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QUANTITATIVE ANALYSIS OF PHYTOCHEMICAL PROFILE IN MARINE MICROALGAE *CHLORELLA VULGARIS*

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ABSTRACT

The present study is aimed to investigate the quantitative phytochemical profile of C. vulgaris from Vellar estuary. The microalgae was isolated by phytoplankton net and identified through microscopic observations. The isolated strain was mass cultured in Conway medium in laboratory condition and the biomass was harvested by filtration method. The dried biomass was carried out for metabolite extraction by methanol and the results was found to be 19%. Totally seven phytochemicals were estimated quantitatively, and phenols was found to be higher followed by alkaloid, terpenoid and glycoside and the tannin was found to be lesser amount. It is inferred that the C. vulgaris having more pharmaceutically potent chemical substances than the other species.

KEY WORDS

Phytochemicals, Secondary metabolites, C. vulgaris.

1. INTRODUCTION

Phytochemicals namely alkaloids, flavonoids, terpenoids, saponins, glycosides, phenols, sterols etc, are the major secondary metabolites synthesized by plants and animals [1]. The secondary metabolites are natural chemical substances which are produced from the metabolisms for defense purposes from the predators [2]. The phytochemicals played a vital role in several activities such as antioxidant, anticancer, antiviral, antibacterial, enzyme inhibitors etc. Marine derived phytochemicals substances having more potential biological activities than the terrestrial origin and approximately 4,000 health benefits phytochemicals have been documented from marine and terrestrial origin [3]. Microalgae are being important phytochemical source for phytochemicals and nutritional supplements and it has rich content of phenols and terpenes, several researchers have been identified the active secondary metabolites from microalgal species [4]. But few studies have been found in quantitative estimation of phytochemicals from microalgae. Based on the fact, the present study is aimed to isolate and identified the microalgae *C. vulgaris* from the Vellar estuary and to investigate the phytochemical components in quantitative level.

2. MATERIALS AND METHODS

2.1. Collection and identification

The marine microalgae *C. vulgaris* was collected from the Vellar Estuary, Parangipettai, Tamil Nadu, by phytoplankton net following the standard protocol. The *C. vulgaris* was cultured in Conway medium and distinguished by morphological key features through microscopic observations and it was cultured mass scale in laboratory with optimum conditions such pH, temperature, time etc.

2.2. Extraction of secondary metabolites

The mass cultured *C. vulgaris* was assessed the metabolite extraction, 100g of *C. vulgaris* was extracted with methanol at the ratio of 2:1 and then the mixture was allowed to stand for 48 hrs at room temperature. Then the solvent from the mixture was evaporated by reduced under vacuum pressure and the final product was kept in the 4°C condition for further analysis.

2.3. Quantitative phytochemical screening

The phytochemical such as alkaloids, flavonoids, terpenoids, glycosides, tannins, triterpenes and phenols were quantitatively estimated in *C. vulgaris* methanolic extract by spectroscopic method.

2.3.1. Alkaloids

The alkaloid was estimated in *C. vulgaris* methanolic extract by Martinez Nadal et al. (1963). The 1 ml of methanolic extract (1 mg/ml) was dissolved in water with 0.1 ml of FeCl (2.5 mM FeCl in 0.5 M HCl) followed by the addition of 0.1 ml 1,10-phenanthroline. The mixture was incubated for 30 min at 70°C the absorbance was measured at 500 nm.

2.3.2. Flavonoids

The flavonoid was determined by following the method of Hazra et al. [6] in *C. vulgaris* methanolic extract. The 0.1 ml sample was mixed with 0.03 ml 5% $NaNO_2$. After incubation for 5 min at 25°C, the AlCl (0.03 ml, 10%) was added and then added with 0.2 ml 1 mM NaOH to mixture. Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm.

2.3.3. Terpenoids

The terpenoids composition of *C. vulgaris* methanolic extract was carried out as described by Ghorai et al. [7]. The sample for was transfer red from assay tube to read the absorbance at 538 nm.

2.3.4. Glycosides

The total glycoside estimation of C. vulgaris methanolic extract was assessed through spectroscopically by following the method of Ejikeme et al. [8].

2.3.5. Tannins

The tannin composition of methanolic extract of *C. vulgaris* was estimated through spectroscopic method by following the method of Chaudhuri et al. [9]. The 0.1 ml methanolic extract (1 mg/ml) was added with 0.5 ml vanillin hydrochloride reagent and incubated for 20 min at room temperature condition and then the absorbance of the resulting magenta-pink colour was read at 500 nm.

2.3.6. Triterpenes

The triterpene content of *C. vulgaris* methanolic extract was screened spectroscopically by following the procedure of Chaudhuri et al. [10].

2.3.7. Phenols

The phenol composition of *C. vulgaris* methanolic extract was done by following the method of Folin-Ciocalteu (FC) as described by Hazra et al. [6]. The 0.1 ml mthanolic extract was added with 0.75 ml FC reagent, followed by the addition of 0.06% Na_2CO_3 (0.75 ml) solution. After the micture was incubated at 22°C for 90 mins then the absorbance was read at 725 nm.

3. RESULTS AND DISCUSSION

Several researchers have been reported the secondary metabolites from microalgae having plenty of pharmaceuticals applications. In the present study the yield percentage was calculated by the extracted matrix from dry sample and it was found to be 19%. Marine derived phytochemicals have wide variety of biomedical applications such as anticancer, antiviral, nueroprotective, antioxidant, cardiorotective effect etc, and it concentrations was differed from one species to another species and in extract was depends upon the solvent polarity [11, 12]. In this study, the phytochemical composition *C. vulgaris* was estimated quantitatively and the results have been showed in Table 1. The phytochemicals such as alkaloids, flavonoids, triterpenes, glycosides, tannins, triterpenes and phenols have recorded for 71.6, 50.9, 62.4, 57.5, 23.17, 47.2 and 90.9µg/mg. In the present findings is an agreement with earlier report of Rajendran et al. [13] who reported the maximum content of flavonoids in the secondary metabolites form *Tetraselmis* and *Oscillatoria* extracted by four types of solvents namely, acetone, ethanol, methanol and chloroform respectively. Similarly, Adhoni et al. [14] screening the phytochemical profile of C. vulgaris various polar and non-polar solvents extracts and reported 14 phytochemical constituents, the high concentration of phytochemicals was recorded in polar solvents extracts. Thus, the earlier findings of phytochemicals profile revealed that the phytochemical composition was depends on the chemical diversity and solvent system. Likewise, Chaudhuri et al. [10] investigated the 11 phytochemicals composition of *E. tuba* qualitatively and quantitatively and recorded the phytochemicals namely, phenol, flavonoids, carboxylic acid, tannin,

alkaloid, ascorbic quantitatively and the flavonoids have showed the maximum range 100mg/100g and alkaloid was recorded minimum level 2.95mg/100g respectively. Correspondingly, Geetha et al. [15] investigated the phenols and flavonoids content in *Chlorella* species quantitatively and reported the maximum composition of total phenols and flavonoids for 2.47 and 1.86mg/g dry weight respectively.

Table 1. Quantitative Phytochemical analysis of *C. vulgaris*

Name of the sample	Phytochemicals (µg/mg)						
	Alkaloids	Flavnoids	Terpenoids	Glycosides	Tannins	Triterpenes	Phenols
C. vulgaris	71.6 ± 0.16	50.9 ± 0.08	62.4 ± 0.16	57.5 ± 0.23	23.17 ± 0.32	47.2 ± 0.16	90.9 ± 0.09

4. CONCLUSION

The present study, the microalgae *C. vulgaris* was collected and identified and cultured in mass scale level in optimized condition. The pharmaceutical important phytochemicals have been noticed in higher level. the study also concluded that, the *C. vulgaris* have more potent chemical constituents than the other alagal species and it could be renewable source of phytochemical for development for potent drug in pharmaceutical industry.

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