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Structural characterization and biological activity of polar lipids from microalgae

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October 24, 2016 | Truro, NS



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Microalgae

- Diverse group of organisms consisting of both prokaryotic and eukaryotic forms.
- Potential tool to reduce the proliferation of greenhouse gasses in the atmosphere through the biological fixation of industrial CO₂ emissions. Extraordinary potential for cultivation as energy crops.
- Source of high value products including pigments, ω -3 fatty acids (EPA & DHA), proteins and other bioactives.
- Aquaculture and animal feed application, source of pigments e.g. astaxanthin, ω -3 fatty acids and protein.



Commercial products derived from microalgae

Algal carbon conversion (ACC) flagship program

Goal: to provide Canadian industry with a cost-competitive, value-generating solution to divert CO₂ emissions into algal biomass, which can then be processed into biofuels and other marketable products.



A demonstration-scale algal biorefinery

- The primary objective of the ACC Flagship is for NRC and its partners to establish a pilot-scale algal bio-refinery at an industrial site.
- The biorefinery demonstration facility will grow algal biomass while remediating wastewater and recycling industrial CO₂ emissions.
- Partners: St Mary's Cement, Pond Technologies, Canadian Natural Resources Limited.



Microalgae – superfoods?

10 healthy foods you're not eating
Eating the same meals day after day is not only boring—you could be missing out on foods that offer vital nutrients and disease-fighting antioxidants. Here's a list of healthy foods you should be eating—but probably aren't

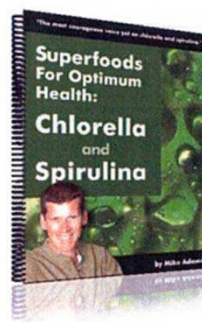
Slide 2 of 11



<http://www.besthealthmag.ca>

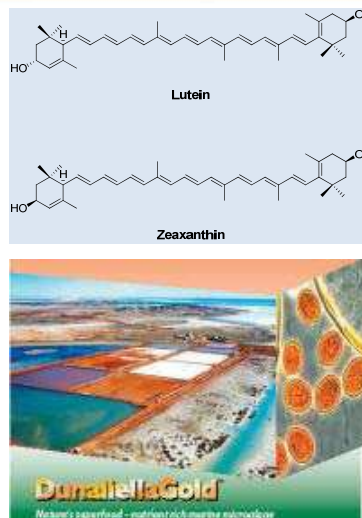
2. Algae

Spirulina, a blue-green algae available at health food stores, is a promising immune-booster. In a May 2008 study, German researchers found that spirulina preparations containing zinc and powdered acerola (a vitamin C-rich tropical fruit) have high anti-inflammatory and antioxidant potential. Further testing is required to determine whether long-term use of spirulina preparations strengthens immune defence and reduces cold and flu risk. (Consult with a doctor or a trained practitioner before trying any dietary supplements.)
Tip: Add spirulina powder to your morning smoothie.



High value product - Pigments

- Global carotenoid market is about \$887 million to \$1 billion. Astaxanthin (AstaPure™), β -Carotene (DunaliellaGold™) are already in market from microalgal source.
- Lutein is another carotenoids found in the macula, a small area of the retina responsible for central vision.



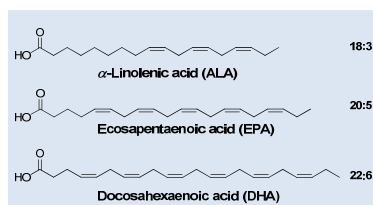
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High value products - Omega-3 Fatty Acids

- The global omega-3 ingredient market is about \$1.2-\$1.5 billion. 1901 ω -3 food product were launched between 1988-2008.
- Current growth of ω -3 market is about 10-13% per year, 85% oils comes from fish and 3% from algal plus higher plants.



<https://www.google.ca> - images of EPA DHA



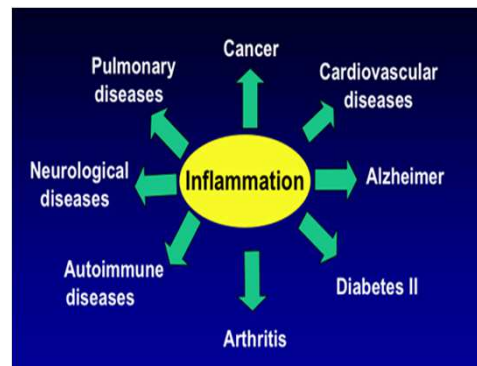
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- **Polar lipids (MGDG, DGDG & betain lipid)**
 - NO inhibitory activity (anti-inflammation)
 - Pancreatic lipase inhibitory activity (anti-obesity)
- **Antioxidative activities of microalgae & lipid composition**

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Inflammation

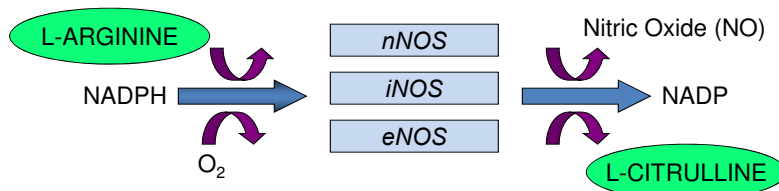
- Inflammation is a complex biological response of body tissue to harmful stimuli.
- Associated with nearly all diseases including cancer, arthritis, obesity, autoimmune diseases and neurological disorders.
- New anti-inflammatory drugs or supplement may help for the treatment of chronic diseases.



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Nitric oxide (NO) and inflammation

- Nitric oxide (NO) is an important signaling molecule synthesized by the enzymatic oxidation of the amino acid, L-arginine into L-citrulline.
- Among three types of nitric oxide synthase (NOS) neuronal (nNOS) and epithelial (eNOS) are constitutively expressed in mammalian cells and synthesis NO in response to the increase in intracellular calcium levels.
- Inducible nitric oxide synthase (iNOS) is only expressed when certain cells are activated by specific pro-inflammatory agents such as endotoxin, LPS, TNF, IFN- γ and IL-1. Once the iNOS is induced, it produces NO in uncontrollable amount. Excessive production of NO has detrimental effects on many organ systems of the body leading to tissue damage, even leading to a fetal development (septic shock).



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Nitric oxide inhibitory activity of microalgae

Microalgae	Strain ID	MeOH extract % Yield	% NO inhibition at 50 μ g/mL
<i>Bothryococcus braunii</i>	UTEX 572	19.3	-22.3 \pm 3.5
<i>Chlorella sorkiniana</i>	UTEX 1230	23.4	12.1 \pm 1.7 *
<i>Chlorella vulgaris</i>	UTEX 26	19.3	0.1 \pm 1.7
<i>Isochrysis galbana</i>	CCMP 1324	35.3	9.8 \pm 2.6 *
<i>Nannochloropsis granulata</i>	CCMP 535	51.3	1.2 \pm 2.0
<i>Neochloris oleabundans</i>	UTEX 1185	26.2	15.0 \pm 11.8
<i>Pavlova lutheri</i>	CCMP 1325	30.1	19.9 \pm 8.7 *
<i>Pavlova pinguis</i>	CCMP 609	36.9	29.9 \pm 7.7 *
<i>Phaeodactylum tricornerutum</i>	CCMP 1327	37.5	7.0 \pm 3.1 *
<i>Porphyridium aerugineum</i>	UTEX 755	12.1	20.3 \pm 7.2 *
<i>Scenedesmus dimorphus</i>	UTEX 746	11.8	8.3 \pm 2.5 *
<i>Tetraselmis chui</i>	PLY 429	32.4	47.7 \pm 0.3 *

NO inhibitory activity were tested on lipopolysaccharide (LPS)-induced NO production in RAW264.7 macrophage cells. Each value represents the mean \pm of three determinations. Significantly different from the LPS treated control: * $p < 0.05$, ** $p < 0.01$

UTEX = The Culture Collection of Algae, University of Texas at Austin, USA, CCMP = National Centre for Culture of Marine Phytoplankton, Maine, USA, PLY = Plymouth Culture Collection of Marine Microalgae, Plymouth, UK.

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Tetraselmis chui

- *T. chui* is a marine unicellular alga.
- Studied well for the algal-biofuel production.
- Not much chemistry has been done except fatty acid and pigments analyses.



1000 L Brite Box, lid removed showing microalgae culture



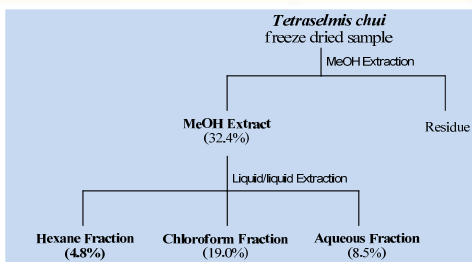
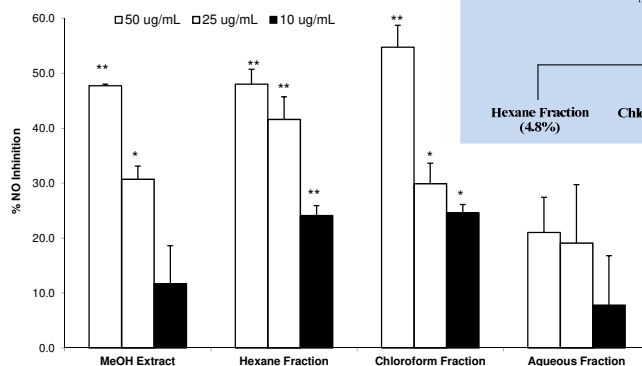
Tetraselmis chui

Anti-inflammatory agents

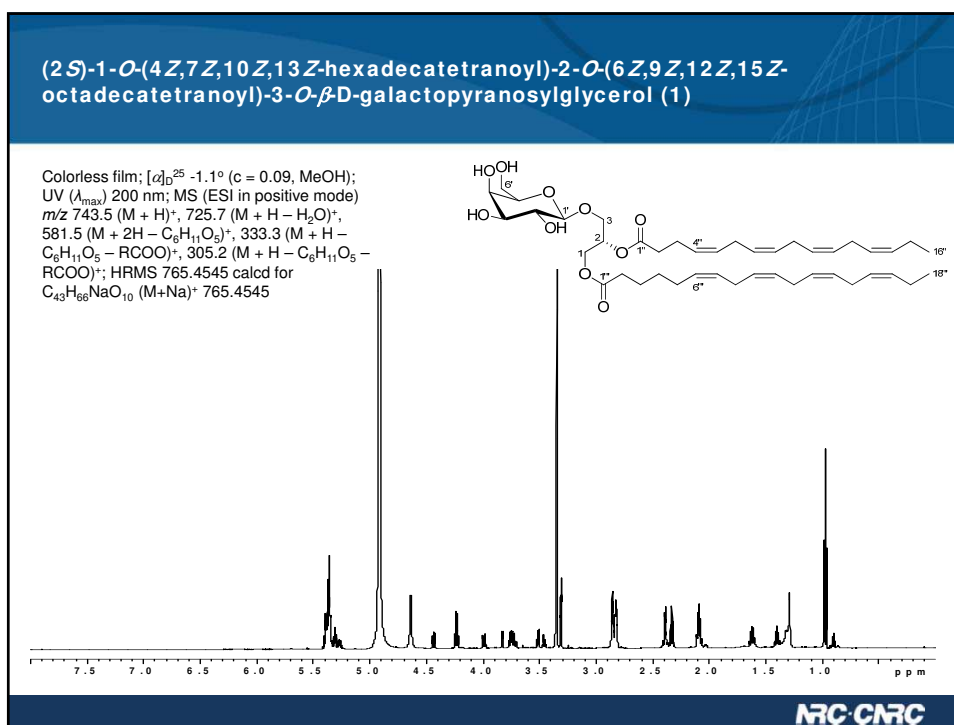
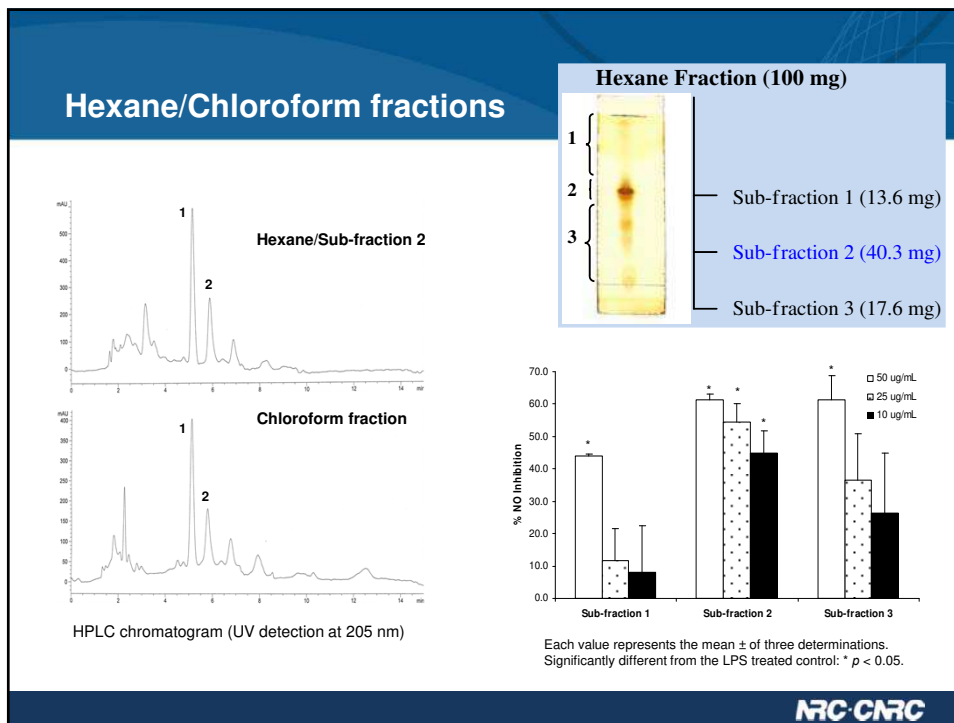


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T. Chui – Extraction and fractionation

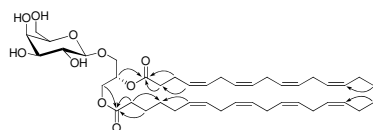


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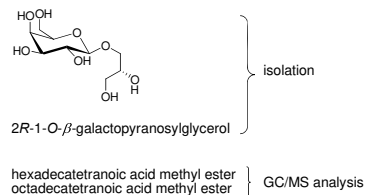


(2*S*)-1-*O*-(4*Z*,7*Z*,10*Z*,13*Z*-hexadecatetraenyl)-2-*O*-(6*Z*,9*Z*,12*Z*,15*Z*-octadecatetraenyl)-3-*O*-β-D-galactopyranosylglycerol (1)

Key HMBC Correlations of 1



NaOMe/MeOH

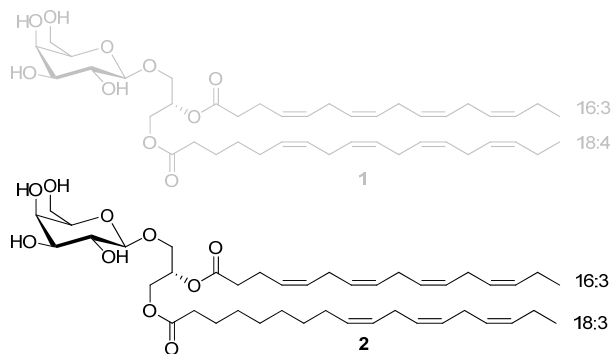


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(2*S*)-1-*O*-(4*Z*,7*Z*,10*Z*,13*Z*-hexadecatetraenyl)-2-*O*-(9*Z*,12*Z*,15*Z*-octadecatetraenyl)-3-*O*-β-D-galactopyranosylglycerol (2)

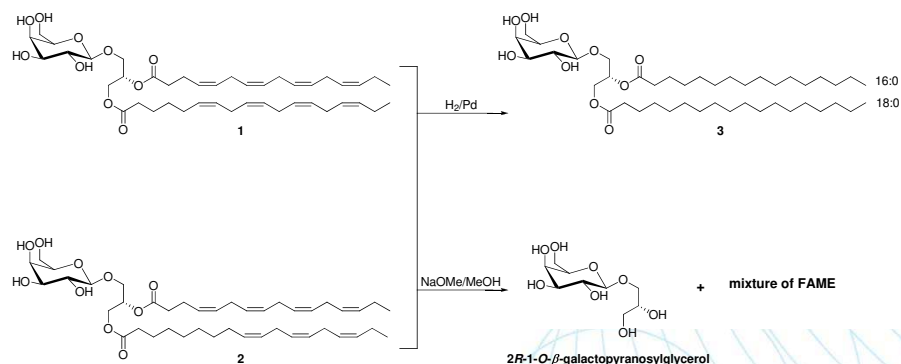
$[\alpha]_D^{25}$ -3.7° (c = 0.08, CHCl₃); UV (λ_{max}) 200 nm; IR n_{max} (neat) 3400, 2920, 1720, 1080, 1020 cm⁻¹; MS (ESI in positive mode) m/z 745.5 (M + H)⁺; HRESIMS 767.4685 calcd for C₄₃H₆₈NaO₁₀ (M+Na)⁺ 767.4710.



Greca M. D. et al., *Gazzeta Chimica Italiana* 1989, 119, 549-551

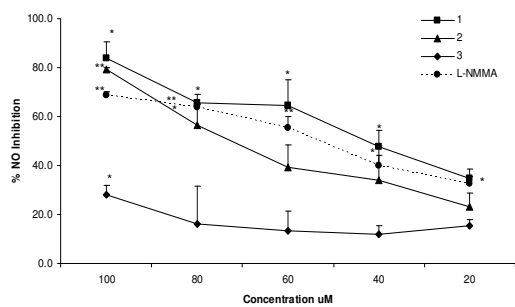
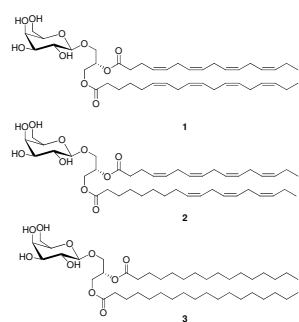
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Chemical modification of MGDGs



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NO Inhibitory activity of MGDGs



Each value represents the mean \pm of three determinations. Significantly different from the LPS treated control: * $p < 0.05$, ** $p < 0.01$

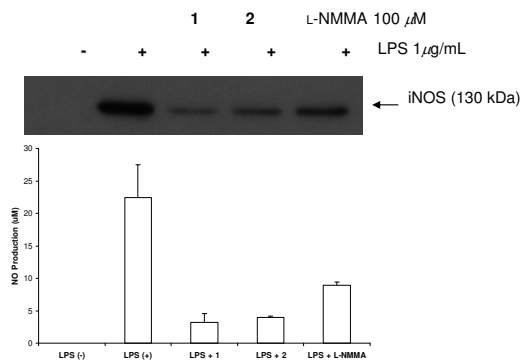
2R-1-O-β-Galactopyranosylglycerol showed no NO inhibition.

Mixture methyl esters of PUFAs showed cytotoxic effects towards RAW264.7 macrophage cells.

N^G-methyl-L-arginine acetate salt (L-NMMA), a well known NO inhibitor used as a positive control.

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iNOS Expression of MGDGs

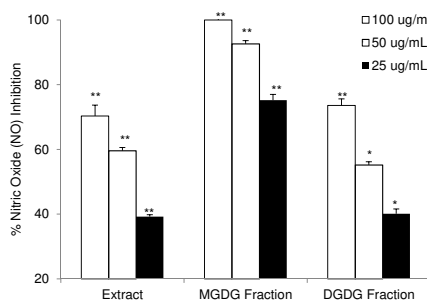


Raw 264.7 cells were treated with LPS (1 µg/mL) for 24 h with or without tested compounds.

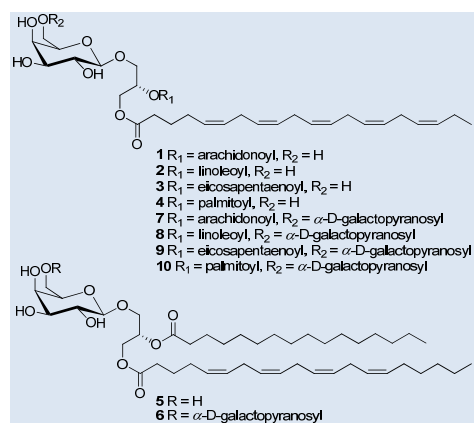
Banskota et. al., *Natural Product Research* 2013, 27, 1084-1090.

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Galactolipids from *Porphyridium aerugineum*

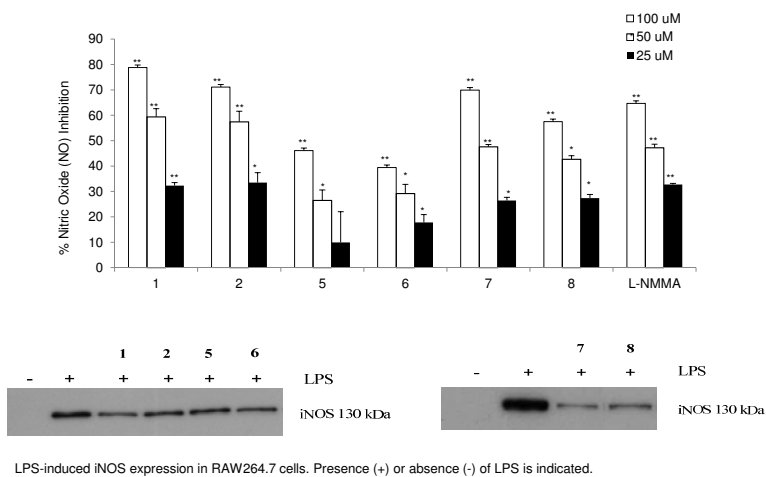


Nitric Oxide (NO) inhibitory effect of the MeOH/CHCl₃ (1:1) extract and galactolipids rich fractions. Significantly different from the LPS treated control: * $p < 0.05$, ** $p < 0.01$



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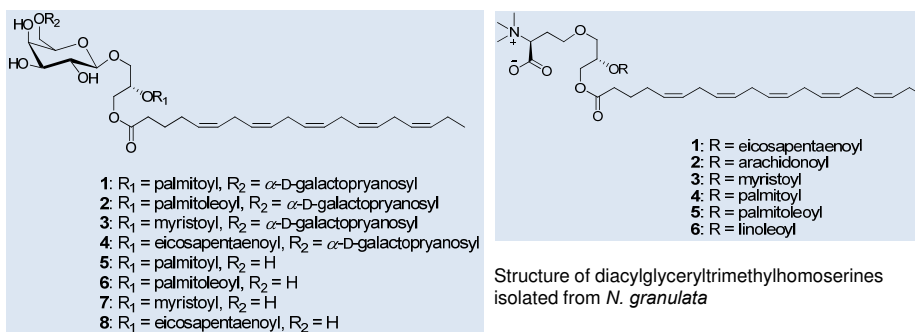
Galactolipids from *Porphyridium aeruginosum*



Banskota et al., *Journal of Applied Physiology* 2013, 25, 951-960.

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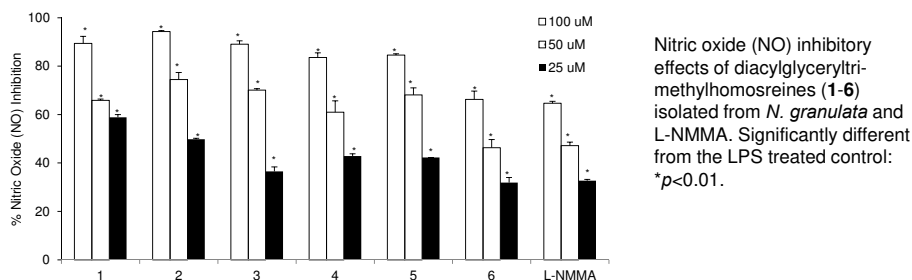
Polar lipids from *Nannochloropsis granulata*



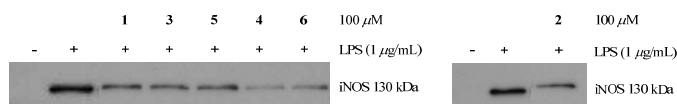
Banskota et al., *Journal of Applied Physiology* 2013, 25, 349-357.

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NO inhibitory activity of betain lipids



Inhibitory effects of diacylglyceryltrimethylhomoserines (1-6) isolated from *N. granulata* on iNOS expression



Banskota et al., *Journal of Applied Phycology* 2013, 25, 1513-1521.

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Microalgal polar lipids – possible new anti-inflammatory agents!!!



- Galactolipids from higher plants reported to have anti-inflammatory activity and in rose-hip preparation use for treatment of arthritis galactolipid is considered as active component.
- Polar lipids isolated from microalgae showed NO inhibitory activity through down-regulation of iNOS, strongly suggested their possible use as anti-inflammatory agents.

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Lipase Inhibition - Obesity

- Obesity is a growing global health problem; more than 1.1 billion people worldwide are above their ideal weight, and 312 million of them are obese.
- Obesity is associated with many diseases, including diabetes, hypertension, and heart disease.
- Inhibition of pancreatic lipase activity is one of the promising targets for the development of new anti-obesity nutraceutical/pharmaceutical products by reducing energy intake through gastrointestinal mechanisms.
- One of the few drugs that is currently available for the treatment of obesity is orlistat, which reduces intestinal fat absorption via inhibiting pancreatic lipase.

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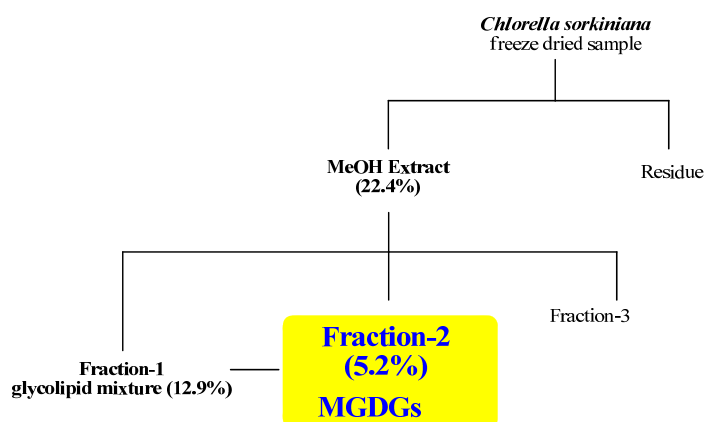
Chlorella sorokiniana – Lipase Inhibitory activity

- *C. sorokiniana* is a freshwater unicellular microalga.
- Reproduce at an extremely fast rate, renewing into four cells in every 17- 24 h. Used extensively as a model system to study enzymes involved in higher plant metabolism.
- Nutraceutical products as a whole algal biomass (Crypto Power, Chlorella Bio Clearing, FEBICO) or aqueous extract.



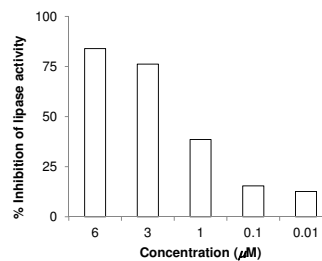
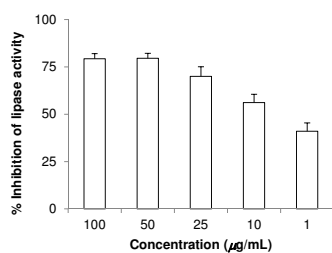
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C. Sorokiniana - Extraction and fractionation



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Lipase inhibitory activity of galactolipids fraction derived from *Chlorella sorokiniana*

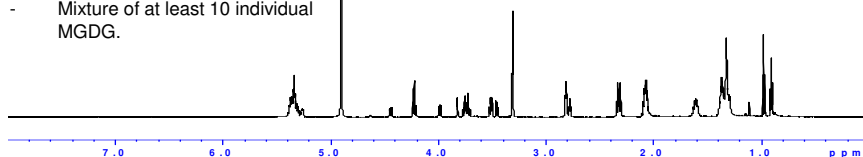
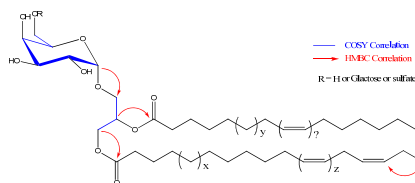


Lipase inhibitory effect of monogalactosyldiacylglycerols (MGDGs) rich fraction derived from *C. sorokiniana* (R) and orlistat (L).

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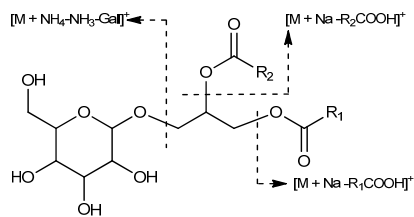
C. Sorokiniana - Monogalactosyldiacylglycerols (MGDGs)

- Preliminary NMR study suggested that the presence of significant amount of monogalactosyldiacylglycerol (MGDG) in the glycolipid rich fraction.
- Presence of both essential fatty acids *i.e.*, linoleic acid [LA 18:2 (n-6)] and α -linolenic acid [ALA 18:3 (n-3)] in individual MGDG.
- Mixture of at least 10 individual MGDG.

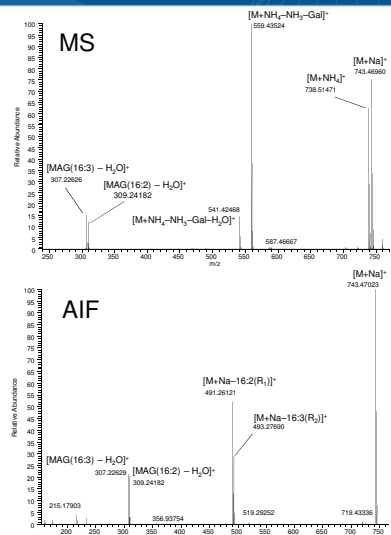


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Structure elucidation of MGDGs by LC/MS analysis

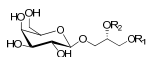


Fragmentation pattern of MGDG following different pathways for ammonium or sodium adducts. Top-Right) positive-ion MS spectrum. Bottom-Right) positive-ion all-ion fragmentation (AIF) spectrum.



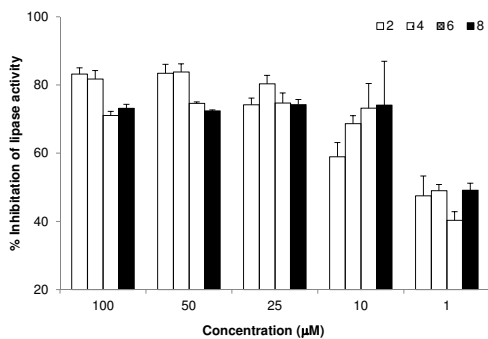
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Lipase inhibitory activity of galactolipids



- 1 R₁ = 7Z,10Z-hexadecadienyl, 16:2, R₂ = 7Z,10Z,14Z-hexadecatrienyl
- 2 R₁ = ω -linolenyl, R₂ = 7Z,10Z,14Z-hexadecatrienyl
- 3 R₁ = 7Z,10Z-hexadecadienyl, R₂ = 7Z,10Z-hexadecadienyl
- 4 R₁ = linoleyl, R₂ = 7Z,10Z,14Z-hexadecatrienyl
- 5 R₁ = ω -linolenyl, R₂ = 7Z,10Z-hexadecadienyl
- 6 R₁ = linoleyl, R₂ = 7Z,10Z-hexadecadienyl
- 7 R₁ = linoleyl, R₂ = palmitoleyl
- 8 R₁ = oleoyl, R₂ = 7Z,10Z-hexadecadienyl
- 9 R₁ = linoleyl, R₂ = palmitoyl
- 10 R₁ = oleoyl, R₂ = palmitoleyl

Galactolipids especially MGDGs may be a new anti-obesity agent - further *in-vivo* efficacy test is needed to know the anti-obesity potency.



Lipase inhibitory effect of major MGDGs present in *C. sorokiniana*

Journal of Applied Phycology 2016, 28 169–175.

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