Available online on 15.08.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Antioxidant Activity and total Phenolic Content of Defatted *Scenedesmus quadricauda* KDPSC2 Biomass Extract by Microbial Fermentation

V. Sarumathi[1], Krishnan Dhandayuthapani[2]* and Mazher Sultana[3]

[1] Research and Development centre, Bharathiar University, Coimbatore, Tamil Nadu, India

[2] Assistant Professor of Plant Biology & Biotechnology, Arignar Anna Govt. Arts College Cheyyar, Tamil Nadu, India.

[3] PG & Research Department of Advanced Zoology and Biotechnology, Presidency College, Chennai- 600 005, Tamil Nadu, India

ABSTRACT

Microalgae biomass are considered as sustainable sources for various bioactive compounds. The objective of this study to evaluate the defatted biomass of green microalga *Scenedesmus quadricauda* KDPSC2 as a source for enhancement of total phenolic content (TPC) and antioxidant activity by microbial fermentation. Ultrasonic assisted hydrolysate was prepared from defatted biomass using ultrasound power density of 0.3 Wcm^{-2} at 10 min. Then the prepared defatted biomass hydrolysate (DBH) was used as soul medium for fermentation by five different microbial strains such as *Bacillus amyloliquefaciens*, *B. stearothermophilus*, *B. subtilis*, *Saccharomyces cerevisiae* NITTS1 and *Cyberlindnera jadinii* MMS7. Among these microbial strains, *C. jadinii* MMS7 was selected for further study because it was enhanced the TPC and antioxidant activity of DBH by fermentation. Besides, the fermentation conditions were optimized by classical method of optimization and found the pH 5.5, temperature 35 ^{o}C, agitation speed 150 rpm and 36 h as optimum physical parameters for enhancing the TPC and antioxidant activity of DBH by fermentation using *C .jadinii* MMS7. At these optimum conditions, 4.67 fold DPPH radical scavenging activity was obtained from DBH after fermentation. Therefore, this study demonstrates that the defatted biomass of *S. quadricauda* KDPSC2 could be a useful source for production of TPC by *C. jadinii* MMS7 fermentation.

Keywords: *Cyberlindnera jadinii*, Defatted biomass, Fermentation, Hydrolysate, *Scenedesmus quadricauda*, Total phenolic content.

Article Info: Received 06 June 2019; Review Completed 22 July 2019; Accepted 29 July 2019; Available online 15 August 2019

Cite this article as:

Sarumathi V, Dhandayuthapani K, Sultana Mazher, Antioxidant Activity and total Phenolic Content of Defatted *Scenedesmus quadricauda* KDPSC2 Biomass Extract by Microbial Fermentation , Journal of Drug Delivery and Therapeutics. 2019; 9(4-s):258-263 **http://dx.doi.org/10.22270/jddt.v9i4-s.3314**

***Address for Correspondence:**

Krishnan Dhandayuthapani, Assistant Professor of Pl. Bio. & Biotechnology, Arignar Anna Govt. Arts College Cheyyar, Tamil Nadu, India

INTRODUCTION

Nowadays, there is a growing interest day by day to explore the substances having antioxidant properties, which are used as food supplements or as specific preventative pharmaceuticals for humans and animals[1]. Because, the antioxidants which are considered as an important protection agents to reduce oxidative damage in human body, when the internal enzymatic mechanisms fail or are inadequately efficient[2]. However, currently the synthetic antioxidants includes tertiary butylhydroquinone (TBQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl-gallate have been used in various food industries to improve the shelf life of foods which contained polyunsaturated fats. Nevertheless, the incorporation of synthetic antioxidants in foods has been questioned due to their potential health risks and toxicity[3]. To overcome this problem various plants are using as a source for natural antioxidants preparation, since the plant kingdom is a good source to produce a wide range of natural antioxidants. Exogenous antioxidants are mainly derived from photosynthetic organisms and belong to different families such as polyphenols (phenolic acids, flavonoids, anthocyanins, lignans, and stilbenes), carotenoids (xanthophylls and carotenes), sterols, or vitamins (vitamins B, D, E, and C)[4].

However, still there is not enough knowledge and data about the practical usefulness of various plants to meet out the requirement due to rapid population development. Investigating new natural antioxidants for nutraceuticals and pharmaceuticals industries is a relevant key-research topic[5]; microalgae are highly promising in this context[6,7]. Microalgae are characterized by a high biodiversity and

richness in terms of adaptive traits allowing them to colonize all kind of aquatic ecosystems. The metabolic diversity of microalgae, stemming from the adaptive flexibility of the microalgal world, makes them promising candidates to be exploited in biotechnological applications[8]. The advantages of microalgae compared to higher plants or fruits the actual main source of antioxidants for human-derive from the combination of being photosynthetic, mainly unicellular, displaying high growth rate, and occupying reduced space for their large cultivation[7].

The antioxidant power of microalgae is comparable, and even higher than, the antioxidative activity of higher plants or fruits. Interestingly, the antioxidant potential of some classes of microalgae such as Chlorophyta and Eustigmatophyceae were recorded as highest values ranged from 214 to 258 Trolox equivalents µmol g^{-1}DM than the antioxidant activity of 224 Trolox equivalents µmol g^{-1} DM by *Rubus* sp. (raspberry) fruits[9]. These results point to the reason that there is such great interest in the highly promising microalgae as antioxidant providers for nutraceuticals and human wellness, and invoke the necessity of further exploring this great potential. The relevant antioxidant activity is probably related to the high content and diversity of antioxidant molecules in microalgae, which are a source of a wide range of antioxidant molecules[10, 11, 12, 13].

Recently interest has been increased considerably in producing antioxidants from various natural sources by fermentation for use in foods or medicinal products to replace chemically extracted natural antioxidants, which are being restricted due to their cost. Fermentation is one of the oldest and most effective strategies of food production and preservation. It provides a natural way to reduce the volume, to destroy undesirable components, to enhance the nutritive value of foods through the biosynthesis of vitamins, essential amino acids and appearance of the food, to reduce the energy required for cooking, and to make safer products[14]. Furthermore, fermentation improves micronutrient bioavailability and aids in the degradation of anti-nutritional factors[15].

Microalgal biorefinery approach offers a dual benefit in production of biofuel and animal feed from microalgae biomass and which can prevent the diversion of major food crops using for production of biofuel and animal feed[16]. According to Sander and Murthy[17] for every 24 kg microalgal biodiesel produced, 34 kg of co-products are generated such as defatted biomass, glycerol and unsaponifiable lipids. Among these co-products, the defatted algal biomass (DAB) using for production of biomethane[18], bioethanol[19], biohydrogen and short chain carboxylic acids[20] and animal feed supplement[21]. However, to the best of our knowledge, there is no publication on the antioxidant activities on ultrasonic pretreated defatted *S. quadricauda* KDPSC2 biomass extract by microbial fermentation. Hence, the major objectives of this study are to (i) prepare ultrasonic assisted hydrolysate from defatted biomass, (ii) screen the microbial strain for fermentation of DBH, (iii) optimize the DBH fermentation conditions using selected strain for enhancement of polyphenol content, (iv) to perform the 2,2,-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay.

MATERIALS AND METHODS

Materials

2,2,-Diphenyl-1-picrylhydrazyl (DPPH), nutrient agar, yeast peptone dextrose (YPD) agar and other chemicals with highest purity or analytical grade used in this study were purchased from Himedia Chemicals (Mumbai, India), Sigmae-Aldrich (Bommasandra, India) and Merck Chemicals Ltd.,(Mumbai, India).

Defatted algal biomass preparation

Green microalga *Scenedesmus quadricauda* KDPSC2 (GenBank accession number: H238504) was isolated from waterfront of Mamandur Lake (12°45'14" °N and 79°39'24" °E) Cheyyar Taluk's, Tiruvannamalai District, Tamil Nadu, India and used in this study to prepare the defatted algal biomass. *S. quadricauda* KDPSC2 was cultivated in leather industry effluent for 10 days at 25±1 °C with illuminated using fluorescent light (33 µmol photons m^{-2} s^{-1}) with a 12:12 h light : dark cycle. On the 11th day, biomass was harvested by centrifugation at 14,000 rpm for 15 min using a centrifuge (RM-12C BL centrifuge, REMI Laboratory Instruments, India). Subsequently, the microalga biomass was dried using a hot air oven at 60 °C until attaining zero weight loss. The lipid in biomass was extracted using a standard method of Bligh and Dyer[22]. Then the lipid removed (=defatted) biomass was air-dried at room temperature and used it for further study.

Hydrolysate preparation from defatted biomass

Ultrasound assisted hydrolysate preparation from defatted biomass was carried out using an ultrasonic bath (Lark Innovative Fine Teknowledge, Chennai, India). The ultrasonic pretreatment was carried out in 250 mL stainless steel beaker containing of 20 g defatted biomass in 100 mL distilled water at ultrasound power density of 0.3 Wcm^{-2} for 10 min according to the literature[23]. The bath has a fixed frequency of 25 kHz. During sonication, the sample was stirred gently and the temperature was maintained between 25 to 26 °C. The prepared DBH was used for microbial fermentation.

Microbial strain and Inoculum preparation

Five different microorganisms such as *Bacillus amyloliquefaciens* (GenBank Accession Number: KT276356), *B. stearothermophilus* (GenBank Accession Number: KT282130), *B. subtilis* (GenBank Accession Number: KT282131), *Saccharomyces cerevisiae* NITTS1 (GenBank Accession Number: MG255132.1) and *Cyberlindnera jadinii* MMS7 (GenBank Accession Number: MK942589) were used in this study for fermentation. The bacterial and yeast strains were stored at 4 °C in a nutrient agar slant and yeast peptone dextrose agar slant (Peptone 20 gL^{-1}, yeast extract 10 g L^{-1}, dextrose 20 gL^{-1}, Agar 20gL^{-1}) respectively. All the microorganisms were revived every month.

Inoculum was prepared by transferring a loop full of bacterial and yeast strains into 50 mL of NB broth and YPD broth respectively, at aseptic condition. Then the inoculated broth was maintained under aerobic conditions at 30 °C with agitation speed at 120 rpm in an orbital shaking incubator (REMI laboratory Instruments, India) for 48 h. The newly prepared inoculum was used as seed culture for fermentation process.

Microbial fermentation of DBH

The ultrasonic assisted prepared DBH was used as sole medium for microbial fermentation. Batch fermentation was carried out in 250 mL Erlenmeyer flask contained 100 mL of DBH. Prior to microbial inoculation, the pH of DBH was adjusted to 7.0 and 5.5 for bacterial strains and yeast strains respectively, using 1N NaOH or HCl. Then the media were autoclaved at 121 °C for 15 min with 15 psi and cooled at

room temperature. Finally, the medium was inoculated with 10 % v/v fresh inoculum of each microbial strains individually, and incubated in an orbital shaker incubator at 28±2 °C for 72 h under constant shaking at 120 rpm. Every 12 h once the sample was withdrawn and estimated the biomass and the TPC. The untreated defatted biomass water extract was used as a control. Based on the maximum TPC and antioxidant activity of fermented DBH the microbial strain was selected and used for further study.

Optimization of fermentation of DBH by selected microbial strain

In order to find the optimum fermentation conditions for enhancing the antioxidant activity of DBH by fermentation using selected strain was studied. In this study, the following four fermentation process conditions such as pH (pH 4 to 6), temperature (25 to 40 ^{o}C), agitation speed (100 to 300 rpm) and fermentation time (0 to 72 h) were optimized by classical method of optimization (One parameter at-a-time). All the experiments were carried out in triplicate to check the reproducibility.

Biomass Estimation

The biomass was estimated by gravimetrical method. 10 mL of samples was added to pre-dried (105 °C in oven, overnight) and pre-weighed conical bottom glass centrifuge tube and then centrifuged at 6000 rpm for 10 min. The pellet was washed twice with deionized water and repeated the centrifugation process. Then washed biomass was dried at 105 °C until getting the constant weight. After drying, allowed to cool in a desiccator and the final weight was recorded using an analytical balance (S234, Denver Instrument, Bohemia, NY). The loss of weight was calculated as grams of dry weight per litre. The biomass estimation was performed in triplicate and all values are represented as mean ± SD of three replications.

Estimation of TPC

The TPC of fermented hydrolysate was estimated by Folin-Ciocalteu method[24]. The samples were diluted to match the measurable range of the spectrophotometer. A 200 µL sample was mixed with 1 mL of 1:10 diluted Folin-Ciocalteu reagent. After 4 min, 800 µL of saturated Na_2CO_3 (75 gL^{-1})) was added to this mixture and incubated at room temperature for 2 h. Then the sample was centrifuged at 6000 rpm for 10 min and the collected supernatant was measured at 765 nm using UV-Visible spectrophotometer (PC-Based Double Beam UV-VIS Spectrophotometer 2206, Systronics India Ltd, Gujarat). Gallic acid range from 0-500 mgL^{-1} was used for standard calibration curve. The optical density (OD) value of the sample was interpolated in the standard graph and calculated the TPC of sample. The results were expressed as gallic acid equivalent (GAE) per gram dry weight of defatted biomass of *S. quadricauda* KDPSC2 and calculated as mean value ±SD (n=3)

In Vitro antioxidant activity

2,2,-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was performed as described by Qureshi et al[25]. 1 mL of fermented hydrolysate was mixed with 1 mL of ethanolic solution of DPPH (0.2 mM). To this mixer, 1 mL of ascorbic acid (200 μgmL^{-1}) in ethanol was added. After mixing vigorously for 30 s, the solution was incubated at 37 °C for 25 min in dark. The DPPH radical scavenging activity of the solution was measured at 517 nm using UV-Visible spectrophotometer (PC-Based Double Beam UV-VIS Spectrophotometer 2206, Systronics India Ltd, Gujarat) using mixture of 1 mL ethanol and 1 mL of DPPH as blank. The synthetic antioxidant Butylated Hydroxy Toluene (BHT) used as positive control. Assay was performed in triplicates and the mean value was used to calculate the DPPH radical scavenging activity using the following equation,

Radical scavenging activity = [(Abs control - Abs samples)/Abs control] X 100

Statistical analysis

The results of all the experiments were presented as the mean ± standard deviation (SD) values of three independent replicates. The one-way analysis of variance (ANOVA) also done for the obtained data using MINITAB 12 software at the significant level of $p \leq 0.05$.

RESULTS AND DISCUSSION

TPC and antioxidant activity of DBH extract by microbial fermentation

Microalgae biomass are considered as sustainable sources of bioactive compounds because they have wide range of applications from animal feed to human health products[26]. Among the various bioactive compounds, the phenolics are most important one to prevent oxidative stress-related diseases like cancer and neurological disorders in human body[27]. Currently, there is an increasing interest in exploring the phenolics compound in microalgae since very few reports on the microalgae phenolics compound[28]. In the present study, ultrasonic assisted DBH was prepared from defatted biomass of *S. quadricauda* KDPSC2 and it was used as a source for extracting the phenolics compounds by fermentation using five different microbial strains. As shown in the Figure 1, the highest TPC was obtained from DBH after fermentation by five different microbial strains than before fermentation. All the five microbial strains were showed positive results of enhancement of TPC in DBH. However, among the five different microbial strains, the maximum TPC of 6.31±0.07 mg GAE g^{-1} DW was obtained from DBH by *C. jadinii* MMS7 fermentation. This may be a change in the properties of ultrasonic assisted DBH could occur during the fermentation by *C. jadinii* MMS7. But, Bulut et al[29] reported that the highest TPC of 5.40±0.28 mg GAE g^{-1} WD was obtained from *Scenedesmus* sp. ME02 by ethanol/water extraction. In this study not used any solvent to extract the phenolics compounds from DAB. We did it only by microbial fermentation.

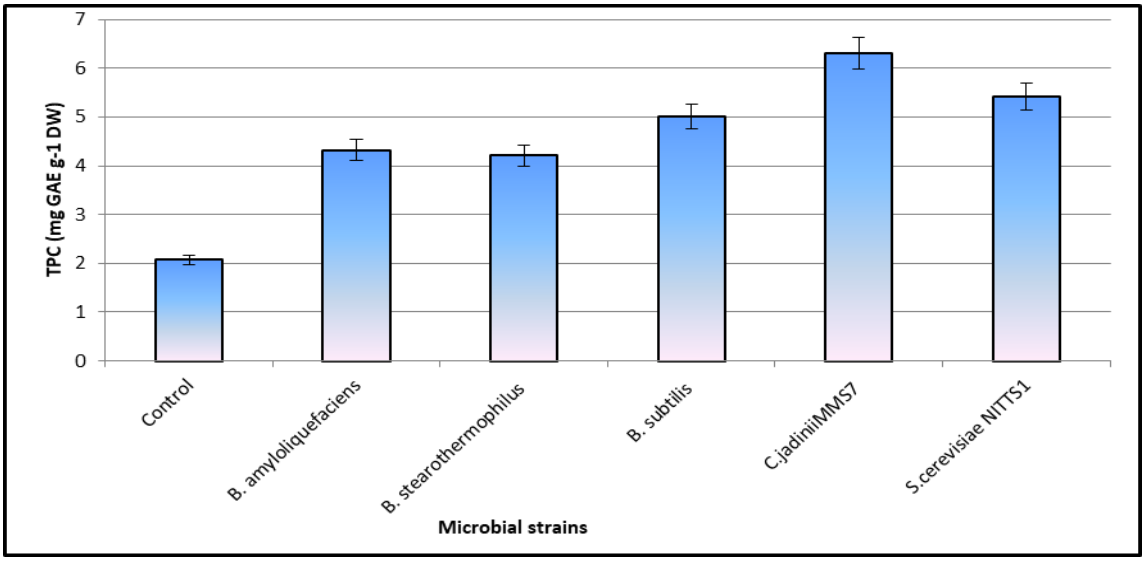

Figure 1: Concentration of TPC in DBH after microbial fermentation

In order to estimate the antioxidant capacity of fermented DBH, the DPPH assay was used. This assay measures the reducing capacity of tested antioxidants toward the DPPH radical either by direct reduction via electron transfer or by radical quenching by H atom transfer[30]. In this study, we recorded a wide range of 49.12 ± 0.11% to 62.31 ± 0.12% DPPH radical scavenging activity in fermented DBH. As shown in the Figure 2, maximum DPPH radical scavenging activity of 62.31 ±0.12% was recorded in DBH after fermentation by *C. jadinii* MMS7. Hence, this strain was selected and used for further study.

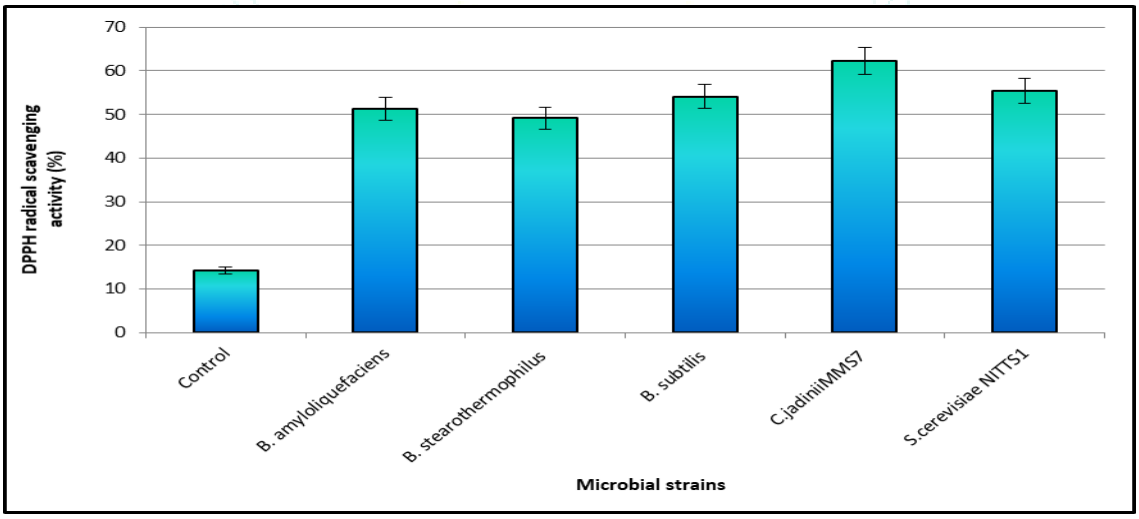

Figure 2: Antioxidant capacity of DBH in different microbial fermentation

Effect of pH on enhancement of antioxidant activity of DBH

In order to find the optimum pH for enhancement of DPPH radical scavenging activity of DBH by fermentation using *C. jadinii* MMS7 was studied. The pH range from 4 to 6 with an increment of pH 0.5 was used for this study. The pH of DBH was maintained using 1N NaOH/HCl. As shown in Figure 3, the highest DPPH radical scavenging activity of 64.21±0.13% was obtained from DBH by fermentation at pH 5.5 because this pH was well favoured the growth of *C. jadinii* MMS7. The present study agreed with the work of Ezekiel and Aworh[31]. Based on the TPC of DBH, pH 5.5 was considered as an optimum pH for further optimization study.

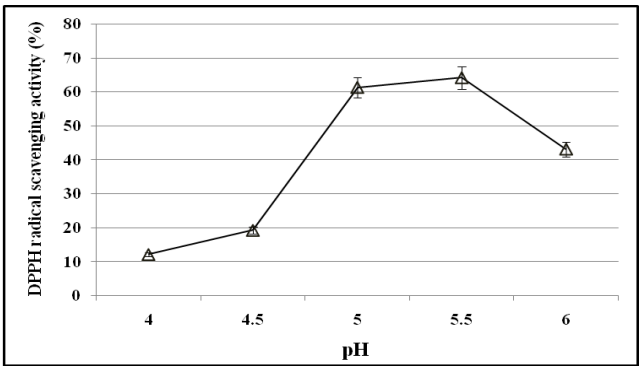

Figure 3: Effect of pH on enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation

Effect of temperature on enhancement of antioxidant activity of DBH

The physical factor such as temperature directly can influence on product yield and productivity in the microbial fermentation[32]. Effect of the temperature on enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation was studied with various temperature ranges from 25 °C to 40 °C with an increment of 5 °C. As shown in Figure 4, the maximum DPPH radical scavenging activity of 66.24 ± 0.12% was enhanced in DBH by fermentation using *C. jadinii* MMS7 at 35 °C. Further increase in the temperature there was a reduction in antioxidant activity because it was unfavourable for the growth of *C. jadinii* MMS7. Hence, the fermentation gets affected. When increasing the temperature from 25 °C to 35 °C an increased in the biomass of *C. jadinii* MMS7 from 13.11±0.04 g dwL^{-1} to 18.13±0.07 g dwL^{-1}. Whereas low biomass of 5.03±0.05 g dwL^{-1} was obtained at 40 °C. These results suggesting that the temperature above 35°C does not support the growth of *C. jadinii* MMS7 in the fermentation broth DBH. Therefore, the temperature 35 °C was found as an optimum temperature and it was used for further optimization study. Similarly, Zhao et al[33] found that the temperature 35 °C as an optimum temperature for single cell protein production from *C. utilis* by fermentation.

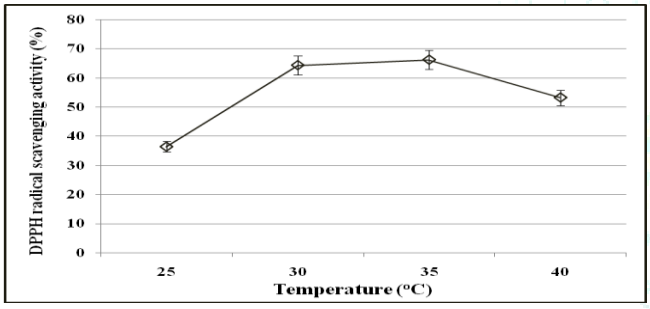

Figure 4: Effect of temperature on enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation

Effect of agitation speed on enhancement of antioxidant activity of DBH

During the fermentation, the agitation will provides an adequate mixing of fermentation medium for facilitating the assimilation of nutrients by yeasts cells[34]. However, excess the agitation speed creates shear forces, which will affect the product formation during microbial fermentation due to morphological changes and damaging the cell structure of microorganisms[35]. In the present study, in order to find the optimum agitation speed for enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation was studied with different agitation speed range from 100 to 300 rpm with an increment of 50 rpm. The maximum DPPH radical scavenging activity of 68.22±0.11% and biomass of 21.45±0.06 g dwL^{-1} were observed at agitation speed of 150 rpm. Further increase in the agitation speed there was a significant reduction in the antioxidant activity (Figure 5).

Effect of fermentation time on enhancement of antioxidant activity of DBH

The fermentation of DBH was carried at optimum temperature 35 °C for 72 h under constant shaking at optimum agitation speed of 150 rpm. Prior to start the fermentation, DBH pH was adjusted to optimum pH 5.5 using 1N NaOH or HCl and then autoclaved at 121 °C for 15 min with 15 psi. Every 12 h once the fermented extract was used to measure the DPPH radical scavenging activity. As shown in Figure 6, the highest DPPH radical scavenging activity of 70.12±0.08% was observed at fermentation time 36 h. Further increase in the time the DPPH radical scavenging activity was gradually decreased. The DPPH radical scavenging activity of fermented extract was in accordance with their amount of TPC. Polyphenolic compounds are known to possess antioxidant activity since it was considered as most effective antioxidants[36, 37, 38]. The microbial fermentation enhanced the phenolic compounds and antioxidant capacity of DBH prepared by ultrasonic pretreatment. The results of this study suggest that the microbial fermentation is the best method to extract the phenolic compounds from microalgal biomass than the solvent extraction. Some of the study shows that the solubility of natural antioxidants mainly depends on the polarity of the solvent[29,39,40].

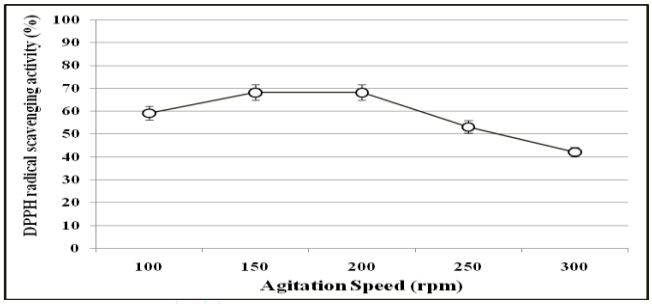

Figure 5: Effect of agitation speed on enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation

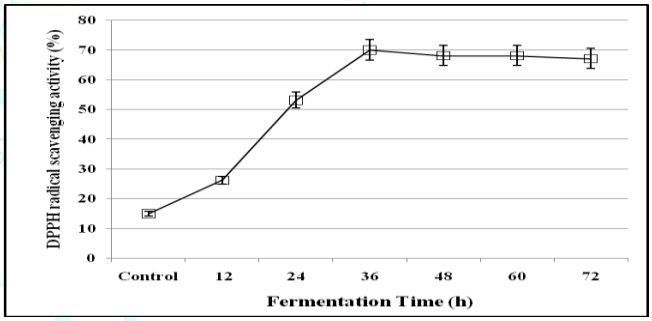

Figure 6: Effect of fermentation time on enhancement of antioxidant activity of DBH by *C. jadinii* MMS7 fermentation

CONCLUSIONS

The ultrasonic assisted hydrolysate was prepared from defatted biomass of microalga *S. quadricauda* KDPSC2 and used as feedstock for microbial fermentation for enhancing the TPC and antioxidant activity. Based on the yield of TPC the yeast *C .jadinii* MMS7 was selected as best microbial strain to extract TPC and enhancing the antioxidant activity in DBH by fermentation process. The pH 5.5, temperature 35 °C and agitation speed 150 rpm were found to be the optimum conditions for enhancing antioxidant activity of DBH by fermentation using *C .jadinii* MMS7. At these optimum conditions, the DPPH radical scavenging activity of 70.12 ± 0.08% was observed in fermented extract at 36 h, which is 4.67 fold higher than unfermented water extract. Hence, defatted biomass of microalga *S. quadricauda* KDPSC2 considered as potential natural source for production of antioxidant.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest to publish this paper.

REFERENCES

1. Sarikurkcu C, Arisoy K, Tepe B, Cakir A, Abali G, Met E. Studies on the antioxidant activity of essential oil and different solvent extracts of *Vitex agnus-castus* L. Fruits from Turkey. Food and Chemical Toxicology, 2009; 47 (10): 2479–2483.
2. Halliwell B, Antioxidant characterization methodology and mechanism. Biochemical Pharmacology, 1995; 49: 1341–1348.
3. Wong SP, Leong LP, Koh JHW, Antioxidant activities of aqueous extracts of selected plants. Food Chemistry, 2006; 99: 775-783.
4. Xu DP, Li Y, Meng X, Zhou T, Zhou Y, Zheng J, Li HB. Natural antioxidants in foods and medicinal plants: Extraction, assessment and resources. International Journal of Molecular Sciences, 2017; 18: 96.
5. Asif M. Chemistry and antioxidant activity of plants containing some phenolic compounds. Chemistry International, 2015; 1: 35–52.
6. Galasso C, Gentile A, Orefice I, Ianora A, Bruno A, Noonan DM, Sansone C, Albini A, Brunet C. Microalgal derivatives as potential nutraceutical and food supplements for human health: A focus on cancer prevention and interception. Nutrients, 2019; 11: 1226.
7. Sansone C, Brunet C. Promises and Challenges of Microalgal Antioxidant Production Antioxidants, 2019; 8: 199.
8. Yusoff FM, Nagao N, Imaizumi Y, Toda T. Bioreactor for microalgal cultivation systems: Strategy and Development. In Prospects of Renewable Bioprocessing in Future Energy Systems. Biofuel and Biorefinery Technologies; Rastegari A, Yadav A, Gupta A, Eds.; Springer: Cham, Switzerland, 2019; p. 10.
9. Mojaddar Langroodi A, Tajik H, Mehdizadeh T. Antibacterial and antioxidant characteristics of Zataria multiflora Boiss essential oil and hydroalcoholic extract of *Rhus coriaria* L. Journal of Food Quality and Hazards Control, 2019; 6:16-24.
10. Assuncao MFG, Amaral R, Martins CB, Ferreira JD, Ressurreicao S, Santos SD, Varejao JMTB, Santos LMA. Screening microalgae as potential sources of antioxidants. Journal of Applied Phycology, 2017; 29: 865–877.
11. Banskota AH, Sperker S, Stefanova R, McGinn PJ, O'Leary SJB. Antioxidant properties and lipid composition of selected microalgae. Journal of Applied Phycology, 2019; 31: 309–318.
12. Li H, Cheng K, Wong C, Fan K, Chen F, Jiang Y. Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry, 2007; 102: 771-776.
13. Khan MI, Shin JH, Kim JD. The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. Microbial Cell Factories, 2018; 17: 36.
14. Qian ZJ, Kang KH, Ryu B, Je JY, Heo SJ, Oh C. In vitro antioxidant activities of the fermented marine microalga *Pavlova lutheri* (Haptophyta) with the yeast *Hansenula polymorpha*. Journal of Phycology, 2012; 48: 475–482.
15. Bae HN, Kim YM. Improvement of the functional qualities of sea tangle extract through fermentation by *Aspergillus oryzae*. Fish Aquat Science, 2010; 13: 12-17.
16. Lum KK, Kim J, Lei XG. Dual potential of microalgae as a sustainable biofuel feedstock and animal feed. Journal of Animal Science and Biotechnology, 2013; 4:53.
17. Sander K, Murthy GS. Life cycle analysis of algae biodiesel. International Journal of Life Cycle Assess, 2010; 15:704–714.
18. Sarat Chandra T, Suvidha G, Mukherji S, Chauhan VS, Vidyashankar S, Krishnamurthi K, Sarada R, Mudliar SN (2014) Statistical optimization of thermal pretreatment conditions for enhanced biomethane production from defatted algal biomass. Bioresource Technology, 2014; 162: 157–165.
19. Sivasankar P, Dhandayuthapani K, Shanthi K. Production of ethanol using hydrolysate derived from acid pretreated defatted biomass of *Nannochloropsis limnetica,* International Journal of Recent Scientific Research, 2017; 8(8): 19392-19395.
20. Kumar N, Min B, Venkata Mohan S. Defatted algal biomass as feedstock for short chain carboxylic acids and biohydrogen production in the biorefinery format, Bioresource Technology, 2018; 269:408- 416.
21. Sorensen M, Gong Y, Bjarnason F, Vasanth GK, Dahle D, Huntley M, Kiron V. *Nannochloropsis oceania*-derived defatted meal as an alternative to fishmeal in Atlantic salmon feeds. PLoS ONE, 2017; 12(7): e0179907.
22. Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 1959; 37:911- 917.
23. Liu H, Yan YX, Wang WY, Yu YY. Improvement of the activity of activated sludge by low intensity ultrasound, Environmental Science, 2005; 26 (4): 124-128.
24. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture, 1965; 16: 144–153.
25. Qureshi MN, Kuchekar BS, Logade NA, Haleem MA. In-vitro antioxidant and in-vivo hepatoprotective activity of *Leucas ciliata* leaves. Records of Natural Products, 2010; 4: 124-130.
26. Guedes AC, Amaro HMF, Malcata X. Microalgae as sources of high added-value compounds- A brief review of recent work. Biotechnology Progress, 2011; 27:597–613.
27. Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. Molecules, 2010; 15:7313-7352.
28. Custodio L, Justo T, Silvestre L, Barradas A, Vizetto C, Pereira H, Barreira L, Rauter AP, Alberício F, Varela J. Microalgae of different phyla display antioxidant, metal chelating and acetylcholinesterase inhibitory activities. Food Chemistry, 2012; 131:134–140.
29. Bulut O, Akın D, Sonmez Ç, Oktem A, Yucel M, Oktem HA. Phenolic compounds, carotenoids, and antioxidant capacities of a thermo-tolerant *Scenedesmus* sp. (Chlorophyta) extracted with different solvents. Journal of Applied Phycology, 2019; 31(3):1675-1683.
30. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. Journal of Agricultural and Food Chemistry, 2005; 53:4290- 4302.
31. Ezekiel O, Aworh O. Simultaneous saccharification and cultivation of *Candida utilis* on cassava peel. Innovative Food Science & Emerging Technologies, 2018; 49:184-191.
32. Reihani SFS, Khosravi-Darani K. Influencing factors on single cell protein production by submerged fermentation: A review. Electron Journal of Biotechnology, 2019; 37:34-40.
33. Zhao G, Zhang W, Zhang G. Production of single cell protein using waste capsicum powder produced during capsanthin extraction. Letters in Applied Microbiology, 2010; 50(2):187–191.
34. Rollero S, Roberts S, Bauer FF, Divol B, Agitation impacts fermentation performance as well as carbon and nitrogen metabolism in *Saccharomyces cerevisiae* under winemaking conditions. Australian Journal of Grape and Wine Research, 2018; 24(3), 360–367.
35. Mittal GS, Food Biotechnology: Techniques and Applications. Lancaster: Technomic Publishing Co., New York, 1992.
36. Jimenez-Escrig A, Jimenez-Jiménez I, Pulido R, Saura-Calixto F, Antioxidant activity of fresh and processed edible seaweeds. Journal of the Science of Food and Agriculture, 2001; 81: 530-534.
37. Luo HY, Wang B, Yu CG, Qu YL, Su CL. Evaluation of antioxidant activities of five selected brown seaweeds from China. Journal of Medicinal Plants Research, 2010; 4: 2557-2565.
38. Zakaria NA, Ibrahim D, Sulaiman SF, Supardy A. Assessment of antioxidant activity, total phenolic content and in-vitro toxicity of Malaysian red seaweed, *Acanthophora spicifera*. Journal of Chemical and Pharmaceutical Research, 2011; 3: 182-191.
39. Jerez-Martel I, Garcia-Poza S, Rodriguez-Martel G, Rico M, Afonso-Olivares C, Gomez-Pinchetti JL. Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. Journal of Food Quality, 2017; 2924508. doi.org/10.1155/2017/2924508
40. Machu L, Misurcova L, Vavra Ambrozova J, Orsavova J, Mlcek J, Sochor J, Jurikova T. Phenolic content and antioxidant capacity in algal food products. Molecules, 2015; 20:1118–1133.