Phytochemical Screening: *Nanochlorum spp.*

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Abstract

This study attempted to screen selected phytochemicals in the ethanolic, methanolic and acetone extracts of *Nanochlorum spp.* The extracts were subjected to the following tests: Test tube method for Alkaloid Analysis (Confirmatory test, test for quaternary bases and/or amine oxide), Test Tube Reaction Methods for Steroids (Keller-Kiliani test: test for 2-deoxysugars), the Bornträger's Test for Anthraquinones, Test tube Screening Methods for Flavonoids (Test for leucoanthocyanins: Bate-Smith and Metcalf method), and the Test tube Screening Method for Tannins (Ferric chloride test). Results showed that in all *Nanochlorum spp.* extract, steroids, anthraquinones and tannins were positive (with slight precipitation) but negative with alkaloids and flavonoids.

Keywords: *Nanochlorum spp.*, phytochemicals, screening

Plants produce a great number of compounds of various chemical structures and can be considered as the most prolific laboratories in the world. Guevara (2006) stated that present interest is on the secondary metabolites (including the alkaloids, steroids, flavonoids, etc.), and though relatively less abundant in plants than the primary metabolites (carbohydrates, proteins, and lipids) are of higher value especially because of their pharmacological activities (Chen, et. al., 2004).

Algae, particularly green algae, are widely distributed throughout the world, and living in different water ecosystems, rocky coastline, and terrestrial environments with ample moisture. They are also found in damp soil, attached to land plants (few are parasites), and even in snow or ice (Steinman, 2005). Each has its own morphological and physical characteristics but somehow they have similar effects on environments and living things.

Nanochlorum spp. are phytoplanktons that belong to and same phylum as microalgae (e.g. *Chlorella sorokiniana*, *Chlorella vulgaris*) are among the major producers of food in the sea. Even though individually small in size,

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they contribute an enormous quantity to the world's supply of organic matter (Trainor, 1978). Like the microalgae, they may have primary metabolites like carbohydrates, lipids, and proteins but the presence of secondary metabolites or phytochemicals have not been reported.

Phytochemical research has progressed as more and more plant constituents are being isolated and identified. With more systematic methods of screening bioactive constituents in plants (Guevara, 2006), it is hoped that rapid success will be realized in the phytochemical studies of Philippines plants since it is estimated that new compounds are being discovered and described at a rate of one per day (Aguinaldo, Ramos, Santos, & Nonato, 2004). These compounds could be either harmful or valuable for living organisms. Record studies showed that phytochemicals have hundreds of uses but productivity cost was the main problem for these researches. Researchers continue to find ways to lessen this problem. It was the aim of this study to screen for selected but valuable phytochemicals present in *Nanochlorum spp*.

Methodology

This descriptive study was conducted at the West Visayas State University Chemistry laboratory using *Nanochlorum spp.* from the Aquaculture department (AQD) of the Southeast Asian Fisheries Development Center (SEAFDEC) located in Tigbauan, Iloilo, Philippines. Water source of *Nanochlorum spp.* was filtered using suction filtration, and the filter papers were soaked in three different solvents for 48 hours in a shaker. The solvents were then made to evaporate using rotary evaporator until syrupy, and then left and kept in a refrigerator for analysis. Duplicates of each of the three extracts (ethanolic, methanolic, and acetone) were tested for the presence or absence of the five secondary metabolites such as alkaloids, steroids, anthraquinones, flavonoids and tannins.

While waiting for 48 hours on the shaker, the following tests reagents were prepared.

Alkaloid Reagents

Mayer's reagent. 1.4 g of Mercuric oxide was dissolved in 60 mL water. The resulting solution was then poured into a solution of 5.0 g KI in 10 mL of water. Enough water was then added to make 100 mL.

Dragendorff's reagent. Equal parts of solution A (0.85 g bismuth(III) nitrate dissolved in a mixture of 10 mL acetic acid and 40 mL water) and solution B (8.0 g of KI dissolved in 20 mL of water) were mixed well and stored in dark bottle at room temperature. This solution served as the stock solution.

One ml of the stock solution was mixed with 2 mL acetic acid and 10 mL water to prepare the Dragendorff's reagent.

Steroid Reagent

For Iron (III) chloride reagent, 3 mL of 1% $FeCl_3$ was dissolved in 50 mL of glacial acetic acid.

Test Proper

Two replicates of each of the extract were analyzed using standard procedures: laboratory test tube method for Alkaloids analysis (confirmatory test, and test for quaternary bases and/or amine oxide); test tube reaction methods for Steroids (Keller-Kiliani test for 2-deoxysugars); Borntrager's test tube screening method for Anthraquinones; test tube screening methods for Flavonoids (test for leucoanthocyanins: Bate-Smith and Metcalf method); and the test tube screening method for Tannins (Ferric chloride test) (Guevarra, 2006). Results of the tests were recorded and tabulated in a table.

Results and Discussion

Table 1 below shows positive results for steroids anthraquinones and tannins, and negative results for alkaloids and flavonoids. The table further shows that the degree of precipitation (+) are the same for all secondary metabolites present and for all the extracts.

Results showed that alkaloids and flavonoids were absent in *Nanochlorum spp.* extracts, but steroids, anthraquinones, and tannins were present though in very minimal amount as shown by the slight precipitation. The slight precipitation may be attributed to the fact that the amount of *Nanochlorum spp.* used for analysis was also minute, or the presence of these secondary metabolites may have been affected by the maturity of the plant used.

Table 1

Phytochemical Analysis of Nanochlorum spp.

Extracts	Phytochemicals Tested				
	Alkaloids	Steroids	Anthraquinones	Flavonoids	Tannins
Methanol					
(R1)	(-) (PA)	blue (+) (PP)	Red coloration (+) (PP)	(NC) (-) (PA)	Brownish green (+)(PP)
(R2)	(-) (PA)	blue (+) (PP)	Red coloration (+) (PP)	(NC)(-) (PA)	Brownish green (+)(PP)
Ethanol					
(R1)	(-) (PA)	slightly bluish (+) (PP)	Red coloration (+) (PP)	(NC) (-) (PA)	Brownish green (+) (PP)
(R2)	(-) (PA)	slightly bluish (+) (PP)	Red coloration (+) (PP)	(NC) (-) (PA)	Brownish green (+) (PP)
Acetone					
(R1)	(-) (PA)	slightly bluish (+) (PP)	Red coloration (+) (PP)	(NC) (-) (PA)	Brownish green (+) (PP)
(R2)	(-) (PA)	slightly bluish (+) (PP)	Red coloration (+) (PP)	(NC) (-) (PA)	Brownish green (+) (PP)

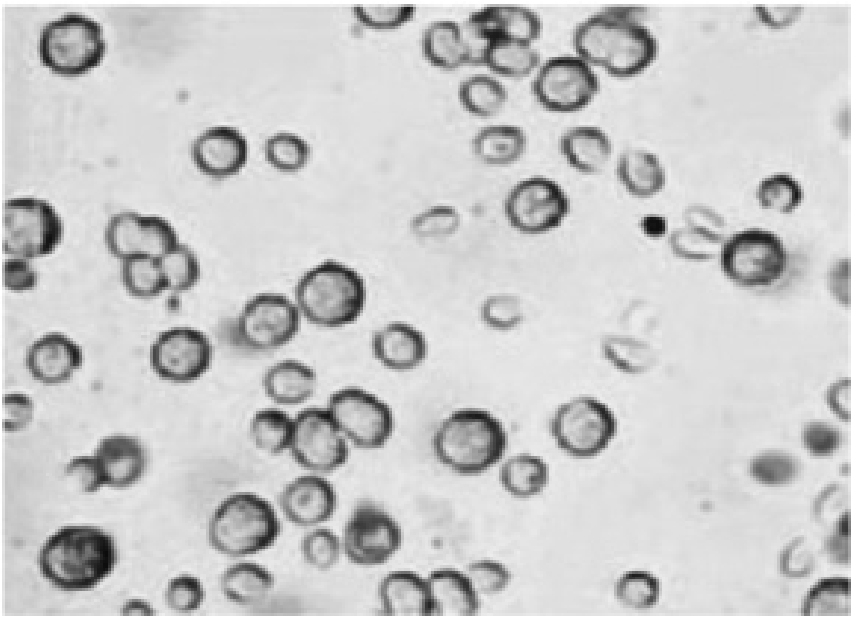
Note: (-) =negative precipitation/negative for test, (+) = slight precipitation/positive on test, (++) =definite precipitation, (+++) =heavy precipitation, NC=no color change, PA=phytochemical absent, PP= phytochemical present

Conclusions and Recommendations

It was concluded that *Nanochlorum spp.* contain, though in minute amounts, steroids, anthraquinones, and tannins and can therefore be a potential source of chemicals of pharmacological and medicinal values. It is further concluded that these phytochemicals can be extracted using any of the solvents used in this study. But it is recommended, that the test should be conducted further in a larger scale using different maturity of *Nanochlorum spp.* and using crude aqueous extract.

Appendices

Appendix 1. Picture of the set-up in the study of *Nanochlorum spp.*cultivation



Appendix 2. Microscopic Picture of *Nanochlorum spp.*

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