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Effect of UV irradiation on *Chlorella vulgaris* to induce Vitamin D

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Abstract
Vitamin D plays significant role in the regulation of calcium. Vitamin D deficiency is increasing problem throughout the globe. Vitamin D deficiency leads to the rickets in children's and osteocalcemia in adults. *Chlorella vulgaris* is aquatic unicellular green microalgae mainly utilized as the dietary supplements. *Chlorella vulgaris* does not consist vitamin D_2 but has significant amount of ergosterol. Ergosterol is the precursor of the ergocalciferol (vitamin D_2). Our objective was to induce the vitamin D in *C. vulgaris* by UV irradiation. *C. vulgaris* cultured in the Bold's Basal media (BBM) on orbital shaker under controlled conditions. The culture was irradiated by UV radiations. The irradiated sample was saponified by refluxing through methanolic KOH. Vitamin D_2 was extracted using Hexane. Extracted samples were analyzed by thin layer chromatography. TLC results of extracted samples were compared with standards of Vitamin D_2. TLC plate was developed in Hexane –Diethyl ether (4:1) solvent system. TLC proved the ergocalciferol i.e. vitamin D_2 induced in *C. vulgaris* due to irradiation of UV light.

Keywords: Ergocalciferol, ergosterol, *Chlorella vulgaris*, UV irradiation, etc.

1. Introduction
Vitamin D plays a significant role in maintenance of human health. Vitamin D is synthesized in human skin on exposure to sunlight. But the amount is considerably low as we don't expose over selves adequately to direct sun light and hence to compensate this, dietary intake is required [1]. Chlorella is unicellular aquatic green alga, used widely as supplement for nutritional dietary intake. It is one of the significant sources with composition of high amount of proteins, chlorophylls, dietary fiber in addition to minerals and vitamins [2]. Chlorella mainly produces vitamin A along with folic acid (vitamin B9). Chlorella contains ergosterol and 7-dehydroporiferasterol as major sterols [3]. Ergosterol is also taxonomically important in distinguishing "Chlorella" species [4].
Regulation of calcium homeostasis is main biological function of vitamin D. It also aids in regulation of cell proliferation and differentiation [1]. Vitamin D deficiency causes rickets in children and osteoporosis in adults. In cardiovascular, autoimmune disease and cancer prevention vitamin D possibly play significant role because in broad range of tissues vitamin D receptors are detected [5]. Vitamin D is also used as general health marker due to its link between deficiency and several diseases. Vitamin D is available in 2 forms: Vitamin D2 (Ergocalciferol) and Vitamin D3 (Cholecalciferol) [6]. Mushrooms are important dietary source of vitamin D. when mushrooms are irradiated with UV radiations, ergosterol get converted into ergocalciferol i.e. pre-vitamin D2 [7]. Microalgae used in aquaculture content of vitamin D were lower than limit of detection [8]. Deficiency of vitamin D is a pandemic. It has increased the worldwide probability of skeletal and chronic disease caused due to vitamin D deficiency. Therefore, maintaining vitamin D from exposure to sun, dietary intake of a food source rich in vitamin D content and from supplements of vitamin D is essential to support healthy lifestyle [9].

2 Material Methods
2.1 Microalgae Sample Collection, Identification and Culture
The algal sample was collected from the Kala Talav, Kalyan in sterile test tube. Kala Talav is freshwater lake in which algal growth observed due to eutrophication. Chlorella was abundant in collected sample. '*Chlorella vulgaris'* uni cultured species isolated from water sample by serial dilution method. Isolated sample was cultivated in the Bold's Basal Medium (BBM) on orbital shaker under controlled conditions [10]. Isolated *Chlorella* species was identified as *Chlorella vulgaris*. Authentication was done from 'Botanical Survey of India' (BSI), western Regional Centre, Pune.

2.2 Irradiation of UV light
UV-B lamp of 9 watt was used to irradiate *C. vulgaris* samples with 3060 mw/cm^2. *C. vulgaris* Sample 1 was irradiated by UV-B lamp for 6 minute and sample 2 for 8 minute. The irradiated samples were oven dried at 40ºC. The oven dried sample was used for extraction of vitamin D.

2.3 Extraction of Sample
Saponification was carried out by refluxing 100 mg sample in methanolic KOH. The saponified material was extracted in hexane. Hexane layer was separated. Separated hexane layer evaporated at 37 ºC in oven. Dried extract reconstituted in ethanol.

2.4 Thin Layer Chromatography
Pre coated aluminum TLC Silica Gel F254 of MERCK was used as sorbent. A pre coated thin-layer chromatography plate activated 1 hour at 110ºC. Vitamin D_2 tablet of Health Aid containing 500 IU Vitamin D_2 used as standard for comparing the spot. Samples and standard were loaded on the TLC plate and run in saturated chromatography chamber with Hexane – diethyl ether (4:1) [11]. A solution of 50% H_2SO_4 was used as developing agent. The spot of separated vitamin D were visualized in direct 366 nm CAMAG Repro star 3 UV light chamber.

3 Results and Discussion
The TLC plates were visualized in 366nm UV light chamber. Rf values of both the samples and standard were calculated. The Rf values of sample 1 and sample 2 were found to be matching with the Rf values of standard (Table 1), indicating presence of vitamin D_2 in the samples.

Table 1: TLC Rf values: - Sample 1(6 minutes irradiated), Sample 2(8 minutes irradiated) and vitamin D_2 standard

Solvent System	Spots	Distance travelled by solute	Distance travelled by solvent	Rf
Hexane - Diethy ether (4:1)	Sample 1 spot	10.5 cm	11.0 cm	0.95
	Sample 2 spot	10.5 cm	11.0 cm	0.95
	Standard spot	10.4 cm	11.0 cm	0.94

UV radiations are known for increase the production of vitamin D_2. Studies have shown that in White button mushroom (*Agaricus bisporous*), the amount of vitamin D_2 is enhanced significantly when exposed to UV light [12, 13]. Long-history of UV light use considered safe for vitamin D production in foods. Therefore the use of UV light on mushrooms for production of vitamin D_2 was found suitable and safe [14]. Pulsed UV light source was found to be safe and more effective to enrich mushroom with high amount of vitamin D [13]. Research in pilot studies revealed that UV light treatment on *Solanum. Glaucohyllum* Desf. and *Solanum. lycopersicum* L. results into production of Vitamin D_3 [15]. Ergosterol is one of the significant sterol detected in *C. vulgaris* [4]. Irradiation of UV radiations bring about photolytic changes in B ring of ergosterol to form intermediate product i.e. pre-vitamin D_2, which undergoes further thermal rearrangement to synthesis vitamin D_2 [16]. Thus by irradiating with UV radiations, Vitamin D_2 content in *C. vulgaris* can be increased.

4. Conclusions
C. vulgaris is one of the significant vegan supplementary sources of the proteins and vitamins. Changing lifestyle fail to obtain required amount of sun exposure**,** to produce sufficient amount of vitamin D. There are less numbers of vitamin D natural sources which can be used as supplement. Those are available most are from animal products. There is a need to provide an alternative source of Vitamin D. *Chlorella* consist of ergosterol as one of the major sterol which is the precursor of ergocalciferol i.e. Vitamin D_2. It proved ergocalciferol a form of Vitamin D_2 produced in the *C. vulgaris* by UV light irradiation. *Chlorella vulgaris* can be used as one of the cost effective and vegan supplement source of Vitamin D. To our knowledge, this is the maiden attempt of using UV irradiation on microalgae for production of Vitamin D.

5. Conflict of Interest
On behalf of all authors, the corresponding author states that there is no conflict of interest.

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