide complex used in this method to the ferrous form. Therefore by measuring the formation of pearls Prussian blue spectroscopically, a higher absorbance indicates a higher reducing power.[24]

Reducing power of activity in the Agaricus bisporous extracts indicated that some components in the extract were electron donors that could react with the free radicals to convert them into more stable products to terminate radical chain reaction.[25]

Superoxide anion radical is generated by four electron reduction of molecular oxygen in to water. This radical also formed in aerobic cells due to electron leakage from the electron transport chain super oxides are generated from molecular oxygen of oxidative enzymes and as well as non-enzymatic reactions such as auto oxidation by catecholamines.[26] The studies also proved on super oxide free radical scavenging activity of methanolic extract of Agaricus bisporous was noticed significant reduction of the super oxide anions.[27]

Hydrogen peroxide is a biologically relevant, non radical oxidizing species may be formed in tissues through oxidative processes. Hydrogen peroxide which in turn generate hydroxyl radicals (•OH) resulting in initiation and propagation of lipid peroxidation. The ability of the extracts to quench (•OH) seems to be directly related to the prevention of the lipid peroxidation.[28] Appears to be moderate scavenger of active reactive oxygen species.

Methanolic extract of Agaricus bisporous showed good hydrogen peroxide scavenging activity but it was less effective than standard ascorbic acid.[29]

Nitric oxide is a potent pleiotropic inhibitor of physiological processes such as smooth muscle relaxation, neuronal signaling inhibition of platelet aggregation and regulation of cell mediated toxicity .It is a diffusible free radical that plays many roles as an effectors molecule in diverse biological systems including neuronal messenger, vasodilatation and antimicrobial and antitumor activities.[30]

Methanolic extracts of Agaricus bisporous showed more nitric oxide scavenging activity but it was less effective than standard ascorbic acid.[31]

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for profit sectors.

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