Medicinal & Aromatic Plants
ISSN: 2167-0412

Powers et al., Med Aromat Plants (Los Angels) 2017, 6:3
DOI: 10.4172/2167-0412.1000293

Short Communication Open Access

Activity of Green Algae Extracts against *Toxoplasma gondii*

Jonathan L Powers[1#], Xuejin Zhang[1#], Chi Yong Kim[1], Daniel A Abugri[2] and William H Witola[1*]

[1]*Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL 61802, USA*

[2]*Department of Chemistry, College of Arts and Sciences, Tuskegee University, Tuskegee, Alabama, USA*

[#]*Contributed equally*

[*]**Corresponding author:** William H Witola, Department of Pathobiology, College of Veterinary Medicine, University of Illinois, Urbana-Champaign, 2001 South Lincoln Avenue, Urbana, IL 61802, USA, Tel: 217-300-3439; Fax: +217-244-7421; E-mail: whwit35@illinois.edu

Received date: June 07, 2017; **Accepted date:** June 20, 2017; **Published date:** June 25, 2017

Abstract

Toxoplasma gondii is a zoonotic protozoa of economic significance in livestock. Infected livestock meat and products act as a source of *T. gondii* infection in humans. Current drugs against *T. gondii* are limited by hypersensitivity and toxicity, and are not effective against the encysted bradyzoite stage of *T. gondii*. Thus, there is urgent need for safe and effective therapeutic agents against *T. gondii*. Marine algae possess potent antifungal and antibacterial properties, but there are no reports on its anti-protozoal activity. Therefore, in this study we obtained n-hexane and methanol extracts of green algae (*Chlorophyceae*) and analyzed their content by high performance liquid chromatography coupled with mass spectrometry, as well as tested their *in vitro* anti-*Toxoplasma* activities. Compared to the n-hexane extract of *Chlorophyceae*, the methanol extract contained higher content of flavonoids/polyphenols, alkaloids (elaeocarpidine and auramine), and artemisic acid. Importantly, the methanol extract had more potent anti-*Toxoplasma* activity (IC_{50}=4.43 ± 1.26 µg/mL) than the n-hexane extract (IC_{50}=23.32 ± 3.97 µg/mL), corroborating the higher content of flavonoids, alkaloids, and artemisic acid in methanol extracts than in n-hexane extract. The anti-*Toxoplasma* IC_{50} values of the methanol and n-hexane extracts were 34-fold and 7-fold lower than their respective cytotoxic IC_{50} values in human fibroblast cell line. Consistent with our findings, flavonoids, alkaloids and artemisic acid have previously been shown to have potent anti-*Toxoplasma* activity. Together, our results show that *Chlorophyceae* contains significant amounts of bioactive compounds with potent anti-*Toxoplasma* activity.

Keywords: Green algae chlorophyceae; *Toxoplasma gondii*; Antiparasitic; Bioactive compounds

Short Communication

Toxoplasma gondii is zoonotic protozoa that is very prevalent worldwide and a major cause of abortions and neonatal deaths in sheep and other livestock, resulting in significant economic losses [1]. Infected livestock meat and products act as a source of *T. gondii* infection in humans [2]. Despite these challenges, there is currently no medicine that can eliminate *T. gondii* infection, particularly the encysted stage. Thus, there is urgent need to develop a generation of safe and efficacious drugs for use in controlling *T. gondii* infections. Marine algae contain significant amounts of bioactive compounds [3] and as a result has been shown to possess potent antifungal and antibacterial properties [4]. However, there has been no reports on its anti-protozoal properties. Therefore, in this study we endeavored to evaluate the *in vitro* anti-*Toxoplasma* activity of green algae extracts.

Dried, pure cultured green algae, *Chlorophyceae* was generously provided by Dr. Wei Liao of the Anaerobic Digestion Research and Education Center, Michigan State University. Dried algae samples were finely milled and soaked in methanol or n-hexane for 24 hours with agitation. The extraction solution was filtered, evaporated to dryness and the extracts reconstituted in dimethyl sulfoxide (DMSO) to desired concentration. *T. gondii* RH strain, engineered to constitutively express cytosolic yellow fluorescent protein (RH-YFP) [5] was cultured in human foreskin fibroblasts (HFF) maintained in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Life Technologies), 1% (v/v) Glutamax and 1% (v/v) penicillin-streptomycin-fungizone (Life Technologies) at 37˚C with 5% CO_2. *T. gondii* tachyzoites were extracted from HFF cells by passing the cell suspension twice through a 25-gauge needle, and extruded parasites isolated from cell debris by passing through a 3 µM filter, followed by washing the filtered parasites in PBS.

To determine the effect of the algae extracts on the growth of *T. gondii*, HFF cells were grown to confluence in 96-well plates. Prior, to infection, old medium was replaced with fresh medium, and freshly extracted *T. gondii* added to the HFF cultures at 1000 tachyzoites per well. Test algae extracts at increasing concentrations were added immediately after parasite inoculation. Cultures without compounds (but with similar volume of DMSO) were maintained as controls. At, 24, 48 and 72 hour post-infection, parasite proliferation was determined using an image Xpress micro automated fluorescence microscope by measuring the parasite YFP fluorescence followed by quantification of YFP intensity using ImageJ version 1.37v software (NIH). We found that at 72 hours of culture, the methanol and n-hexane extracts of *Chlorophyceae* algae exhibited dose-dependent inhibitory effect on the intracellular growth of *T. gondii* tachyzoites (Figure 1A and 1B). However, at earlier times of 24 and 48 hours post-infection, there was no significant difference in parasite growth between the algae extract-treated and the DMSO-treated parasites. This suggested that the bioactive compounds from algae could have acted by inhibiting the invasion of host cells by egressed parasites, assuming that during the first 48 hours post-infection, the parasites would mostly be growing only in cells infected initially. Between 48 and 72 hours post-infection, it is expected that the first egress would

have occurred and the egressed parasites would have infected new host cells and started growing intracellularly [6], thereby increasing the number of parasites in the culture substantially. However, in the presence of the algae bioactive compound, the egressed parasites would be directly exposed and internalize considerable amounts of the compounds, which would then compromise the parasites' infectivity and ability to proliferate in host cells.

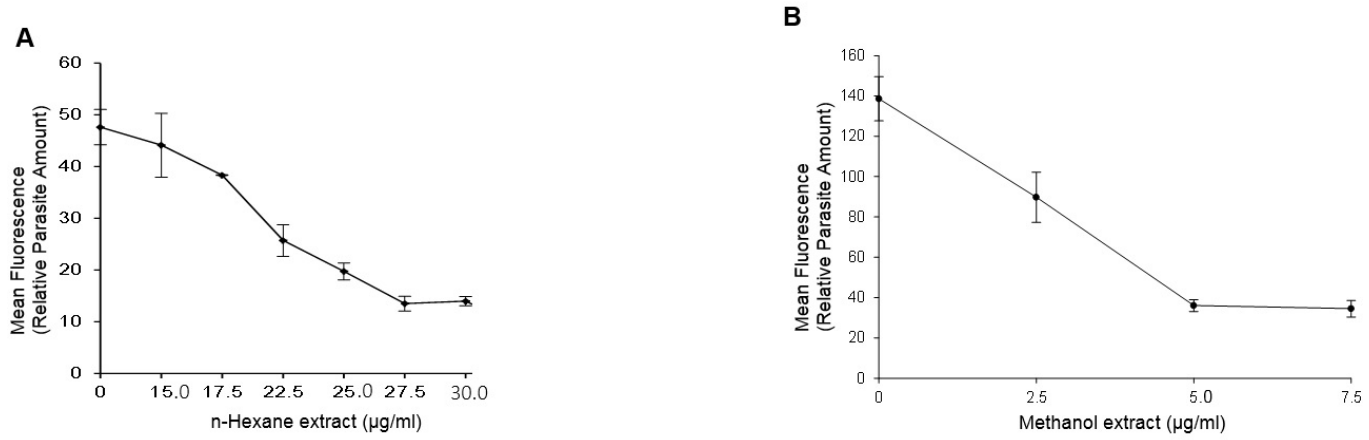

Figure 1: Growth curves for *T. gondii* tachyzoites in cultures treated with varying concentrations of *Chlorophyceae* extracts. Confluent human foreskin fibroblasts (HFF) were inoculated with *T. gondii* tachyzoites and immediately treated with varying concentrations (µg/ml) of (A) n-hexane extract, and (B) methanol extract for 72 hours. The fluorescence generated by the parasites is proportional to the amount of parasites in culture and is depicted as the mean fluorescence on the Y-axis. The data shown represent means of three independent experiments with standard error bars.

By nonlinear regression analysis, using Graph Pad Prism software version 6.0 program (CA, USA), we found that the *T. gondii* growth inhibitory concentration (IC_{50}) values for the methanol and n-hexane *Chlorophyceae* extracts were, 4.43 ± 1.26 µg/mL and 23.32 ± 3.97 µg/mL, respectively. This indicated that, the methanol extract had more potent activity than the n-hexane extract, suggesting that the methanol extract was more enriched in bioactive compounds content than the n-hexane extract. We, therefore, performed mass-spectrometry analysis to determine the major constituents in the n-hexane and methanol. The extracts were analyzed by High Performance Liquid Chromatography (HPLC) and Electrospray Ionization-Mass spectrometry (ESI-MS) at the Mass Spectrometry facility, University of Illinois at Urbana-Champaign. Briefly, equal amounts of the extracts were run on HPLC using a 2.1 mm ID reverse phase C-18 column with the mobile phase composed of 5% acetonitrile/95% water/0.1% formic acid, followed by ESI-MS analysis in negative mode, operated by Masslynx software v.4.1. Based on the elemental composition of the extracts, as determined by ESI-MS analysis, the methanol extract of *Chlorophyceae* contained higher content of flavonoids/polyphenols, alkaloids (elaeocarpidine and auramine), and artemisic acid with mass numbers of 256.95, 2667.17 and 296.24, respectively, than the n-hexane extract (Figure 2A and 2B).

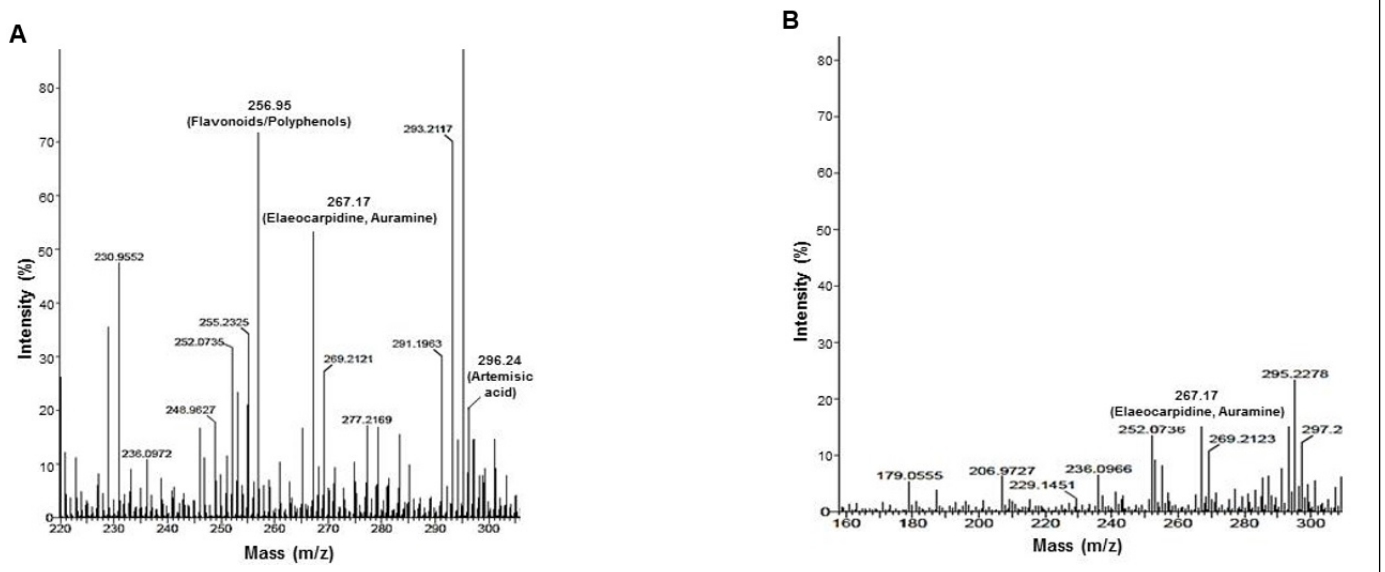

Figure 2: ESI-LC/MS chromatogram of *Chlorophyceae* extracts. (A) Methanol extract of *Chlorophyceae* contained high intensity of flavonoids, alkaloids (Eaeocarpidine/auramine) and artemisic acid that have been reported to have antiprotozoal activity. (B) The n-Hexane extract contained detectable levels of alkaloids (Eaeocarpidine/auramine) but not flavanoids nor artemisic acid.

Consistent with our findings in the present study, in our previous study, we have found that Sorghum bicolor red leaf extract fraction that was rich in flavonoids content, had potent *in vitro* activity against *T. gondii* at dose levels that were non-toxic to mammalian cells [7]. Corroborating our observations in the present study, alkaloids have also been previously shown to have anti-*Toxoplasma* activity *in vitro* [8]. Additionally, artemisic acid has been documented to have potent activity against a broad range of protozoan parasites including *Plasmodium, Leishmania, Trypanosoma, Toxoplasma, Neospora, Eimeria, Acanthamoeba, Naegleria, Cryptosporidium, Giardia* and *Babesia* [9]. Therefore, the anti-*Toxoplasma* activities of the methanol *Chlorophyceae* extracts can be attributed to the high content of flavonoids, alkaloids and artemisic acid.

To determine the cytotoxic IC_{50} values of the *Chlorophyceae* extracts in mammalian cells, HFF cells were cultured in supplemented IMDM medium (without red phenol) in 96-well plates and varying concentrations of the extracts added. Wells to which equivalent volumes of DMSO only were added were included as negative control. At 72 hours of culture, a colorimetric assay using the cell proliferation reagent WST-1 (Roche) for the quantification of cell viability was performed on the cultures by adding 20 µL of the WST-1 reagent to each well. After mixing, the plates were wrapped in aluminum foil and incubated for 1 hour at 37 ˚C with 5% CO_2. After 1 hour of incubation, 150 µL of the medium from each well was transferred to a new 96-well plate and quantification of the formazan dye produced by metabolically active cells was read as absorbance at a wavelength of 420 nm using a scanning multi-well spectrophotometer (Spectra Max 250; Molecular Devices). We generated dose–response curves using GraphPad PRISM software and found that the HFF cytotoxicity values for the methanol and n-hexane *Chlorophyceae* extracts were 150.83 ± 12.94 and 159.52 ± 15.71, respectively. Thus, the n-hexane and methanol *Chlorophyceae* extracts had *T. gondii* IC_{50} values that were 34-fold and 7-fold lower than their respective cytotoxic IC_{50} values in HFF host cells. This indicated that both extracts have potent *in vitro* inhibitory activity against *T. gondii* parasites at concentrations that are non-toxic to mammalian cells suggesting that they may not have adverse side-effects *in vivo*. Together, our findings demonstrate that green algae, *Chlorophyceae*, contains significant amounts of bioactive compounds that possess potent anti-*Toxoplasma* activity.

Acknowledgements

This study was funded in part by the University of Illinois at Urbana-Champaign. We are grateful to Dr. Furong Sun of the Mass Spectrometry facility, School of Chemical Sciences at the University of Illinois at Urbana-Champaign for the help in ESI-MS analysis of the of the extracts used in this study. We thank Dr. Wei Liao of the Anaerobic Digestion Research and Education Center, Michigan State University, for generously providing the *Chlorophyceae* algae.

References

1. Lindsay DS, Dubey JP (2014) Toxoplasmosis in wild and domestic animals. In: Weiss LM, Kim K (eds). Toxoplasma gondii: the model apicomplexan 2nd edn. Elsevier, Amsterdam, The Netherlands pp: 194-209.
2. Cook AJ, Gilbert RE, Buffolano W, Zufferey J, Petersen ES, et al. (2000) Sources of Toxoplasma infection in pregnant women: European multicenter case-control study. European research network on Congenital Toxoplasmosis. BMJ 321: 142-147.
3. Lordan S, Smyth TJ, Soler-Vila A, Stanton C, Ross RP (2013) The α-amylase and α-glucosidase inhibitory effects of Irish seaweed extracts. Food Chem 141: 2170-2176.
4. Kellam SJ, Cannell RJP, Owsianka AM, Walker JM (1988) Results of a large-scale screening programme to detect antifungal activity from marine and freshwater microalgae in laboratory culture. Brit Phycol J 23: 45-47.
5. Gubbels MJ, Li C, Striepen B (2003) High-throughput growth assay for Toxoplasma gondii using yellow fluorescent protein. Antimicrob. Agents Chemother 47: 309-316.

6. Radke JR, White MW (1998) A cell cycle model for the tachyzoite of Toxoplasma gondii using the Herpes simplex virus thymidine kinase. Mol Biochem Parasitol 94: 237-247.
7. Abugri DA, Witola WH, Jaynes JM, Toufic N (2016) *In vitro* activity of Sorghum bicolor extracts, 3-deoxyanthocyanidins, against Toxoplasma gondii. Exp Parasitol 164: 12-19.
8. Alomar ML, Rasse-Suriani FA, Ganuza A, Cóceres VM, Cabrerizo FM, et al. (2013) *In vitro* evaluation of β-carboline alkaloids as potential anti-Toxoplasma agents. BMC Notes 6: 193.
9. Loo CS, Lam NS, Yu D, Su XZ, Lu F (2017) Artemisinin and its derivatives in treating protozoan infections beyond malaria. Pharmacol Res 117: 192-217.