Pharmacognosy Research
A publication of Phcog.Net
A Rapid Publication Journal
Home
Current issue
Instructions
Submit article

Pharmacognosy Res. 2016 Apr-Jun; 8(2): 118–122. PMCID: PMC4780137
doi: 10.4103/0974-8490.172660

Inhibition of Pro-inflammatory Mediators and Cytokines by *Chlorella Vulgaris* Extracts

G. Sibi and Santa Rabina[1]

Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bengaluru, Karnataka, India
[1]Department of Genetics, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bengaluru, Karnataka, India
Correspondence: Dr. G. Sibi, Department of Biotechnology, Indian Academy Degree College, Centre for Research and Post Graduate Studies, Bengaluru, Karnataka, India. E-mail: gsibii@gmail.com

Abstract

Objective:

The aim of this study was to determine the *in vitro* anti-inflammatory activities of solvent fractions from *Chlorella vulgaris* by inhibiting the production of pro-inflammatory mediators and cytokines.

Methods:

Methanolic extracts (80%) of *C. vulgaris* were prepared and partitioned with solvents of increasing polarity viz., *n*-hexane, chloroform, ethanol, and water. Various concentrations of the fractions were tested for cytotoxicity in RAW 264.7 cells using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, and the concentrations inducing cell growth inhibition by about 50% (IC_{50}) were chosen for further studies. Lipopolysaccharide (LPS) stimulated RAW 264.7 cells were treated with varying concentrations of *C. vulgaris* fractions and examined for its effects on nitric oxide (NO) production by Griess assay. The release of prostaglandin E_2 (PGE_2), tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6) were quantified using enzyme-linked immunosorbent assay using Celecoxib and polymyxin B as positive controls.

Results:

MTT assay revealed all the solvent fractions that inhibited cell growth in a dose-dependent manner. Of all the extracts, 80% methanolic extract exhibited the strongest anti-inflammatory activity by inhibiting NO production ($P < 0.01$), PGE_2 ($P < 0.05$), TNF-α, and IL-6 ($P < 0.001$) release in LPS induced RAW 264.7 cells. Both hexane and chloroform fractions recorded a significant ($P < 0.05$) and dose-dependent inhibition of LPS induced inflammatory mediators and cytokines *in vitro*. The anti-inflammatory effect of ethanol and aqueous extracts was not significant in the study.

Conclusion:

The significant inhibition of inflammatory mediators and cytokines by fractions from *C. vulgaris* suggests that this microalga would be a potential source of developing anti-inflammatory agents and a good alternate for conventional steroidal and nonsteroidal anti-inflammatory drugs.

SUMMARY

- *C. vulgaris* extracts have potential anti-inflammatory activity
- Solvent extraction using methanol, hexane, and chloroform has exhibited significant effect in LPS activated RAW 264.7 cells
- *C. vulgaris* extracts reduce the production of NO, PGE_2, TNF-α, and IL-6 in LPS activated RAW 264.7 cells.

Abbreviations Used: COX-2: Cyclooxygenase-2, DMSO: Dimethyl sulfoxide, FBS: Fetal bovine serum, IL-6: Interleukin 6, iNOS: Inducible nitric oxide synthase, L-NMMA: NG-methyl-L-arginine acetate salt, LPS: Lipopolysaccharide, MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, NO: Nitric oxide, PBS: Phosphate buffered saline, PGE_2: Prostaglandin E_2, TNF-α: Tumor necrosis factor-α

Keywords: Anti-inflammatory, *Chlorella vulgaris*, Microalgae, Pro-inflammatory cytokines, Pro-inflammatory mediators

INTRODUCTION

Inflammation is an important host defense mechanism and is characterized by a complex of interactions between mediators of inflammation and inflammatory cells.[1,2] Uncontrolled inflammation can lead to tissue injury and chronic diseases.[3] In general, treatment for inflammation is aimed at either inhibiting the activity of inflammatory cells or inhibiting the production of inflammatory mediators.[4] At present, most inflammatory diseases are treated with steroidal and nonsteroidal anti-inflammatory drugs which suppress the levels of pro-inflammatory cytokines, inducible nitric oxide synthase (iNOS), cyclooxygenase-2, and prostaglandin E_2 (PGE_2).[5] However, prolonged use of these conventional drugs may produce adverse side effects[6] in addition with long-term steroid use that suppresses the immune system.[7]

New anti-inflammatory agents from natural sources with fewer adverse effects are alternates that could be developed for long-term administration. Microalgae form the part of natural sources that could be a sustainable source of bioactive compounds, and anti-inflammatory activity of microalgae has been reported widely.[8,9,10,11,12,13,14] Pro-inflammatory cytokines activate immune cells to up-regulate inflammation and are, therefore, useful targets in the development of new anti-inflammatory drugs.[15,16] For this reason, we investigated the inhibitory activity of *Chlorella vulgaris* solvent fractions on pro-inflammatory cytokines and inflammatory mediators.

MATERIALS AND METHODS

Chemicals and reagents

Chemicals 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), lipopolysaccharide (LPS), NG-methyl-L-arginine acetate salt (L-NMMA), celecoxib, polymyxin B, and enzyme-linked immunosorbent assay (ELISA) kits were purchased from Sigma-Aldrich (India). All other solvents/chemicals used were of reagent or analytical grade.

Preparation of algal extract

C. vulgaris was grown on Bold's medium for a period of 15 days under illumination and centrifuged at 10,000 ×g for 10 min and the pellet was washed with distilled water. The pellet was lyophilized and the dried powder was extracted by ultrasonication with 80% methanol (2 L) at room temperature for 30 min. The methanolic extracts were filtered by Whatman filter paper and the filtrate was concentrated using rotary vacuum evaporator. The evaporated extract was suspended in water (1 L) and partitioned with solvents of increasing polarity. At first, *n*-hexane (1 L) was added to the methanolic extract, agitated for 24 h at room temperature, and separated as hexane fraction. This was followed by extraction with chloroform and ethanol. Each fraction was dried under a rotary vacuum evaporator, lyophilized in a freeze drier and dissolved in dimethyl sulfoxide (DMSO), and stored at 4°C for further studies.

RAW 264.7 cell culture

RAW 264.7 cells, purchased from American Type Culture Collection (ATCC), were grown on Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 µg/mL). The cells were incubated and maintained in an atmosphere of 5% CO_2 at 37°C. The cells were subcultured every 2 days and exponential phase cells were used throughout the experiments.

Cytotoxicity assessment using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

The cytotoxicity of the crude solvent extracts against the RAW 264.7 cells (ATCC, USA) was determined using the colorimetric MTT assay. Cells were seeded in a 24-well plate at a concentration of 1×10^5 cells/mL. After 24 h, the seeded cells were treated with microalgal extracts and incubated for an additional 24 h at 37°C. MTT stock solution (50 µl; 2 mg/mL in phosphate buffered saline) was added to each well to a total reaction volume of 250 µL. After incubating for 3 h at 37°C under humidified 5% CO_2 atmosphere, the supernatants were aspirated and the formazan crystals in each well were dissolved in 200 µl DMSO. The resulting absorbance was measured with a microplate reader (Tecan Infinite, F 500) at 540 nm. The concentrations inducing cell growth inhibition by about 50% (IC_{50}) were chosen for further studies.

Determination of nitric oxide production

RAW 264.7 cells (1×10^5 cells/mL) were placed in a 24-well plate and after 24 h, the cells were preincubated with different concentrations of *C. vulgaris* fractions at 37°C for 1 h. Further incubation was done at 37°C for another 24 h with LPS (1 µg/mL) and the nitrite that accumulated in the culture medium was measured[17] as indicative of the nitric oxide (NO) production. The culture medium was collected and centrifuged at 750 ×g to precipitate any remaining cell debris. 100 µl supernatant was mixed with equal volume of Griess reagent (1% sulphanilamide and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid) and incubated at room temperature for 10 min. This was followed by measuring the absorbance at 540 nm in a microplate reader (Tecan Infinite, F 500) using L-NMMA (80 µM) as positive control. The nitrite concentration was estimated against a sodium nitrite standard calibration curve.

Assays of pro-inflammatory cytokines

The inhibitory effects of *Chlorella* extracts on the production of cytokines were measured by ELISA using culture supernatants collected from treated cells. RAW 264.7 cells (1.8×10^5 cells/mL) were plated in a 24-well plate containing 1 ml of DMEM medium for 18 h, followed by treatment with LPS (500 ng/ml) in the presence of solvent fractions of *Chorella vulgaris*. After another 24 h of incubation, the PGE_2, tumor necrosis factor-α (TNF-α), and interleukin 6 (IL-6) in the cell culture medium were quantified using ELISA kits (Sigma-Aldrich, India) according to the manufacturer's instructions using Celecoxib (3 µM) and polymyxin B (100 U/mL) as positive controls.

Statistical analysis of data

All values were expressed as mean ± standard deviation. Statistical differences between the treatments and the control were evaluated by analysis of variance and Student's *t*-tests. Values of $P < 0.05$ were considered to be significant.

RESULTS

Cytotoxicity assay

Tetrazolium dye colorimetric test (MTT assay) was performed as a preliminary test to determine whether the solvent fractions from *C. vulgaris* caused cytotoxicity in RAW 264.7 cells. The absorbance of the cells exposed to different concentrations of solvent fractions for 24 h. All the fractions inhibited cell growth in a dose-dependent manner and the concentrations inducing cell growth inhibition by about 50% (IC_{50}) were 125 µg/mL (methanol) and 250 µg/mL (hexane and chloroform) [Figure 1]. Treatment of RAW 264.7 cells with ethanol and aqueous fractions had no significant effect on cell viability after 24 h incubation. Based

on cytotoxicity effects, IC_{50} was chosen as the highest concentration on the production of NO, TNF-α, and IL-6 in LPS stimulated RAW 264.7 cells.

Inhibitory effects on inflammatory mediators

RAW 264.7 macrophages activated by LPS would generate massive inflammatory mediators (NO and PGE_2) and cytokines (TNF-α and IL-6), which would cause kinds of inflammatory diseases.[18,19] Hence, LPS-activated RAW 264.7 cell model was chosen to reveal the anti-inflammatory mechanism of *C. vulgaris* extracts in this study. Treatment of RAW 264.7 cells with LPS alone resulted in significant increases in inflammatory mediators and cytokines. However, methanolic, hexane and chloroform extracts reduced the NO production significantly in a dose-dependent manner and methanol extract of *C. vulgaris* strongly inhibited the NO production ($P < 0.01$) at a concentration of 125 µg/mL. It was observed that ethanol and aqueous fractions had less activity in reducing NO production even at 500 µg/mL. The ability of *C. vulgaris* extracts to modulate the production of PGE_2 was determined by ELISA and was found to significantly inhibit PGE_2 production as compared to the LPS-treated group in a dose-dependent manner. RAW 264.7 cells were stimulated with LPS and then incubated with the solvent fractions of *C. vulgaris* and a significant inhibition of PGE_2 production was observed in cells treated with methanol ($P < 0.05$), hexane and chloroform ($P < 0.05$) extracts.

Inhibitory effects on proinflammatory cytokines

Since *C. vulgaris* was found to potently inhibit the pro-inflammatory mediators, we further investigated its effect on LPS induced TNF-α and IL-6 production. The concentrations of cytokines TNF α and IL-6 in cell supernatants was measured by ELISA and treatment of RAW 264.7 cells with LPS alone resulted in higher cytokine production. Methanol extracts significantly decreased the LPS induced TNF-α at 125 µg/mL, whereas the concentration was 250 µg/mL for hexane and chloroform fractions ($P < 0.05$). However, ethanol and aqueous fractions decreased TNF-α at higher concentrations (500 µg/mL). Similar results were observed in reducing IL-6 production by *C. vulgaris* extracts.

DISCUSSION

Inflammation is the normal physiological and immune response to tissue injury. Macrophages play important roles in inflammation by overproducing inflammatory mediators, including NO and PGE_2.[20] NO is synthesized by the iNOS and has been reported as a mediator of inflammation.[21] It is as an important molecule to regulate the biological activities in vascular, neural, and immune systems.[22] However, its uncontrolled release can cause target tissue destruction during an infection.[23] Inhibition of inflammatory mediators is a useful strategy for the treatment of acute or chronic inflammatory disorders. LPS and proinflammatory cytokines activate immune cells to up-regulate inflammatory states and are therefore useful targets in the development of anti-inflammatory agents. To investigate the effect of *C. vulgaris* on NO production, Griess assay was used to measure the accumulation of nitrite in culture media. RAW 264.7 cells were stimulated with LPS in the presence or absence of *C. vulgaris* extracts and the nitrite levels were increased significantly in LPS induced cells. Further, fractions of 80% methanolic extracts were evaluated on NO production by RAW 264.7 cells. Prostaglandins (PG) are involved in various pathophysiological processes including inflammation and PGE_2 involved in inflammatory responses is generated by the sequential metabolism of arachidonic acid by cycloxygenase.[24] PGE_2 is a pleiotropic mediator that causes pain, swelling, and stiffness.[25] In this study, the release of pro-inflammatory mediators were prevented as the amount of NO and PGE_2 production was reduced as depicted in Figure 2a-e revealing the potential anti-inflammatory activities of *C. vulgaris*.

Pro-inflammatory cytokines (TNF-α, IL-1 β, and IL-6) are important initiators of the inflammatory response and mediators.[26,27] The cytokines IL-1, IL-6, and TNF-α are produced mainly by activated monocytes or macrophages which stimulate cell proliferation in various types of cells.[28] TNF was shown to affect various biological processes including the regulation and the production of other cytokines.[29] TNF-α activates macrophages and promotes inflammation and expression of cell adhesion molecules to inflammatory tissue thereby plays a key role in the induction and perpetuation of inflammation.[30,31] IL-6 is regarded as an endogenous mediator of LPS-induced fever.[32] In this study,

we found that *C. vulgaris* extracts significantly inhibit proinflammatory cytokines production in a dose-dependent manner in LPS stimulated RAW 264.7 cells. Anti-inflammatory activity of bioactive compounds from marine *Chlorella* was reported earlier[33,34,35,36,37,38] however, inhibition of inflammatory mediators and cytokines by fresh water *C. vulgaris* is reported first time in this study.

CONCLUSION

In this study, 80% methanolic extract of *C. vulgaris* was used to prepare various fractions to investigate the *in vitro* anti-inflammatory activity using RAW 264.7 cells. The results showed that treatment with *C. vulgaris* extracts inhibits the inflammatory response by reducing the production of NO, PGE_2, TNF-α and IL-6 in LPS activated RAW 264.7 cells *in vitro*. It suggests that *C. vulgaris* possesses significant anti-inflammatory activity and could be a potential source of anti-inflammatory agents of natural origin.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

ABOUT AUTHORS

Dr. G Sibi, is an Associate Professor at the Department of Biotechnology, Indian Academy Degree College, Bangalore. His research interest is in the field of Phycology, which involves the characterization of microalgal bioactive compounds for health care and biofuel production for sustainable environment. He is the recipient of projects from National agencies and Editorial Board Member of various International Journals.

Santa Rabina, is a Masters Degree Student at Department of Genetics, Indian Academy Degree College, Bangalore.

REFERENCES

1. Villarreal G, Zagorski J, Wahl SM. Encyclopedia of Life Sciences (ELS) London: Nature Publishing Group; 2001. Inflammation: Acut.

2. Sacca R, Cuff CA, Ruddle NH. Mediators of inflammation. Curr Opin Immunol. 1997;9:851–7. [PubMed: 9492989]

3. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008;454:436–44. [PubMed: 18650914]

4. Benjamini E, Sunshine G, Leskowitz S. 3rd ed. New York: John Wiley and Sons, Inc; 1996. Immunology: A Short Course.

5. Pohanka M, Snopkova S, Havlickova K, Bostik P, Sinkorova Z, Fusek J, et al. Macrophage-assisted inflammation and pharmacological regulation of the cholinergic anti-inflammatory pathway. Curr Med Chem. 2011;18:539–51. [PubMed: 21143112]

6. Lin L, Hu K. Tissue plasminogen activator and inflammation: From phenotype to signaling mechanisms. Am J Clin Exp Immunol. 2014;3:30–6. [PMCID: PMC3960759] [PubMed: 24660119]

7. Cheng M, Pan ZY, Yang J, Gao YD. Corticosteroid therapy for severe community-acquired pneumonia: A meta-analysis. Respir Care. 2014;59:557–63. [PubMed: 24046464]

8. El Gamal AA. Biological importance of marine algae. Saudi Pharm J. 2010;18:1–25. [PMCID: PMC3731014] [PubMed: 23960716]

9. Bhakuni DS, Rawat DS. New Delhi, India: Springer; 2005. Bioactive Marine Natural Products.

10. Awad NE. Biologically active steroid from the green alga *Ulva lactuca*. Phytother Res. 2000;14:641–3. [PubMed: 11114004]

11. Tan LT, Williamson RT, Gerwick WH, Watts KS, McGough K, Jacobs R. Cis, cis- and trans, trans-ceratospongamide, new bioactive cyclic heptapeptides from the Indonesian red alga *Ceratodictyon spongiosum* and symbiotic sponge *Sigmadocia symbiotica*. J Org Chem. 2000;65:419–25. [PubMed: 10813950]

12. Gemma C, Mesches MH, Sepesi B, Choo K, Holmes DB, Bickford PC. Diets enriched in foods with high antioxidant activity reverse age-induced decreases in cerebellar beta-adrenergic function and increases in proinflammatory cytokines. J Neurosci. 2002;22:6114–20. [PubMed: 12122072]

13. Deng R, Chow TJ. Hypolipidemic, antioxidant, and antiinflammatory activities of microalgae *Spirulina*. Cardiovasc Ther. 2010;28:e33–45. [PMCID: PMC2907180] [PubMed: 20633020]

14. Jung HA, Jin SE, Ahn BR, Lee CM, Choi JS. Anti-inflammatory activity of edible brown alga *Eisenia bicyclis* and its constituents fucosterol and phlorotannins in LPS-stimulated RAW264.7 macrophages. Food Chem Toxicol. 2013;59:199–206. [PubMed: 23774261]

15. Zeilhofer HU, Brune K. Analgesic strategies beyond the inhibition of cyclooxygenases. Trends Pharmacol Sci. 2006;27:467–74. [PubMed: 16876882]

16. Jachak SM. PGE synthase inhibitors as an alternative to COX-2 inhibitors. Curr Opin Investig Drugs. 2007;8:411–5.

17. Lee MH, Lee JM, Jun SH, Lee SH, Kim NW, Lee JH, et al. The anti-inflammatory effects of *Pyrolae herba* extract through the inhibition of the expression of inducible nitric oxide synthase (iNOS) and NO production. J Ethnopharmacol. 2007;112:49–54. [PubMed: 17395412]

18. Park HH, Kim MJ, Li Y, Park YN, Lee J, Lee YJ, et al. Britanin suppresses LPS-induced nitric oxide, PGE2 and cytokine production via NF-κB and MAPK inactivation in RAW 264.7 cells. Int Immunopharmacol. 2013;15:296–302. [PubMed: 23270759]

19. Barbour SE, Wong C, Rabah D, Kapur A, Carter AD. Mature macrophage cell lines exhibit variable responses to LPS. Mol Immunol. 1998;35:977–87. [PubMed: 9881693]

20. Esposito E, Cuzzocrea S. The role of nitric oxide synthases in lung inflammation. Curr Opin Investig Drugs. 2007;8:899–909.

21. de las Heras B, Abad MJ, Silván AM, Pascual R, Bermejo P, Rodríguez B, et al. Effects of six diterpenes on macrophage eicosanoid biosynthesis. Life Sci. 2001;70:269–78. [PubMed: 12005260]

22. Moncada S, Palmer RM, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev. 1991;43:109–42. [PubMed: 1852778]

23. Kim HW, Murakami A, Williams MV, Ohigashi H. Mutagenicity of reactive oxygen and nitrogen species as detected by co-culture of activated inflammatory leukocytes and AS52 cells. Carcinogenesis. 2003;24:235–41. [PubMed: 12584172]

24. Vane JR, Bakhle YS, Botting RM. Cyclooxygenases 1 and 2. Annu Rev Pharmacol Toxicol. 1998;38:97–120. [PubMed: 9597150]

25. Dinarello CA. Cytokines as endogenous pyrogens. J Infect Dis. 1999;179(Suppl 2):S294–304. [PubMed: 10081499]

26. Männel DN, Echtenacher B. TNF in the inflammatory response. Chem Immunol. 2000;74:141–61. [PubMed: 10608086]

27. Glauser MP. The inflammatory cytokines.New developments in the pathophysiology and treatment of septic shock. Drugs. 1996;52(Suppl 2):9–17. [PubMed: 8869831]

28. Stein B, Sutherland MS. IL-6 as a drug discovery target. Drug Discov Today. 1998;3:202–13.

29. Hume DA, Underhill DM, Sweet MJ, Ozinsky AO, Liew FY, Aderem A. Macrophages exposed continuously to lipopolysaccharide and other agonists that act via toll-like receptors exhibit a sustained and additive activation state. BMC Immunol. 2001;2:11. [PMCID: PMC58839] [PubMed: 11686851]

30. Beutler B, Cerami A. The biology of cachectin/TNF – A primary mediator of the host response. Annu Rev Immunol. 1989;7:625–55. [PubMed: 2540776]

31. Youn GS, Kwon DJ, Ju SM, Choi SY, Park J. Curcumin ameliorates TNF-α-induced ICAM-1 expression and subsequent THP-1 adhesiveness via the induction of heme oxygenase-1 in the HaCaT cells. BMB Rep. 2013;46:410–5. [PMCID: PMC4133911] [PubMed: 23977989]

32. Van Snick J. Interleukin-6: An overview. Annu Rev Immunol. 1990;8:253–78. [PubMed: 2188664]

33. Renju GL, Kurup GM. Anti-inflammatory activity of lycopene isolated from *Chlorella marina* on carrageenan induced rat paw edema. J Res Biol. 2011;3:886–94.

34. Renju GL, Kurup GM, Saritha Kumari CH. Anti-inflammatory activity of lycopene isolated from *Chlorella marina* on type II collagen induced arthritis in Sprague Dawley rats. Immunopharmacol Immunotoxicol. 2013;35:282–91. [PubMed: 23237458]

35. Guzmán S, Gato A, Lamela M, Freire-Garabal M, Calleja JM. Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res. 2003;17:665–70. [PubMed: 12820237]

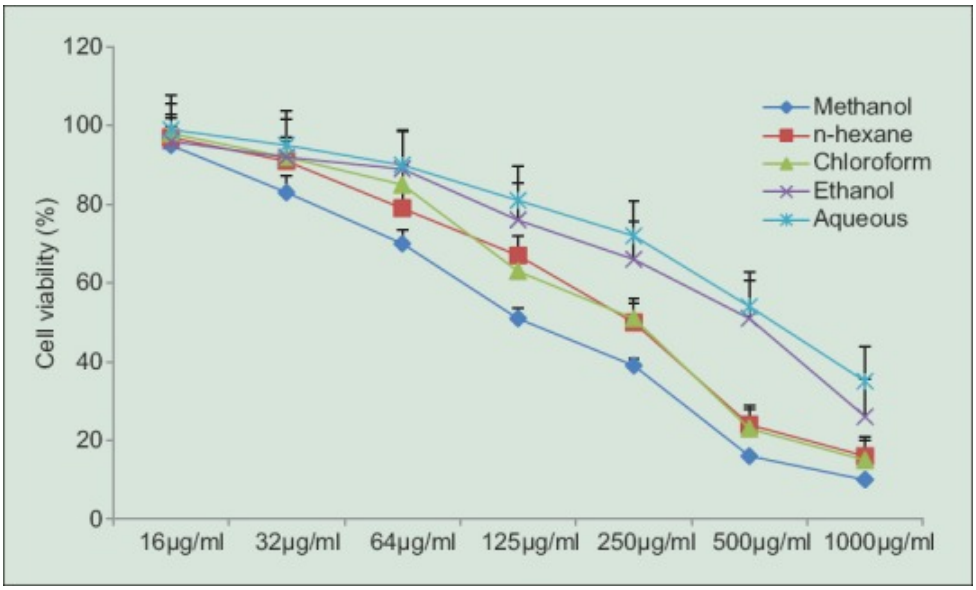
36. Guzmán S, Gato A, Calleja JM. Antiinflammatory, analgesic and free radical scavenging activities of the marine microalgae *Chlorella stigmatophora* and *Phaeodactylum tricornutum*. Phytother Res. 2001;15:224–30. [PubMed: 11351357]

37. Samarakoon KW, Ko JY, Shah MR, Lee JH, Kang MC, Nam KO, et al. *In vitro* studies of anti-inflammatory and anticancer activities of organic solvent extracts from cultured marine microalgae. Algae. 2013;28:111–9.

38. Soontornchaiboon W, Joo SS, Kim SM. Anti-inflammatory effects of violaxanthin isolated from microalga *Chlorella ellipsoidea* in RAW 264.7 macrophages. Biol Pharm Bull. 2012;35:1137–44. [PubMed: 22791163]

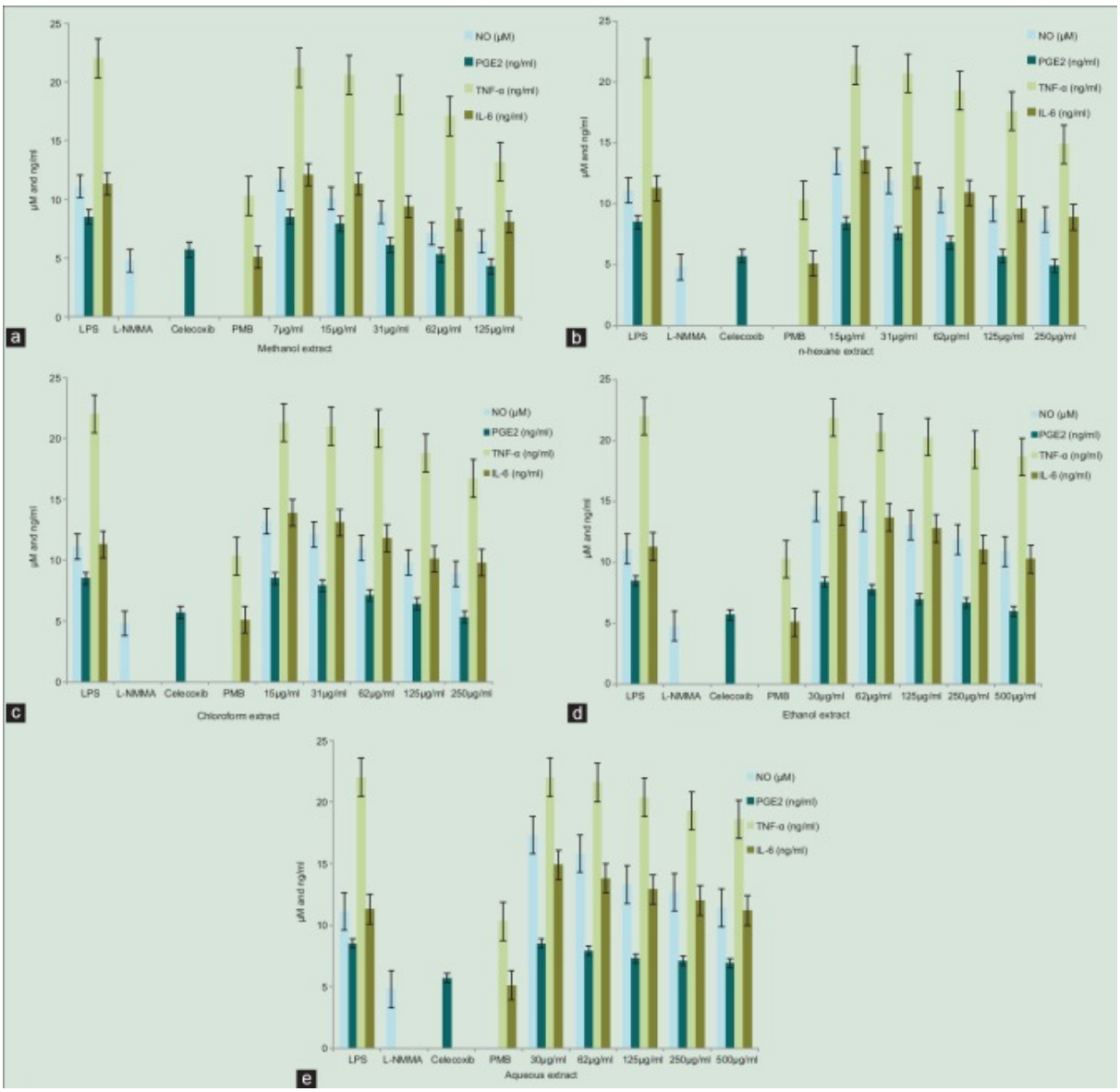
Figures and Tables

Figure 1



Cell viability of RAW 264.7 cells by fractions from *C. vulgaris*. Cells were incubated with various concentration of fractions (16, 32, 64, 125, 250, 500, 1000 µg/ml). Values were expressed as mean ± standard deviation for three independent experiments performed in triplicate

Figure 2



Effects of *C. vulgaris* extracts and fractions on the production of lipopolysaccharide-induced nitric oxide, prostaglandin E_2, tumor necrosis factor-α, interleukin 6. RAW 264.7 cells were incubated with various concentrations of (a) methanolic extracts, (b) hexane extracts, (c) chloroform extracts, (d) ethanol extracts, (e) aqueous extracts in the presence of 500 ng/ml lipopolysaccharide. Three independent assays were performedin triplicate and the data shown are the mean ± standard deviation