

Brine Shrimp Lethality of the Methanol-Dichloromethane Extracts of Anonas (*Annona reticulata*) Leaves and Barks

Alfredo R. Rabena, Ph.D.

Abstract

Leaves and barks of anonas (Annona reticulata) were collected from Wigan City, Ilocos Sur. The samples were chopped into fine pieces and then soaked in methanol. The extract was allowed to pass through a filter paper and the supernatant liquid was concentrated using a rotary evaporator for three hours at 40°C. Dichloromethane was used to get the remaining concentrated extracts in the rotavapor and then evaporated in the fume hood.

Introduction

Rationale

Natural Products Chemistry helps human beings discover solutions and find ways to answer the many questions and problems in life. Natural products range from microorganisms, animals and plants. Plants constitute the highest number of natural products. Such products are results of secondary metabolism that take place in plants particularly in leaves during photosynthesis. Thus, such products are termed secondary metabolites.

Secondary metabolites from plants play a vital role in the lives of people. They are found to be versatile biochemists that lengthen lives, cure diseases, kill microorganisms and even brighten the appearance of materials. On the contrary, they are potent poisons and inhibitions of life.

It is in this context where natural products especially secondary metabolites that are intended for humans are to be screened in order for researchers to determine their ultimate use. Such chemicals will be used in their full potentials.

One of the plants used by people in folk medicine is the *anonas*. Scientifically known as *Annona reticulata*, *anonas* possesses qualities for potency.

The odor of its barks and leaves resembles those of plants previously identified. *Anonas* belongs to the *Anonaceae* family, a group of plants containing drugs potent against cancer.

The main objective of this study was to conduct a bioassay of the dichloromethane extract of both leaves and barks against brine shrimp. Eggs of brine shrimps (*Artemis salina*) were hatched for a day until young stages of the said organism called nauplii were reached. Nauplii stage of *Artemis salina* has cells similar to cancer cells. They are similar in terms of somatic cells particularly in their growth. Such cells are popularly used in the cytotoxicity screening of potential chemicals.

Objectives of the Study

This study aimed to

1. extract the active ingredient found in *anonas* (*nonas reticulata*) leaves and barks;
2. conduct a brine shrimp lethality test of *anonas* extract; and
3. determine the lethality LD₅₀ of the active ingredient of *anonas* extract.

Review of Related Literature

Resurreccion et al (1997) reported studies on the cytotoxic potentials in two *Anabaena* isolates using the disk diffusion method and the brine shrimp lethality test. Lipophilic, hydrophilic and culture medium extracts were assayed against the following microorganisms: *Bacillus subtilis*, *Pseudom aeruginosa* and *Codida albicans* and the brine shrimp *Artemia salina* for two tests, respectively. All extracts were shown to exhibit toxic activity towards the brine shrimp nauplii at varying concentration levels. The lipophilic extracts had the lowest LOS₀ values ranging from 11 mg/ml for Ab 22 to 55 mg/ml for Ab 25.

Sarau and Bermes reported in 1997 that ethanol extracts obtained from fresh seeds of *Raphanus sativus* L and air dried leaves and roots of *Schefflera odorata* B. were tested for their cytotoxic activity on four cell lines; three representing lung pathologist and one cell line representing other categories of hwnan cancer. Results showed that *Raphanus sativus* L., alcoholic, chloroform and aqueous extracts were relatively non-cytotoxic to all cell lines tested with a dose of 1 to 200 mg/ml. The percentage cell survival ranges from 70 to 100% at a dose of 1 to 100 mg/ml while *Schefflera odorata* B. leaves and roots have IC₅₀>20 mg/ml on alcohol, chloroform and aqueous extracts. The required dose on ICS₀ for a potential agent is set at <20 mg/ml as established by the American Nature Cancer Institute.

In 1997, Villasenor and Canlas reported that a new B-sitosteryl glycoride was isolated from *Mentha cordifolia* leaves by solvent partitioning and a bioassay-directed sequential and repeated vacuum liquid chromatography. The acetic acid-induced test showed that B-sitosteryl S D-guloside and its aglycone, B-sitostreyl isolated from the hexane extract of peppermint leaves at a dosage of 100 mg/ml mouse, decreased the number of squamous induced by acetic acid by 73% and 70.0% respectively, statistical analysis used was Kruskal Wallis. One-way Analysis of Variance by ranks showed that these isolates approximate the analgesic activity of mefenamic acid at a 0.01 level of significance. Also in 1997, Guevarra et al found anti-tumor promoters from seeds of *Monija oleifer* L. A new carbonate glycoride and seven km compounds were isolated from the carbon tetrachloride extract. Four of the known compounds; rhamnosyl isothiocyanate, rhamnosyl thocarbonate, acylglycosylterol and glycosylterol significantly inhibited the activation of the Epstein-Barr virus early antigen promoted by the TPA, indicating strong anti-tumor promoting activities. The inhibiting effects of the rhamnosyl thiocarbonate on 2-stage chemical carcinogenesis were eventually tested. The results of the test indicated that the isolates delayed the incidence of formation of papillomas and reduced the number of papillomas per mouse by 40% at 20 weeks promotion, an indication of strong chemopreventive activity.

Methodology

Leaves. Five hundred grams of *anonas* (*Annona reticulata*) leaves were collected and chopped into fine pieces. The chopped leaves were soaked in methanol overnight. The leaves and solvent were filtered and the supernatant liquid was evaporated in a rotary evaporator for three hours at 40°C. The remaining extracts after rotary evaporation were soaked in dichloromethane and placed under fume hood.

Barks. Five hundred grams of barks of *anonas* (*Annona reticulata*) were collected and chopped into fine pieces. Similar procedure was done to the bark extracts.

Brine shrimp lethality test was done on the leaves and bark extracts. *Artemia salina* eggs were hatched in big vials. After growing into nauplii, 10 larvae were put into small vials - 0, 1, 2 and 3 respectively for the leaf extract lethality test. Another four vials - 0, 1, 2, and 3 were filled with 10 larvae for the bark extract lethality tests.

Results and Discussion

Artemia salina nauplii subjected to:

A. *Ananas* Leaves Extract

Concentration (in ppm)	Number of Mortality (Replicates)		
	1	2	3
0	4	2	3
20	5	5	5
200	7	7	6
2,000	9	10	10

R. *Artemia salina* nauplii subjected to *ananas* bark extract.

concentration (in ppm)	Number of Mortality Replicates		
	1	2	3
0	0	0	0
20	2	1	2
200	4	3	4
2,000	8	6	6

The number of mortality in each replicate containing 100r nauplii counted after 24 hours using SAS Probit Analysis, the LD for the leaf extract and for the bark extract was determined.

Brine shrimp lethality of the crude extract obtained from leaves was found higher than the extract from the barks. Higher concentrations resulted to higher lethality on brine shrimps as compared to the lower concentrations (in ppm)

Using SAS PROBIT analysis, the LD for extracts from the *ananas* leaves was 0.1454 ppm and for the bark extract, 25.2882 ppm.

Conclusion and Recommendation

It is concluded that methanol-dichloromethane extract of leaves has a high lethality dose 50 (LD₅₀). It is much higher than its bark extracts with the high value in its leaves, an actual bioassay on different cancer cell lines is to be performed.

It is therefore recommended that another solvent should be used in isolating possible active components.

References

- RABENA, A.R.** 1997. *The Isolation, Characterization and Identification of the Active Components of Kakawate (Gliricidia sepium) LAq. Kentsh and Wal, h Against Termites.* 1997. Proceedings. FAODMB 25th Anniversary Symposium, Manila, Philippines.
- RESURRECCION, D.C. CAO, E.P. and M.V. LOVERIA.** 1997. *Determination of Cytotoxic Potentials in Two Anahaena Isolates (4B 22 and AB 25) using the Disk Diffusion Method and the Brine Shrimp Lethality Test.* Proceedings. FAOBMB 25th Anniversary Symposium. Manila, Philippines.
- SARAU, J.K. and G. DECASTRO-BERNAS.** 1997. *Preliminary Screening of Cytotoxic Activity of Raphanus sativus L. Seed and Scff/era odorata B. Leaves and Roots on Different Human-Derived Cell Lines.*
- VILLASENOR, L.M. and A.P. CANLAS.** 1997. *A New B-Sitosterol Glycosides as an analgesic Constituent from Mentha cordifolia Opiz. Leaves.* Proceedings, FAOBMB 25th Anniversary Symposium. Manila, Philippines.
- GUEVARRA, A.P. et. al.** 1997. *Antitumor Promoters for the Seeds of Monijaoleifera L.* Proceedings. FAOBMB, 25th Anniversary Symposium.