STUDIES ON *SCHISTOSOMIASIS JAPONICA* AND SAPONINS

Dr. Edito G. Garcia, Dr. Benjamin D. Cabrera
and **Prof. Amorita V. Castillo**
Institute of Public Health
and College of Pharmacy

ABSTRACT

The molluscicidal activity of the bark of *Entada phaseoloides* and extracts from tubers of its related specie, *Entada parvifolia* against *Oncomelania quadrasi*, the snail intermediate host of Shistosoma japonicum in the Philippines were determined.

The commercial gogo bark applied to waters with *O. quadrasi* in the proportion of 2 gms/liter will kill 100% of snails within 24 hours. At this concentration miracidia and cercariae of S. japonicum in the same waters will die within one hours, thus, making the water safe or noninfective for some time. At the dose 100 gms/sqm of water-covered terrestial snail habitats, provided previously cleared of vegetations, at least 90% of *O. quadrasi* will die within 24 hours. These measures can be practical on a self-help basis by inhabitants of schistosomiasis endemic areas where gogo plant grows or where its bark is marketed.

At dilution of 1:5,000 saponin extracted from tubers of *E. parvifolia* killed at least 90% of snails after 24 hours exposure while ethanol (crude saponin) and other extracts require at least 1:2,000 concentration to kill at least 90% of *O. quadrasi.* At these concentrations and the expenses and time involved in the preparation of these extracts, they are not economical or practical for large scale use of molluscicides.

It is suggested that methods of extraction and purification which require little time of preparation and a cheaper but of higher recovery rate of molluscicidal principles be developed.

Surveys of barrios in three towns of Leyte, endemic for *Schistosomiasis japonica* using the circumoval-precipitin test (COPT) and stool examination in the same subjects were undertaken. Findings show that the use of blood COPT method is advantageous over that of the stool examination in schistosomiasis surveys as its prescribed procedure is simple, specific and more sensitive. Moreover, the prevalence rate it determined was higher than that obtained by the stool examination in all three towns studied with underestimate prevalence from 9 to 19%.

Approximately 73 per cent of field rats were found infected with schistosomiasis as revealed by the finding of eggs in liver sections; 49 per cent showed eggs in sections of the intestines and about 13 per cent showed eggs in the stool. The fact that field rats frequent rice fields in quest of food and the frequency of their defecation should be considered seriously in trying to assess the role of field rats in the dissemination of the infection. Whereas it is true that humans, in schistosoma endemic areas in Leyte, are still the important source of infection, field rats could play the role of maintaining the infection in nature and may be responsible in contaminating areas where feces are never deposited.

I. INTRODUCTION

Schistosomiasis japonica, a snail-transmitted debilitating and often fatal disease is endemic in all the provinces of Mindanao except Oriental Misamis, in the islands of Leyte, Samar, Mindoro, Bohol, and in the province of Sorsogon[1-5]. With prevalence rates ranging from 10% to 70% obtained in the different endemic foci, it is conservatively estimated that at least 600,000 Filipinos are infected. Although the disease has been recognized as a major public health problem at present, it is not being effectively controlled due to lack of effective, practical and economical methods of control.

The wide range of mammalian hosts of *Schistosoma japonicum* limits the effectiveness of sanitary disposal of human feces as a method of control[6]. Mass treatment is not possible for want of a highly effective and relatively safe or

non-toxic chemotherapeutic agent. Immunization of exposed population is not possible since there is no available method of inducing artificially acquired resistance due to lack of knowledge of basic mechanisms involved in the immunology of the infection.

Present methods of control employed by the Schistosomiasis Control Commission are directed against the snail intermediate host, *Oncomelania quadrasi,* where *S. japonicum* must undergo development before it becomes ineffective for mammalian hosts including man. These methods are ecological and aim to alter the habitat of this snail by clearing the, or removal of, vegetation, drainage, filling of low areas when possible, pending of areas not drainable, and better maintenance and control of existing irrigation system. However, except for small experimental areas, no snail colonies have actually been wiped out. Chemical control or killing snails with imported molluscicides is occasionally done on a limited scale in suitable types of snail habitats. The use of chemicals has been limited due to cost of procurement and application (including preparation of area by clearing of vegetation) and the fact that only certain types of habitat are suited for chemical application[7].

Obviously there is need for locally produced cheap and highly effective molluscicide for application to suitable snail areas and to supplement present ecological measures. Saponins from plants have been demonstrated and used to some extent for controlling snail hosts of human schistosomes[8]. Abcede has extracted saponins from *Entada parvifolia.* This study aims to determine molluscicidal activity of saponins and other extracts or preparations from "gogo," *Entada phaseoloides* and its related specie *Entada parvifolia.* Demonstration of molluscicidal properties of gogo and development of methods of extractions and application would contribute to the solution of schistosomiasis provided these meet certain criteria[9].

The circumoval precipitin test (COPT) was first reported by Oliver-Gonzales in 1954 as a serologic test for the diagnosis of schistosomiasis. He demonstrated the formation of precipitates in the form of bleb-like globules or chain around

schistosoma ova incubated in sera of human cases and experimentally infected animals. The test was first introduced in the Philippines by Yogore, *et al.* (1962, 1968) followed by reports by Garcia, *et al* (1963, 1968). In both reports the authors have found the test adequately sensitive and specific.

To overcome the resistance of blood withdrawal from the vein, Cabrera, *et al.* (1968) evaluated the COPT using blood obtained from finger prick and received on filter paper (toyo type I) to dry. The result showed that dried blood on filter paper very slightly affected the sensitivity but not the specificity of the conventional circumoval precipitin test (COPT) using whole or undiluted serum.

Tanaka, A. (1975) found that blood (COPT) filter paper could pick up 94 per cent of egg positive cases compared to 99 per cent detected by using undiluted serum.

In an evaluation of some laboratory procedure for the diagnosis of schistosomiasis it was shown that among chronic cases with hepatosplenomegaly, almost two-thirds would be negative by stool examination (Garcia, *et al.* 1968). It appears therefore that stool survey would underestimate the prevalence of schistosomiasis while a serologic test like (COPT) would give more accurate estimate of the prevalence of the disease.

The application of blood COPT (filter paper) method in field survey as compared to stool (formalin-ether) examination for schistosomiasis was reported by Cabrera, M.G., *et al* (1973). In her report she found out that about 16 per cent of cases are liable to be missed by stool (formalin-ether) concentration technique.

Pesigan, *et al.* (1958) examined 203 field rats collected at Palo, Leyte with 86 found positive for schistosoma ova or 22.7 per cent, with mean daily egg output of 27 hatchability or 10.6 per cent. Magath, T.B., *et al.* (1948) found in Leyte rats showing schistosoma ova in liver, spleen, lungs and intestines but only in 2 instances in the rat feces. In Pesigan and Magath's work, they found the field rats as playing an unappreciable role in dissemination of the disease because of low mean daily egg output and low hatchability rate.

In Japan, the final hosts of *S. japonicum* in nature besides man are cows, dogs, cats, rats, etc. Although wild rats may not be considered as important reservoir host where schistosomiasis is highly endemic, it is possible that they contribute to the maintenance of the disease in Japan today where schistosomiasis in rats had 30.1 per cent prevalence rate. Recently wild rats were found to be an important source of infection in Tone River Basin where schistosoma infection in rats gave prevalence rate of 16.8 per cent as compared to human (0.4 per cent) and cows (7.6 per cent) (Yokogawa, M. 1975).

This study was undertaken to further evaluate the use of filter paper method in field surveys as compared to stool survey: to determine the current status of schistosomiasis in Leyte and to collect more data on schistosoma infection among field rats.

II. MATERIALS AND METHODS

Gogo bark from *Entada phaseoloides* was obtained from the market as this is sold commercially and still used as shampoo. This bark was powdered by grinding. For preparing aqueous, ethanol and butanol extracts, aliquots of the powdered bark were placed in 10 times their volume of the extracting solution. Extraction was for at least one week in the cold. The mixture was then filtered and the filtrate allowed to dry in a refrigerator. The dried extract was later air-dried until it was powdery. Different concentrations of these were later prepared for molluscicidal activity screening.

Past experience of the College of Pharmacy of the University of the Philippines has demonstrated that on weight to weight basis, the greater yield of saponins from tubers is that of *Entada parvifolia.* This is a related specie of *Entada phaseoloides.* The bark is also marketed like gogo for shampoo in some areas. In view of these, preparation of saponins and sapogenins, alcohol, and other extracts from *E. parvifolia* were prepared for testing molluscicidal activity. The different extraction procedures are as follows:

Preparation of ethanol extract. Tubers of *E. parvifolia* were chopped, air-dried and melted into fine powder. The powder was macerated and percolated with 95% ethanol for 7 days. Successive macerations and percolations were made on the marc of 75%, 60% and 40% ethanol for 7 days. The different extracts were combined and concentrated in vacue until a pasty mass was obtained. This was later air-dried and labeled as ethanol extract or crude saponins. From six kilos of dry powdered tubers about 0.756 kilos of ethanol extract or crude saponin were obtained.

Preparation of saponins. The crude saponins or ethanol extracts were dissolved in 95% ethanol (1 gm. of crude saponin to 2 ml. of ethanol). Insoluble matter was filtered off. The ethanol solution was added drop by drop to ether (1 gm/ 20 ml) producing a light brown precipitate. This process was repeated twice after it was again dissolved in 95% ethanol. This mixture was added drop by drop to acetone (20 ml. of acetone per gram of powder) wherein a light brown precipitate is formed. The saponins were again dissolved in ethanol, treated with activated charcoal, warmed in a water bath for 1 to 2 hours then passed thru a column of neutral alumina. The light yellow eluate was concentrated in vacue; then, air-dried. From 50 grams of crude saponin 4.4 grams of white amorphous powder were obtained.

Isolation of sapogenins. Crude saponin dissolved in alcohol was precipitated in ether. The precipitate was dissolved in 5% hydrochloric acid and refluxed for 3 to 5 hours. Alcohol was then distilled off and the hydrolyzate was cooled to room temperature. It was then added slowly with stirring to cold distilled water resulting in the formation of a precipitate. After standing overnight this was filtered. The precipitated crude sapogenin was washed several times to remove the acid. The crude sapogenins were dried at room temperature, then placed in the timple of a Soxhlet apparatus and

extracted exhaustively using ether. Five per cent sodium hydroxide was then added to the ether extract until two layers were formed. The acid genins fraction (aqueous layer) was treated with 10% hydrochloric acid solution and then warmed on a water bath for 2 hours to decompose the sodium salts of the acid genins. After cooling, the solution was filtered off and the precipitate washed repeatedly with cold distilled water to remove the hydrochloric acid. White amorphous powder was obtained after drying the precipitate which was recrystallized with isobutanol to give white rod-like to prismatic crystals. From 150 grams of crude saponins, 0.4 grams of sapogenins were recovered. An earlier preparation of sapogenin was not recrystallized.

The laboratory screening of the different extracts procedure was recommended by the World Health Organization for testing molluscicides[9]. For each trial 2 to 4 beakers of the solution with 10 snails each were used. A plastic screen is placed in the beaker to keep the snails submerged in the test solution. At the end of the period of observation the snails were washed three times with aerated tap water after which they were placed in petri dishes and allowed to recover for 48 hours before pronouncing them dead or alive.

Since application of molluscicides to aquatic habitats would also expose *S. japonicum*, cercariae and miracidia in water, the effect of different concentrations of gogo extracts on these stages of the parasites was determined.

As *O. quadrasi* is an amphibious snail which is strictly aquatic only during the first two to three weeks of life and more terrestrial later, laboratory tests were done to determine the effect of application of gogo preparations on snails in moist oil in the laboratory. Initially petri dishes were used for these tests; later, large laboratory pans measuring 20 centimeters by 36 centimeters layered with garden soil were used. Snails were made to acclimatize in the pans after which the gogo preparation was applied. After the period of exposure the snails were collected, washed three times in water and observed for 48 hours before declaring them dead.

Field experiments were limited to trials using powdered gogo bark from *Entada phaseoloides* since this needed only minimal processing and because extracts were not available in quantity sufficient for field trials. These included the determination of amount or dose of gogo powder per unit of area of terrestrial habitats with varied ecologic features and of stagnant water habitats or those water habitats with minimal flow.

A few trials with Bayluscide or Niclosamide and copper sulphate, two commercially available molluscides, were done for comparison with gogo derivatives.

A meeting was held prior to the start of the work among the Director, Deputy Director of Schistosomiasis Pilot Project at Palo, Leyte, and the project leaders to brief them of the proposed research work; to ask their support and cooperation; and to gather informations pertinent to the work.

It was decided that the survey make use of the blood COPT (filter paper) and the stool (formalin-ether) methods in three schistosomiasis endemic towns in Leyte. The areas chosen were as follows:

Area I — where all control methods are being applied by the Schistosomiasis Pilot Project represented by villages in the municipality of Pastrana.

Area II — where partial control methods are applied by villages in the municipality of Santa Fe.

Area III — where no control methods are applied, hence considered as virgin area as represented by villages in the municipality of Jaro.

The survey covered two groups of people in the population, namely, school children from grades I to IV and the general population coming from the three areas. Approximately 6 villages or barrios per area were surveyed.

After taking the pertinent data required in the survey, inhabitants from the three areas were requested to submit stool specimen wrapped in waxed paper. However, in order to insure collection of a larger sample, the house-to-house survey method was applied. With an applicator stick, a sufficient amount of stool was transferred and emulsified thoroughly in a screw-cap vial containing 10 per cent formalin. The screw-cap was replaced, sealed with masking tape, labelled and packed for shipment to the Institute of Public Health.

Approximately 3 drops of blood from pricked finger were obtained from the inhabitants and received on filter paper strip (Toyo type I). These were labelled, dried and also packed for shipment. Unpaired specimens of stool and blood were disregarded for study.

The procedure for the blood COPT (filter paper) method was based on the original article reported by Cabrera, *et al.* (1968) while that for the stool (Formalin-Ether concentration) technique was taken from textbooks on parasitology.

Meanwhile field rats from the three areas were caught by rat traps set in the evenings. The rats were dissected the following morning with the liver and large intestines of each rat preserved in 10 per cent formalin and shipped to the Institute of Public Health. The liver and intestines were processed, sectioned, stained and examined microscopically for schistosoma eggs. Two to three pellets of rat stool from the distal portion of the large intestine were examined for eggs using also Formalin-Ether technique. While examining the stool for schistosoma ova, other helminths ova and protozoan cysts from the stool specimen were also recorded from the three areas.

III. RESULTS AND DISCUSSION

The results of laboratory trials to determine molluscicidal activity of the different gogo preparations are presented in Tables I to VII. Tables VIII to X show the results of exposure of miracidia and cercariae of *Schistosoma japo-*

nicum to gogo extracts while Tables XI to XIV show the results of treatment of terrestrial habitats of *O. quadrasi* with powdered gogo bark. Not presented in tabular form but mentioned in the text are results of toxicity determination for mice and fish. Also included in this category are observations on the application of gogo to individual snail colonies.

As seen in Table I, a minimum of 2 grams of powdered gogo bark *(Entada phaseoloides)* in one liter or 1000 ml. of water will kill 100% of snails within 24 hours. Aqueous extract at dilution of 1:2,000 will kill all immersed snails within 24 hours, while 1:5,000 dilution will kill 80% of snails as seen in Table II. Ethanol or butanol extracts have no greater molluscicidal activity than aqueous extract as presented in the same table.

As presented in Table III, saponin, ethanol extract (crude saponin), butanol and ether extracts of *Entada parvifolia* tuber all killed *O. quadrasi.* However, the most potent is saponin which killed 92% of snails at dilution of 1:5,000 and 88% at 1:10,000 dilution. If saponin is the molluscicidal principle, the difference in potency of the different extracts may be explained by the amount of saponin present in each preparation.

In nature, the waters of snail habitats have varied pH or hydrogen in concentration. The effect of alkalinity or acidity of water on the molluscicidal activity of aqueous and ethanol extracts are presented in Tables IV and V. It will be seen from these tables that the activity is slightly reduced as the pH increases or decreases and that optimal pH for the extracts is about neutral.

Miracidia and cercariae of *S. japonicum* were also found in the waters where *O. quadrasi* thrives. Experiments were done to determine the effect of application of gogo extracts on these stages of the parasite. As seen in Table VIII, miracidia died within an hour of exposure to 1:10,000 dilution of aqueous extracts and to 1:20,000 concentration of crude saponins while cercariae died within an hour in 1:5,000 dilution of aqueous and ethanol extracts. The implication is that gogo application at doses to kill snails will render the water

non-inffective stage of the parasite for man and other mammalian hosts.

Many habitat of *O. quadrasi* are not defined water courses but are depressions or low areas which maybe waterlogged throughout or part of the year depending on the rainfall. There may also be adjacent areas of flowing streams which become covered with water during rains and when the stream overflows, but these areas are also often practically dry. For these reasons laboratory experiments were undertaken to determine the dose of gogo preparations for application to such types of snail colonies. The results of these experiments are presented in Tables VI and VII. In Table VI, a higher concentration is needed for ethanol extract solution to kill all snails in moist soil than to immerse them in clear water which suggests that some materials in the soil interfere with the molluscicidal activity. Table VII, shows that a dose of 50 gms/sqm of powdered bark killed all snails in moist filter paper. However, the same dose left many snails alive in moist oil with or without covering layer of water. The dose of 75 gms/sqm is needed to kill at least 90% of snails in soil with covering layer of water is probably due to the fact that the active principle goes into solution to affect the snails which is not possible in moist soil.

The results of field experiments show that to kill snails, it requires larger doses of powdered gogo bark a constant level of covering layer of water, and removal of vegetation (see Tables XI to XIV). A minimum of 100 gms of powdered bark is needed for application to depressions with covering layer of water to kill at least 90% of snails. However, the same dose applied to moist areas without covering layer of water did not kill snails (Table XI). Sprinkling the area with water before or after application of gogo to moisten the colony even with a dose of 300 gms/sqm did not result in significant mortality of snails (Table XII). Apparently, the snails will not feed on gogo.

The results of large scale experiments in the field summarized in Table XIV are similar to the small scale (square meter quadrats) experiments. An area of 184 sqms, covered

with several centimeters of water treated with a dose of 100 gm/sqm after removing vegetation resulted in 96.4% mortality. The banks of a pond treated for five consecutive weeks at the dose of 100 gms/sqm showed only slight reduction of snail density.

In view of the limited amounts of sapogenin and its insolubility in water, laboratory trial with gogo preparation were limited to exposure to snails in petri dishes lined with wet filter paper. The results shown in Table XV show that unrecrystallized sapogenin at dose of 25 mgs killed all snails in 24 hours. This effect is residual and snails subsequently placed in the same dish died at a later date.

The observations show that with minimal processing, the bark of commercially marketed *Entada phaseoloides*, can be used for killing *Oncomelania quadrasi* snails at a dose of 2 gms/liter or 2 kilos/stere of water volume of snail habitats and at a dose of 100 gms/sqm of area with covering layer of water. This can be practiced on a self-help basis by inhabitants of schistosomiasis endemic areas where gogo plant grows or where its bark is marketed.

Aqueous, ethanol and butanol extracts of *E. phaseoloides* bark all kill at least 90% of *O. quadrasi* snails within 24 hours exposure at dilution of 1:2,000. From these results, ethanol or butanol (which are expensive) extracts do not show any greater potency over aqueous extract. In view of this, aqueous extraction and subsequent drying may be more practical for concentrating the molluscicidal principle. Concentration of molluscicide reduces its bulk and reduce problems of transportation in bringing the chemical to extensive snail areas.

Of the different extracts of *E. parvifolia*, saponin show the greatest potency as molluscicides. At concentration of 1:5,000, it will kill at least 90% of snails in water. Nonetheless, procedure for extracting saponins with a high recovery rate is needed to make their use for controlling *O. quadrasi* snails practical and economical. It should be stressed however, that mollusciciding is only supplementary to other snail control measures and that snail control is not always synonymous with schistosomiasis control.

Although all preparations or extracts of *Entada phaseoloides* and *Entada parvifolia*, particularly saponins showed adequate molluscicidal property, a number of points is worth considering. These include the desirable general characteristics of a molluscicide, its place in the control of snails hosts of human schistosomes, and the ecology and biology of *O. quadrasi.*

Generally, it is agreed that an ideal molluscicide should have the following characteristics:

1) low toxicity for man, domestic animals and fish;
2) active at very low concentrations as this reduces transportation cost considerably;
3) cheap so that it can be employed economically;
4) stable in storage and in the snail habitat after use;
5) availability of tests to measure concentrations used in the field; and
6) usable with simple equipment.

No molluscicide is likely to have all these characteristics and it is quite possible for a good molluscicide to lack more than one of them[9].

Chemical control of the snail intermediate hosts of human schistosomes is predicated on the assumption that they are a weak link in the life cycle of the parasite and that molluscicides can be used to produce rapid devastating reductions of snail populations. In practice, molluscicides have not always lived up to their promise[9]. This has been due largely to the diversity and extent of the snail habitats and the ecology of the snail species.

Oncomelania quadrasi as stated earlier is an amphibious snail. It is strictly aquatic only during the first two weeks of its life after which it becomes more terrestrial. It can stand desiccation or survive in dry soil for several weeks. Its habitats may be classified as: 1) dry-moist soil areas (alternating between dry, moist or wet conditions), 2) continuously shallow inundated areas; and 3) flowing waters. All of

these would have varying sizes or extents. From this list, different methods of application and formulation of a molluscicide are necessary to make a chemical suitable for controlling snail hosts of schistosomes.

That molluscicides have an important role in snail control is undeniably true, but the search for better molluscicides should be continued. For, gogo, better methods (not only for experimental purposes) of extraction and purification of molluscicidal principles should be developed.

IV. SUMMARY AND CONCLUSION

The molluscicidal activity of the bark *Entada phaseoloides* and extracts from tubers of its related specie, *Entada parvifolia*, against *Oncomelania quadrasi*, the snail intermediate host of *Schistosoma japonicum* in the Philippines, were determined.

The commercial gogo bark applied to waters with *O. quadrasi* in proportion of 2 gms/liter will kill 100% of snails within 24 hours. At this concentration miracidia and cercariae of *S. japonicum* in the same water will die within one hour thus making the water safe or non-infective for some time. At a dose of 100 gms/sqm of water covered terrestrial snails habitats; provided previously cleared of vegetations, at least 90% of *O. quadrasi* will die within 24 hours. These measures can be practical on a self-help basis by inhabitants of schistosomiasis endemic areas where gogo plant grows or where its bark is marketed.

A dilution of 1:5,000 saponin extracted from tubers of *E. parvifolia* killed at least 90% of snails after 24 hours exposure while ethanol (crude saponin) and ether extract require 1:2000 concentration to kill at least 90% of *O. quadrasi.*

It is suggested that cheaper and less time consuming methods of extraction and purification with higher recovery rate of molluscicidal principles be developed. If this is accomplished, molluscicides from entada species may become useful adjutant for schistosomiasis control.

The survey of villages in three schistosomiasis endemic towns in Leyte using both the stool (Formalin-Ether) and the blood COPT (filter paper) methods revealed these findings: 1) there was a higher prevalence rate in Area III where no control measure applied, and lowest in Area I where all available control measures are applied; 2) fourteen per cent of schistosomiasis cases were missed by stool (Formalin-Ether) method, 3) the prevalence rate among males is higher than females in the 15-19 age group (among school children, boys, again, appear to have higher prevalence rate than girls as grade goes higher), and 4) the blood COPT (filter paper) method is more reliable in schistosomiasis surveys because it gave a higher prevalence rate in the three studied areas. Moreover, its procedure is simple, specific, and more sensitive.

Approximately 73 per cent of field rats were found infected with schistosomiasis as revealed by the findings of eggs in liver sections; 49 per cent showed eggs in sections of the intestines and about 13 per cent showed eggs in the stool (using only 2-3 pellets) of caught wild field rats for the Faust-Malaney egg hatching technique which revealed that 58 per cent of rats showed miracidia with a mean of five per rat. This figure could be higher if more feces were used. Field rats frequent rice fields to look for food and the frequency of their defecation should be considered seriously in assessing their role in disseminating infection. Whereas in schistosoma endemic areas, particularly Leyte, humans are still important source of infection, field rats could play the role of maintaining the infection in nature and may be responsible in contaminating areas where human feces are never deposited.

Table I

Results of immersion of *Oncomelania quadrasi* in different gogo (*Entada phaseoloides*) bark concentration in water.

Concentration	Percent Mortality After 1 hour	6 hours	24 hours
4 Gm/1000 ml (1:250)	0	0	100%
2 GM/1000 ml (1:500)	0	0	100%
1 Gm/1000 ml (1:1,000)	0	0	85%
0.5 Gm/1000 ml (1:2,000	0	0	70%
0.25 Gm/1000 ml (1:4,000)	0	0	60%
Water (Control)	0	0	0

Table II

Results of 24-hours exposure (standard immersion test) of *O. quadrasi* to different concentrations of extracts of *Entada phaseolides* bark.

CONCENTRATION	Mortality after 24-hours Aqueous	Ethanol	Butanol
1:1,000	100%	100%	100%
1:2,000	100%	90%	90%
1:5,000	80%	60%	80%
1:10,000	not done	50%	50%
1:20,000	not done	0%	20%
Control	0%	0%	0%

Table III

Results of 24-hour exposure (standard immersion test) of *O. quadrasi* to different concentrations of extracts of *Entada parvifolia* tuber.

Concentration	Mortality after 24-hour Saponins	Ethanol	Butanol	Ether
1:1,000	100%	100%	100%	100%
1:2,000	100%	90%	80%	90%
1:5,000	92%	80%	60%	74%
1:10,000	88%	40%	50%	70%
1:20,000	76%	10%	0%	20%
Control	6%	0%	0%	0%

Table IV

Results of 24-hour immersion of *O. quadrasi* to aqueous extract of *E. phaseoloides* bark in water with different pH.

pH	Mortality after 24-hour in 1:1,000	1:2,000
pH 4	80%	70%
pH 5.5	90%	80%
pH 7.1	100%	100%
pH 7.6	80%	90%
pH 8.4	80%	80%
pH 10	80%	70%

Table V

Results of 24 hours immersion of *O. quadrasi* in ethanol extract of *E. parvifolia* tuber in water at different pH.

pH	Mortality after 24-hours in 1:1,000	1:2,000
pH4	80%	80%
pH 5.5	90%	80%
pH 7.1	100%	90%
pH 7.6	80%	70%
pH 8.4	80%	70%
pH 10	80%	70%

Table VI

Molluscicidal activity of different concentrations of ethanol extracts of *E. parvifolia* tubers on snails in moist soil.

Concentration	Mortality after 6 hours	Mortality after 20 hours	Mortality after 24 hours
1:1,000	10%	76%	100%
1:2,000	4%	70%	80%
Control	0%	0%	0%

NOTE: Extract applied to provide covering layer of solutions.

Table VII

Results of application of powdered gogo bark (*E. phaseoloides*) on snails in laboratory pans with filter paper and soil.

Dose/sq. m.	Snail mortality after 24 hours		
	Moist Filter Paper	Moist Soil	Moist Soil with Film of H_2O
25 gms.	not done	24%	32%
50 gms.	100%	28%	60%
75 gms.	100%	78%	94%
100 gms.	100%	84%	96%
Control	0%	0%	0%

Table VIII

Results of one-hour exposure of miracidia of *S. japonicum* to aqueous extract of gogo bark *(E. phaseoloides)*.

Dilutions	Results after one-hour exposure	
	Miracidia in test tubes	Miracidia in Petri dish
1:500	all dead	all dead
1:1,000	" "	" "
1:2,000	" "	" "
1:5,000	" "	" "
1:10,000	" "	" "
1:20,000	active	active

Table IX

Results of one-hour exposure of miracidia of *S. japonicum* to alcohol extract of *E. parvifolia*.

Dilutions	Results after one-hour exposure	
	Miracidia in test tubes	Miracidia in Petri dish
1:500	dead	dead
1:1,000	"	"
1:2,000	"	"
1:5,000	"	"
1:10,000	"	"
1:20,000	"	"
Control	active	active

Table X
Results of exposure of *S. japonicum* cercariae to gogo extracts.

Dilutions	Change in activity after one-hour	
	Aqueous extract (bark)	Ethanol extract (bark)
1:500	dead	dead
1:1,000	"	"
1:2,000	"	"
1:5,000	"	"
1:10,000	active	active
1:20,000	"	"
Control		

Table XI
Results of application of gogo powder *(E. phaseoloides)* on *O. quadrasi* in water covered and moist plots* in the field.

Dose/sq. m. layer of water	Plots with 1 cm	Moist plots without surface water
75	79%	0
100	90.9%	0
150	92.3%	0

*square meter quadrats with vegetation removed.

Table XII
Mortality of snails in plots* sprinkled with water before or after application of gogo powder (bark).

Dose/sq. m.	Sprinkled before application*			Sprinkled after application		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
100	0	0	0	0	0	0
200	0	0	0	0	0	0
300	0 %	0	0	0	0	0
600	46.8 %	72.2 %	24.4 %	not done	not done	not done
750	43.75 %	8.69%	0	" "	" "	" "

*square meter quadrats with vegetation removed.

Table XIII
Results of application of gogo bark powder on snails in uncleared (with vegetation) plots with surface water.

Dose/sq. m.	Pre-treatment		24 hours after application		48 hours after application	
	Snail density*	dead	Snail density*	dead	Snail density	dead
75	4.25	0	2	0	2.5	0
150	5.25	0	2.25	0	1.25	0
350	5.50	0	2.5	0	2.25	0
Control	3.25	0	3.5	0	4.50	0

*average number of snails per ring sample.
area of ring is 1/70 sq. m.

NOTE: Observation after a week showed snail density of 4.25 to 4.50 for the treated plots.

Table XIV

Summary of other field experiments on the use of gogo bark powder for controlling *O. quadrasi* snails.

1. Weekly application of 199 gm/sqm on the banks of a pond (approximately 240 sqms) for five weeks did not result in significant reduction of snail density. Density of snails per ring sample before application of gogo was 1.81 and 1 week after last applications was 1.23.

2. A cleared area (vegetation removed) of 184 sqms. with covering stagnant water from 1 cm. to 10 cm. was treated with a dose of 100 gm/sqm and calculated to give dilution of 1:2,000. Snail mortality after 48 hours was 96.4%.

Table XV
Results of exposure to sapogenins of *O. quadrasi* in filter paper lined petri dishes.

Dose/sqm.	Snail mortality after 24 hours				
	Sapogenin (1)	Sapogenin (2)	Sapogenin (3)	Sapogenin (4)	Unrecrystallized sapogenin
7.5 gm	0%	0%	0%	0%	0%
10 gm	0%	0%	0%	0%	8%
15 gm	0%	8%	4%	0%	46%
20 gm	0%	32%	32%	28%	82%
25 gm	32%	32%	44%	44%	100%
Control	0%	0%	0%	0%	0%

NOTE: Sapogenins 1, 2, 3, & 4 were recrystallized.

Table XVI
Prevalence of schistosomiasis by municipality, kind of specimen and laboratory technique used, Leyte, 1973-1975.

Municipalities	Stool (formalin-ether)			Blood COPT (filter-paper)		
	No. Exam	No. +	% +	No. Exam.	No. +	% +
Pastrana (Area I)*	1,222	189	15.4	1,222	294	24.0
Santa Fe (Area II)**	980	244	24.8	920	333	33.9
Jaro (Area III)***	1,664	391	23.4	1,664	713	42.8
TOTAL	3,866	824	21.31	3,866	1,340	34.66

* All control measures applied (snail control, treatment, environmental)
** Partial application
*** Control measures nil

Table XVII

Age and sex distribution of schistosomiasis as detected by stool (formalin-ether) and blood COPT (filter paper) examination, Municipality of Pastrana, Leyte, 1973-1975.

Age	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%No.	No.	%
0- 4	27	4.5	13	2.0	40	3.2	0	0	0	0	0	0	2	7.4	1	7.6	3	7.5
5- 9	191	32.2	186	29.5	377	30.8	24	12.5	26	13.9	50	13.2	35	18.3	38	20.4	73	19.3
10-14	170	28.6	194	30.8	364	29.7	35	20.5	40	20.6	56	32.9	75	38.6	75	38.6	131	35.9
15-19	39	6.5	35	5.5	74	6.0	10	25.6	5	14.2	15	20.2	12	30.7	8	22.8	20	27.0
20-29	45	7.5	67	10.6	112	9.1	7	15.5	7	10.4	14	12.5	13	28.8	14	20.8	27	24.1
30-39	45	7.5	58	9.2	103	8.4	8	17.7	13	22.4	21	20.3	9	20.0	6	10.3	15	14.5
40-49	38	6.4	30	4.7	68	5.5	3	7.8	3	10.0	6	8.8	5	13.1	5	16.6	10	14.7
50-59	18	3.0	37	5.8	55	4.5	1	5.5	4	10.8	5	9.0	3	16.6	7	18.9	10	18.1
60-69	18	3.0	8	1.2	26	2.1	2	11.1	1	12.5	3	11.5	5	27.7	0	0	5	19.2
70 +	2	0.30	1	0.1	3	0.2	0	0	0	0	0	0	0	0	0	0	0	0
Total	593	99.2	629	99.4	1,222	99.5	90	15.1	99	15.7	189	15.4	140	23.6	154	24.4	294	24.0

Table XVIII

Age and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT (filter paper) examination, Municipality of Santa Fe, Leyte, 1973-1975.

Age	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1- 4	39	7.8	28	5.8	67	6.8	5	12.8	2	7.1	7	10.4	4	10.2	4	14.2	8	11.9
5- 9	138	27.6	129	26.8	267	27.2	38	27.5	18	13.9	56	20.9	58	42.0	46	35.6	104	38.9
10-14	102	20.4	90	18.7	192	19.5	34	33.3	28	31.1	62	32.2	44	43.1	36	40.0	80	41.6
15-19	45	9.0	36	7.5	81	8.2	17	37.7	10	27.7	27	33.3	23	51.1	19	52.7	42	51.8
20-29	54	10.8	52	10.8	106	10.8	18	33.3	12	23.0	30	28.3	25	46.2	17	32.6	42.	39.6
30-39	45	9.0	47	9.7	92	9.3	17	37.7	13	27.6	30	32.6	8	17.7	18	27.6	21	22.8
40-49	28	5.6	41	8.5	69	7.0	7	25.0	8	19.5	15	21.7	4	14.2	9	21.9	13	18.8
50-59	32	6.4	32	7.0	64	5.6	7	21.8	1	3.1	8	12.5	7	21.8	5	15.6	12	18.7
60-69	11	2.2	20	4.1	31	3.1	4	36.3	4	20.0	8	25.8	5	45.4	5	25.0	10	32.2
70 +	6	1.2	5	1.0	11	1.1	0	0	1	20.0	1	9.0	1	16.6	0	0	1	9.0
Total	500	100.0	480	99.9	980	99.5	147	29.4	97	20.2	244	24.8	179	35.8	154	32.0	333	33.9

Table XIX
Age and sex distribution of schistosomiasis cases detected by stool (formalin-ether) and blood COPT (filter paper) examination, Municipality of Jaro, Leyte, 1973-1975.

Age	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1- 4	23	2.7	20	2.4	43	2.5	4	17.3	0	0	4	9.3	2	8.6	2.	10.0	4	9.3
5- 9	244	29.2	272	32.7	516	31.0	48	19.6	52	19.1	100	19.3	79	32.3	76	27.9	155	30 0
10-14	240	28.7	195	23.4	432	26.1	69	28.7	50	25.6	119	27.3	133	54.4	87	44.6	220	50.5
15-19	58	6.8	38	4.5	96	5.7	22	27.9	6	15.7	28	29.1	39	67.2	18	47.3	57	59.3
20-29	59	7.0	65	7.8	124	7.4	19	32.2	16	24.6	35	28.2	37	62.7	37	56.9	74	59.6
30-39	88	10.5	104	12.5	192	11.5	16	18.1	22	21.1	38	19.7	42	47.7	45	43.2	87	45.3
40-49	68	8.1	69	8.3	137	8.2	21	30.8	18	26.0	39	28.4	30	44.1	38	40.5	58	42.3
50-59	32	3.8	45	5.4	77	4.6	0	0	13	28.9	19	24.6	11	34.3	23	51.1	34	44.1
60-69	16	1.9	17	2.0	33	1.9	0	0	4	23.5	4	12.1	7	43.7	11	64.7	18	54.5
70 +	6	0.7	4	0.4	10	0.6	0	0	0	0	0	0	3	50.0	2	50.0	5	50.0
Un-known	0	0	1	—	1	0.06	0	0	0	100.0	1	100.0	0	0	1	100.0	1	100.0
Total	834	99.4	830	99.4	1664	99.5	209	25.0	182	21.9	391	24.4	383	45.9	330	37.7	713	42.8

Table XX

Age and sex distribution of schistosomiasis as detected by stool (formalin-ether) and blood COPT (filter paper) examination from Pastrana, Santa Fe and Jaro, Leyte, 1973-1975.

Age	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1- 4	89	4.6	61	3.2	150	3.9	9.	10.1	2	3.3	11	7.3	8	9.0	7	11.4	15	10.0
5- 9	573	29.8	587	30.9	1160	30.4	110	19.2	96	16.3	206	17.7	172	30.0	160	27.2	332	28.6
10-14	512	26.6	479	25.3	991	26.0	138	27.0	118	24.6	256	15.7	233	45.5	198	41.3	431	43.5
15-19	142	7.4	109	5.7	251	6.6	49	34.5	21	19.3	70	27.9	74	52.1	45	41.3	119.	47.4
20-29	158	8.2	184	9.7	342	8.9	44	27.8	35	19.0	79	23.1	75	47.5	68	36.9	143	41.8
30-39	178	9.3	209	11.0	387	10.1	41	23.0	48	23.0	89	23.0	59	33.1	69	33.0	128	33.1
40-49	134	7.0	140	7.4	274	7.2	31	23.1	29.	20.7	60	21.9	39	29.1	52	37.1	91	33.2
50-59	82	4.3	74	3.9	156	4.1	8	9.7	18	23.3	26	16.7	21	25.6	35	47.3	56	35.9
60-69	45	2.3	45	2.4	90	2.3	6	13.3	9	20.0	15	16.7	17	37.8	16	35.5	33	36.7
70 +	8	0.4	7	0.4	15	0.4	0	0	1	14.3	1	6.7	4	50.0	2	28.6	6	40.0
Un-known	0	0	0	—	1	—	0	0	1	—	1	—	0	0	1	—	1	—
Total	1921	99.9	1896	99.9	3817	99.9	436	22.7	378	19.9	814	21.3	702	36.5	635	34.4	1355	35.5

Table XXI

COPT (filter paper) type of reactions by municipality, 1973-1975.

Municipalities	SB	MB	LB	SS	MS	LS
Pastrana	105	21	0	81	71	16
Santa Fe	204	66	0	32	22	10
Jaro	494	63	0	64	73	19
Total	803	150	0	177	166	45

SB = Small Bleb
SM = Medium Bleb
LB = Large Bleb
SS = Small Segment
MS = Medium Segment
Ls = Large Segment

Table XXII

Comparison of Stool (formalin-ether) and Blood COPT (filter paper) techniques in detecting schistosomiasis in the municipalities of Pastrana, Santa Fe and Jaro, Leyte, 1973-1975.

Laboratory Technique Used		Blood COPT (filter paper) (+)	(—)	Total
Stool (formalin-ether)	(+)	392	387	779
	(—)	701	1,318	2,019
Total		1,093	1,705	2,798

Table XXIII. Grade and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT filter paper) examination, Municipality of Pastrana, Leyte, 1974-1975.

Grade	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	79	39.3	59	31.4	138	35.5	13	16.4	9	15.2	22	15.9	15	19.0	10	16.9	25	18.1
II	48	23.9	49	26.1	97	24.9	16	33.3	9	18.4	25	25.8	14	29.2	9	18.4	23	23.7
III	49	24.3	37	19.7	86	22.1	4	8.2	7	18.9	11	12.8	9	18.4	10	27.0	19	22.1
IV	25	12.4	43	22.9	68	17.5	4	16.0	10	23.2	14	20.6	11	44.0	14	32.6	25	36.8
Total	201	100.0	188	100.0	389	100.0	37	18.4	35	18.6	72	18.5	49	24.4	43	22.9	92	23.7

Table XXIV. Grade and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT (filter paper) examination, Municipality of Santa Fe, Leyte, 1974-1975.

Grade	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
1	34	33.3	21	20.2	55	26.7	7	20.6	6	28.6	13	23.6	10	29.4	3	14.3	13	23.6
II	32	31.4	31	29.8	63	30.6	6	18.8	9	29.0	15	23.8	16	52.0	12	38.7	28	44.4
III	23	22.5	19	18.3	42	20.4	10	43.5	2	10.5	12	28.6	10	43.5	6	31.6	16	38.1
IV	13	12.7	33	31.7	46	22.3	4	30.8	10	30.3	14	30.4	8	61.5	17	51.5	25	54.3
Total	102	100.0	104	100.0	206	100.0	27	26.5	27	26.0	54	26.2	44	43.1	38	36.5	82	37.8

Table XXV. Grade and sex distribution of schistosomiasis cases detected by stool (formalin-ether) and blood COPT (filter paper) examination, Municipality of Jaro, Leyte, 1974-1975.

Grade	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	64	38.1	77	40.1	141	39.2	19	29.7	13	22.1	36	25.5	14	21.9	16	20.8	30	21.3
II	65	38.7	68	35.4	133	36.9	26	40.0	16	23.5	42	31.6	22	33.8	21	30.9	43	32.3
III	34	20.2	40	20.8	74	20.6	7	20.6	13	32.5	20	27.0	16	47.1	17	42.5	33	44.6
IV	5	3.0	7	3.6	12	3.3	0	0	1	14.3	1	8.3	3	60.0	4	57.1	7	58.3
Total	168	100.0	192	100.0	360	100.0	52	31.0	47	24.5	99	27.5	55	32.7	58	30.2	113	31.4

Table XXVI. Grade and sex distribution of schistosomiasis cases as detected by stool (formalin-ether) and blood COPT (filter paper) examination from three municipalities in Leyte, 1974-1975.

Grade	Number Examined						No. Positive on Stool (formalin-ether)						No. Positive on Blood COPT (filter paper)					
	Male		Female		Total		Male		Female		Total		Male		Female		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
I	177	37.6	157	32.4	334	35.0	39	22.0	32	20.4	71	21.2	39	22.0	29	18.5	68	20.3
II	145	30.8	148	30.6	293	30.7	48	33.1	34	23.0	82	28.0	52	35.9	42	28.4	94	32.1
III	106	22.5	96	19.8	202	21.1	21	19.8	22	22.9	43	21.3	35	33.0	33	34.4	68	33.7
IV	43	9.1	83	17.1	126	13.2	8	18.6	21	25.3	29	23.0	22	51.2	35	42.2	57	45.2
Total	471	100.0	484	100.0	955	100.0	116	24.6	109	22.5	225	23.6	148	31.4	139	28.7	287	30.1

Table XXVII. Prevalence of helminthic and protozoan infections by municipality, 1973-1975.

Municipality	Number Exam.	Ascaris		Trichuris		Hookworm		Schisto		Paragonimus		Taenia		Others		PROTOZOA E. Histo		E. Coli		Lamblia		B. Coli		E. Nana	
		No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+	No.	%+
Pastrana	1222	692	56.6	761	62.2	457	37.3	189	15.4	1	0.08	4	0.32	2	0.16	1	0.08	16	1.31	7	0.57	0	0	0	0
Santa Fe	980	628	64.0	671	68.4	360	36.7	244	24.8	0	0	4	0.40	2	0.20	5	0.51	62	6.32	11	1.12	0	0	18	18.3
Jaro	1664	1012	60.8	1051	63.1	736	44.2	391	23.5	21	1.26	33	1.98	3	0.18	1	0.06	15	0.90	2	0.12	1	0.06	0	
TOTAL	3866	2332	60.3	2483	64.2	1553	40.1	824	21.3	22	0.57	41	1.06	7	0.18	7	0.18	93	2.4	20	0.51	1	0.02	18	0.46

Table XXVIII. Detection of *Schistosoma japonicum* ova from liver and intestine sections, and stools of rats by municipality, Leyte, 1974-1975.

Municipality	Liver			Intestine			Stool		
	No. Examined	No.	%	No. Examined	No.	%	No. Examined	No.	%
Pastrana	167	122	78.0	166	90	54.2	166	35	21.1
Santa Fe	126	82	65.1	126	57	45.2	126	6	4.8
Jaro	264	201	76.1	258	123	47.7	93	7	7.5
Total	557	405	72.7	550	270	49.1	385	48	12.5

Table XXIX

Detection of *Schistosoma japonicum* ova in liver and intestine sections and stools of rats caught in Pastrana, Santa Fe, Jaro, Leyte, by sex, 1974-1975.

	Male	Positive		Female	Positive		Both Sexes	Positive	
	No. Examined	No.	%	No. Examined	No.	%	No. Examined	No.	%
LIVER Rough	149	135	90.6	202	181	89.6	351	316	90.0
LIVER Smooth	71	32	45.1	125	51	40.8	196	83	42.3
LIVER Total	220	167	75.9	327	232	70.9	[a] 547	399	72.9
INTESTINE	218	111	50.9	322	153	47.5	[a] 540	264	48.9
STOOLS	161	21	13.0	219	26	11.9	b 380	47	12.4

a — Sex of 10 rats undetermined
b — Sex of 5 rats undetermined

Table XXX

Distribution of schistosoma ova in liver, intestine and stool of 380 rats caught in Pastrana, Santa Fe and Jaro, Leyte, by sex, 1974-1975.

Material	Male		Female		Both Sexes	
	No.	%	No.	%	No.	%
Positive in:						
Liver	33	20.5	47.	21.5	80	21.0
Intestine	1	0.6	1	0.5	2	0.5
Stools	1	0.6	3	1.4	4	1.0
Liver & intestine	67	41.6	77	35.2	144	37.9
Liver & stools	2	1.2	4	1.8	6	1.6
Liver & intestine & stools	18	11.2	19	8.7	37	9.7
Negative	39	24.2	68	31.0	107	28.2
Total	161	100.0		100.0	380	100.0

Table XXXI

Recovery of other helminth ova iron stools of 380 rats, by sex, Leyte, 1974-1975.

ova	Male (161) Positive		Female (219) Positive		Both Sexes (380) Positive	
	No.	%	No.	%	No.	%
Ascaris sp.	15	9.3	15	6.8	30	7.9
Trichuris sp.	7	4.4	15	6.8	22	5.8
Hookworm sp.	58	36.0	57	26.0	115	30.3
Echinostoma sp.	43	26.7	72	22.9	115	30.3
M. Diminuta	16	9.9	21	9.6	37	9.7
Capillaria sp.	13	8.1	12	5.5	25	6.6

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