MOLLUSCICIDAL AND OTHER ECONOMIC POTENTIALS OF ENDOD*

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Abstract

Ended is the Ethiopian name for *Phytolacca dodecandra,* a full shrub closely related to the American pokeweed, *Phytolacca americana*. The young green leaves and shoots are edible protein sources after they are boiled and the water is discarded. An extract of saponin from P. *dodecandra* berries can be used as a molluscicide in the control of schistosomiasis and other snail-borne diseases. The berries have long been used as soap for washing clothes, and roots and leaves, although well-known for their toxicity, have been used as medicine for various ailments and as abortifacients. Recently, ended berries also have been discovered to possess potent spermicidal properties useful for birth control; aquatic insect larvicidal properties potentially useful in the control of mosquitoes and other water-breeding insects; trematodicidal properties for control of the larval stages of *Schistosoma* and *Fasciola* parasites; hirudinicidal properties for control of aquatic leeches; and fungicidal properties for the potential topical treatment of dermatophytes. Most of these studies are in experimental stages and need further support.

Extensive studies of molluscicidal properties of ended since 1964 suggest a new approach to the control of schistosomiasis and other snail-borne diseases by using locally produced ended on a community self-help basis. Commercially available chemical molluscicides are beyond the reach of developing countries because of their high cost, so ended or similar plant molluscicides that can be developed locally may be very valuable to poorer countries affected by these diseases. Ended's other newly discovered properties might provide additional economic incentives to develop it.

Careful agronomic studies of ended over a 5-year period led to the selection and experimental cultivation of 3 out of 65 strains collected from different ecological zones in Ethiopia. The three strains selected had high molluscicidal potency (two to three times more than previously used unselected strains), produced large yields of berries, and were highly resistant to insect pests. Application of new tissue culture techniques at the Plant Products Institute in Salt Lake City, Utah, is providing methods of cloning and cultivation for mass propagation of the plant. Ended is being used as a model plant by a biotechnology development program investigating in vitro biosynthesis of an active principle through a continuous calus-cell culture system. The cloned ended plantlets from Utah and the seeds of selected strains from Ethiopia are being grown experimentally in Ethiopia, Zambia, Swaziland, Brazil, and the Philippines.

The World Health Organization (WHO) Collaborative Centre for Traditional Medicine at the University of Illinois, Chicago, tested ended for mutagenic properties to determine its safety for widespread use. The results were negative, confirming an earlier study that ended has no mutagenic activity under the different experimental conditions used.

Encouraged by these developments, an International Workshop on Ended was convened in Lusaka, Zambia, in March 1983. The workshop reviewed all studies on this plant, identified gaps in knowledge, and developed specific projects that could be undertaken as collaborative ventures by a worldwide network of interested institutions and individuals.

Description of the Plant

Ended is the Ethiopian name for the soapberry plant *Phytolacca dodecandra* (L' Herit) (Synonyms: *P. abyssinica* Hoffin., *Pircunia abyssinica* Moq.), a member of the Phytolaccaceae family (fig. 1). The distribution of this plant is east, west, central, and southern Africa and parts of South America and Asia (l).

Ended has small berries which, when dried, powdered, and mixed in water yield a foaming detergent traditionally used in Ethiopia and elsewhere for washing clothes. In Ethiopia, ended exists as two main varieties, the more powerful *arabe* with pink berries and *ahiyo* with grey berries.

The plant is a rapidly growing climber with hanging branches. The plant's average height is 2z to 3 meters, although it can reach a height of up to 10 meters. Under favorable climatic conditions in Ethiopia, the plant bears fruit twice a year, in January and July.

Phytolacca dodecandra L'Herit and the closely related *Phytolacca americana* L., commonly known in the United States as pokeweed, long have been recognized for their varied uses. Different parts of the plant, including the leaves, fruit, and roots, are

***The opinions expressed in this paper are those of the author and do not necessarily reflect those of the United Nations.**

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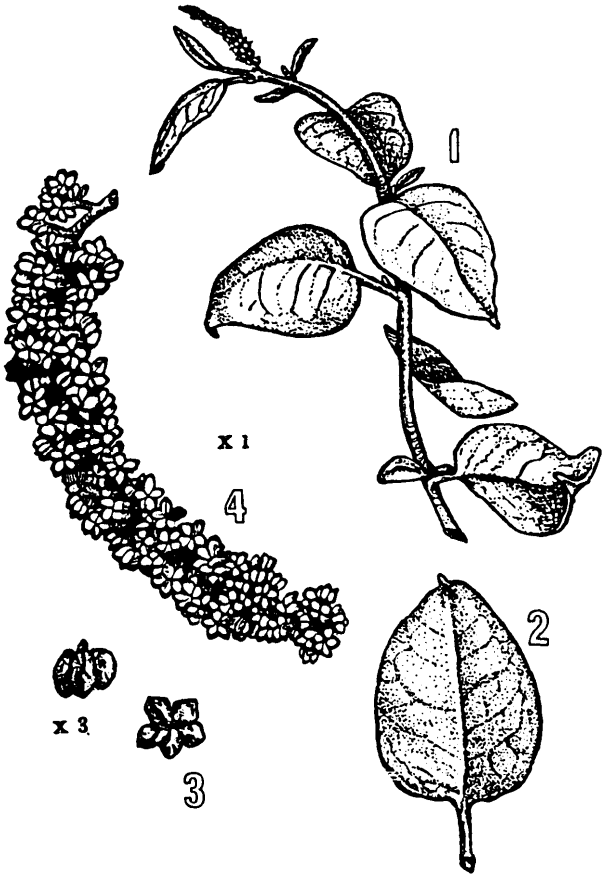
Figure 1 .—Phytolacca dodecandra (Ended plant)



widely regarded for their emetic, purgative, taeniafugal, abortifacient, detergent, antisyphilitic, and other properties (2),

Discovery and Early Studios

In *1964,* while doing an ecological study *to* determine the distribution of schistosome-transmitting snails in a small stream in Adwa, northern Ethiopia, large numbers of dead snails were found in areas immediately downstream of local people using ended to do their laundry. Live snails were abundant in areas further upstream and downstream from laundry sites. This led to subsequent studies of the molluscicidal property of this plant (3,4,5). The results obtained from these studies indicate that this or similar local plant products could be used as molluscicides to control schistosomiasis and other snail-borne diseases on a sustainable community self-help basis.

About 10 to 20 parts per million (ppm) of the sun-dried, ground ended berries diluted in water kill schistosome-host snails within hours of exposure. Ended's molluscicidal potency remains stable over a wide range of water acidity/alkalinity (pH) and with varying concentrations of organic and/or inorganic matter in the water, and is not significantly affected by solar ultraviolet light, In contrast, copper sulphate, the well known and widely used molluscicide, is adsorbed by organic and inorganic matter in treated bodies of water; sodium pentachlorophenate, another important molluscicide, is rapidly destroyed by ultraviolet light; and the widely used Shell product Frescon® is affected by the pH of the water in which it is applied (4,6).

Since the discovery of the molluscicidal properties of ended in 1964, more than 40 scientific articles have been published and several patents registered on different aspects of the plant (7,8,9). Unfortunately, because most of these publications are in journals with relatively limited distribution, such as the Ethiopian Medical *Journal,* the research results have not been accessible to many scientists and potential users in different parts of the world (5).*

Role Of Molluscicides

The role molluscicides can play in effective control of schistosomiasis and other snail-borne diseases of humans and livestock has been well established. Numerous schistosomiasis control projects in Egypt, the Sudan, the Middle East, Japan, the Philippines, China, Brazil, and other countries have shown that snail control by molluscicides, either alone or in combination with other methods (e.g., chemotherapy, environmental measures, health education, etc.), can rapidly reduce or eliminate disease transmission. In recent years, there has been a marked change in snail control strategy from "blanket" or areawide treatment to seasonal and site-specific treatment. For more efficient, economical, and long-range results, focal mollusciciding efforts should be coupled with selected population

***To make this information available in a single** source, we have compiled the widely scattered reprints and publications on this plant and published them in a 522-page book along with a review of the status of ended research as of the publication date. Copies can be obtained free of charge from the authors (Aklilu Lemma, 145 Broadview Ave., New Rochelle, N.Y. **10804;** or Dr. Donald Heyneman, Department of Epidemiology and International Health, University of California, San Francisco, Calif. 94143).

chemotherapy and appropriate health education and community involvement. Such an integrated control strategy will continue to be the most effective approach until an appropriate vaccine against this disease is developed.

Comparison with Other Molluscicides

Several chemical molluscicides have been used for control of schistosomiasis and other snail-borne diseases over the last few decades. Among the most notable are copper sulphate and other copper salts which in the past have played major roles but largely have been discarded because of low efficiency and inactivation by various organic and inorganic matter in water. Another popular chemical molluscicide was sodiumpentachlorophenate, also discarded because of its irritant effect on human skin and rapid decomposition by solar ultraviolet light. The shell chemical product Frescon® (N-tritylmorpholine), known to be highly sensitive to variations in water pH, and the Japanese product *Yurimin* are no longer produced. The Bayer Co. product Bayluscide (niclosamide) is the best molluscicide commercially available and the only one recommended by WHO for widespread use. Because of the high cost of this product (more than $25,000 per metric ton (MT) in 1981), only a few developing countries are using it and on a limited scale with external financial assistance. The lack of market for molluscicides discourages private enterprise from searching and developing other products, including the organo-tin compounds, which have some promise for slow release application.

Plant Molluscicides

In recent years, as a consequence of the constraints to chemical molluscicidal use and of the encouraging results obtained from ended in Ethiopia, interest in plants with molluscicidal properties has increased. Thousands of plants have been screened for molluscicidal activities using a standard WHO procedure. The comparative potencies of ended and some of the leading chemical and plant molluscicides are shown in tables 1, 2, and 3.

Many plant molluscicides, such as from the fruits of *Sapindus saponaria, Swartzia madagascariensis, Balonites aegyptica,* and the bark of *Entada phaseoloides,* contain saponins. The roots of *Derris elliptica,* the pulp of *Agava susakaba,* and the leaves of *Schima argenta* have been reported in the literature to have molluscicidal properties. All of these are suspected to be harmful to the environment; there is long-established knowledge that they are potent fish poisons. Ended is the most exhaustively studied of the known plant molluscicides and provides a model for similar studies. *Croton tiglium, C. macrostachys, Jatropha curcas,* and *Ambrosia maritima* also deserve special consideration (10,11).

The seeds of two species of croton, *Croton tiglium* and C. *macrostachys,* that grow abundantly in the Philippines, India, and the Sudan, have high molluscicidal potencies, For an unknown reason, croton seeds are more active against *Bulinus* species of snails (transmitter of urinary schistosomiasis) than against *Biomphalaria* species (transmitter of intestinal schistosomiasis). A more serious drawback of croton is that it is carcinogenic and highly toxic to humans (12,13).

The seeds of *Jatropha curcas,* a plant that grows abundantly in the Philippines and produces seeds almost year-round, have a relatively high toxicity against *Oncomelania* snails and more moderate toxicity against *Bulinus* species (14,15). WHO is sponsoring studies to identify the active ingredient and determine the product's stability under various physical-chemical conditions and may undertake further field trials in the Philippines, The seeds and all other parts of the *Japtropha* plant showed no effect against *Lymnaea* snails, which transmit the economically important major animal disease, facioliasis (16). *Lymnaea* snails are major agricultural pests that multiply rapidly in rice paddies and destroy blue-green algae that are essential for nitrogen fixation (47). In an attempt to control these snails, scientists at the International Rice Research Institute in Los Banes, the Philippines, are introducing and testing ended, which is very potent against *Lymnaea's* adult and oval stages.

The leaves and flowering tops of the Egyptian plant, *Ambrosia maritima* (locally called damsissa), have some molluscicidal properties that have been studied for a long time. An infusion of the leaves at a concentration of 1,000 ppm kills planorbid snails in 24 hours (17); ended kills the same snails at less than 10 ppm in 24 hours, A positive feature of damsissa is that it seems to grow easily and reach maximum growth at the peak of schistosomiasis transmission in Egypt (11).

Nonovicidal Nature of Ended

While some of the chemical molluscicides such as Bayluscide (niclosamide) are known to penetrate egg masses and kill unhatched snails, others such as *Ended* and *Frescon*® (N-tritylmorpholine) are

Table 1.—Comparative Potencies of Different Molluscicides

Molluscicide	Material Tested	Snails Tested	LC_{100} Exposure time	Authors
Chemical Molluscicides				
Bayluscide	Emulsion conc.	Biomphalaria spp.	0.3 ppm/24 hrs.	Günnert and Strufe, 1962(19)
Frescon	16.5% Emulsion	Biomphalaria spp.	0.2-0.5 ppm/24 hrs.	Lugt, 1981 (18)
Copper sulphate	crystals	Biomphalaria spp.	3 ppm/24 hrs.	Günnert and Strufe, 1962(19)
NaPcP	powder	Biomphalaria spp.	3 ppm/24 hrs.	Günnert and Strufe, 1962(19)
Plant Molluscicides				
Endod	berries Water extract Butanol extract	 Biomphalaria spp. Biomphalaria spp. Oncomelamia spp.	 6.5ppm/24 hrs. 1.6ppm/24 hrs. 1.85 ppm/48 hrs.	 Lugt, 1981 (18) Lugt, 1981 (18) Yasuraoka, 1971(15)
Jatropha	seeds Water extract Butanol extract	 Onchomelamia spp. Onchomelamia spp.	 27-48 ppm/48 hrs.* 45 ppm/48 hrs.	 Yasuraoka, 1976 (14)
Croton	seeds Water extract	 Biomphalaria spp. Bulinus spp.	 20 ppm/24 hrs.** 1 ppm/24 hrs.	 Doffalla and Amin, 1976 12)
Ambrosia	leaves and flowers in water	Biomphalaria spp.	1,000 ppm/24 hrs.	El-Sawy et al, 1981 (17)

[a]No action against *Lymnaea* and low activity against *Bulinus* spp. (16).
[b]No activity found against *Biomphalaria* by Lugt, 1981 (18).

Table 2.—Effects of Various Molluscicides on the Egg and Different Sizes of *Bromphalaria globrata* Expressed as 24-Hour LC_{90} (ppm.)[a]

Molluscicides	Eggs	small (l-3 mm. diam.)	Large (10-15 mm. diamm.)
Ended**	100.00	4.70	3.00
Bayluscide	0.20	0.20	0.26
Frescon	40.00	0.20	0.21
Copper sulphate	4.00	0.70	0050
Pentaclorophenol	1•00	0.65	1.00

[a]Adopted from Lemma and Yau (20)
[b]Endod is active against *Lymnaea eggs* at concentrations of 3-5 ppm in 24 hours
See text for possible explanation

Table 3.—Molluscicidal Potency of Butanol Extract of Ended Against Various Species of Snails[a]

Snail Species (adults)	24-hr. LC_{90} (ppm.)	Investigators
Biomphalaria glabrata **(NIH)**	3.0 ± 0.25	Lemma *et* al., 1972
Biomphalaria glabrata (Brazil)	***2.3 ± 0.25***	Paulini, 1971
Biomphalaria alexandrine (Egypt)	3.0 ± 0.25	Heynemann and Limm,)971 Lemma et al., 1972
Biomphalaria pfeifferi (Ethiopia)	2.8 ± 0.25	Yohannes ***et al.,*** 1970
Bulinus truncatus **(Egypt)**	**3.4 ± 0.25**	**Lemma** et d., 1972
Bulinus truncatus (Ethiopia)	1.4 ± 0.25	Yohannes et al., 1970
Lymnaea natalensis **(Ethiopia)**	**2.8 ± 0.25**	**Yohannes** et al., ***1970***
Oncomelania nosophora **(Japan)**	**1.6 ± 0.25**	**Wagner, 1971** **Yasuraoka, 1971**

aAdopted from Lemma and Yau(20)

known to be nonovicidal at molluscicidal concentrations. This has often been cited as a disadvantage of ended (table 2). This was particularly valid in the earlier days when "blanket" treatment or areawide application of molluscicides was the recommended procedure for controlling snails. Under those conditions, unless the molluscicide had ovicidal properties, repeated applications were necessary to kill the young snails hatched from unaffected eggs.

However, recent field studies in Egypt, the Sudan, Brazil, and St. Lucia indicate that the ovicidal property of a molluscicide may not provide the advantage attributed to it earlier. Repeated molluscicding of such water bodies is necessary because treated areas are rapidly repopulated by snails from untreated parts of rivers or irrigation canals, even areas treated with the highly ovicidal Bayluscide. Under these circumstances, the ovicidal property, or the lack of it, may not be a significant advantage because the molluscicide has to be applied repeatedly on a regular basis,

In an attempt to determine whether ended was nonovicidal to only certain species of snails, eggs of different species of *Biomphalaria, Bulinus,* and *Lymnaea* snails were tested at the Harvard School of Public Health. Whereas 100 ppm or more were needed to kill *Biomphalaria* and *Bulinus eggs* in 24 hours of exposure, 3 to 5 ppm killed all *Lymnaea eggs*. These findings were further substantiated in field trials with ended in Puerto Rico where *Lymnaea, Marisa,* and *Physa eggs* and adult snails were destroyed by a concentration of about 5 ppm in 24 hours (5).

WHO Workshop on Plant Molluscicides

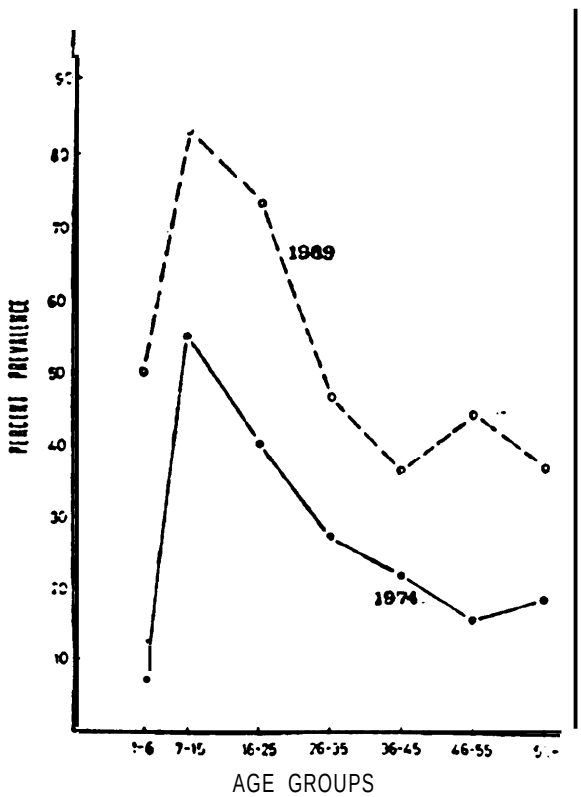
In response to the growing interest in molluscicides of plant origin, the scientific working group of schistosomiasis of the UNDP/World Bank/WHO special program of research and training on tropical diseases convened a workshop on plant molluscicides in Geneva, Switzerland, from January 31 to February 2, 1983. The workshop evaluated and recommended some specific actions for developing plant molluscicides. It recognized ***Phytolacca dodecandra*** (ended) as the most promising plant molluscicide studied to date. Subsequently, an international scientific workshop on ended was convened in Lusaka, Zambia, in March 1983. This workshop reviewed all past and present work on ended and recommended future areas for collaborative work among developing countries and among developing and developed countries.

Field Evaluation of Endod

In a 5-year schistosomiasis pilot control study with ended in Adwa, Ethiopia, the prevalence of S. *mansoni* in children between the ages of 1 to 6 was reduced from 50 percent at the start of the project (1969) to 7 percent after continuous control for 5 years (1973). The incidence of disease throughout the population in Adwa (17,000) dropped from 63 to 34 percent in 5 years. This included many incurable chronic cases in older individuals. This was achieved by systematic application of crude ground ended berries collected from the immediate neighborhood of Adwa (21).

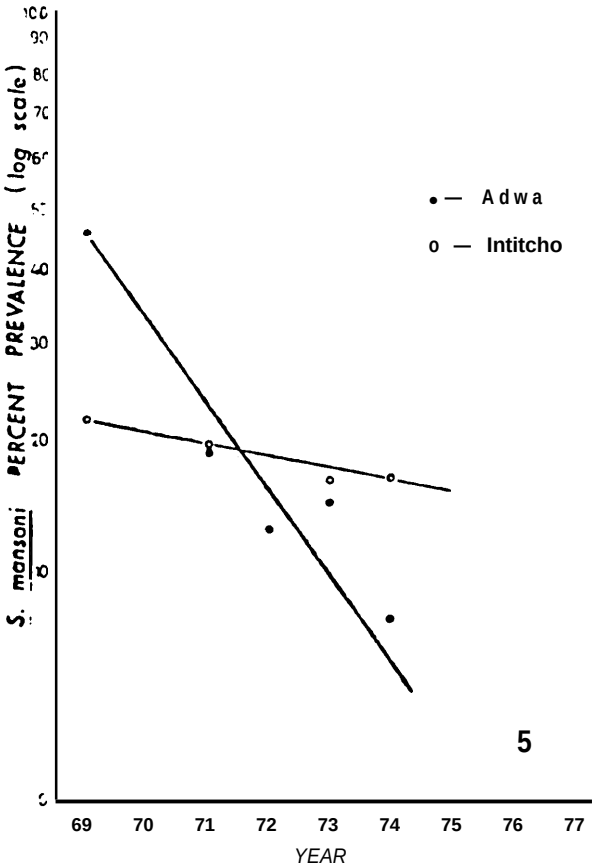
Annual disease surveys during the study showed a progressive reduction both in prevalence and new reports of the disease in Adwa, while these figures remained almost constant in the untreated nearby village of Inticho, suggesting that the action primarily was due to ended applications reducing the snail population (figs. 2 and 3). Ecological observations

Figure 2.— Prevalence Rates of Schistosoma mansoni in Adwa Before and After Control Measures by Application of Ended



SOURCE: Adapted from Lemma, et al. (21).

Figure 3.— Prevalence of Schistosoma mansomi in Children 1-6 Years Old in Adwa and in the Control Area of Intitcho



SOURCE' Adapted from Lemma, et al. (21).

during these 5 years indicated that ended had no obvious adverse effects on the microflora and fauna of treated streams (21).

Significance of Application of Schistosomiasis Control

Schistosomiasis is one of the most important and rapidly spreading parasitic diseases of mankind, a growing threat to large-scale irrigated agriculture and hydroelectric power development schemes in many tropical and subtropical parts of Africa, the Middle East, the Far East, and many parts of South America. An estimated 200 million to 300 million people are affected by this debilitating disease, and another 300 million to 400 million potentially are exposed to it.

The spread of schistosomiasis is a tragic example of man's actions. Well intended irrigation and other water-related development projects in tropical countries often have created new breeding habitats for the snails that transmit this disease. Infected and noninfected people working in such areas often must drink from, bathe in, and labor in the same canals. Human wastes often are excreted, dumped, or washed into the same water, leading to the rapid spread of this disease, especially among highly vulnerable children.

Inasmuch as schistosomiasis is a social disease, its control poses a major social challenge. There seems to be no single method to control schistosomiasis effectively. Ideally, effective control should involve the combined uses of mass treatment of all infected people, snail control using molluscicides to interrupt disease transmission, health education, and improved standard of living.

In spite of recent promising developments, there still is no safe, effective, and affordable drug suitable for mass treatment. Treatment by drugs (containing toxic compounds) sometimes was more tortuous than the disease itself. An excellent new drug, Praziquantel, still is too expensive for mass use. However, even if an ideal drug were available and cured an individual, he or she probably would be reinfected as long as the source of infection remained uncontrolled. Unfortunately, health education and improved environmental sanitation are long-range measures, closely interwoven with general socioeconomic and educational structures of a community. They must rely on governmental policies, plans, and available resources to increase the standard of living of the affected population. Under present circumstances, the most effective and practical method of controlling schistosomiasis is through a combination of selective treatment of infected individuals and control of new transmission by killing the host snails at each proven site of infection.

Community Participation

One essential component of the Adwa project was active community participation. The provincial governor-general, the mayor of Adwa, and the Municipal Council all were involved throughout the duration of the program. Their roles in planning and executing the project were essential for its ultimate success. The budget for the 5-year pilot-control project was provided by the Adwa Municipal Council. The Council also provided necessary facilities and manpower, including a headquarters for the project and local staff to run the field work, collect ended from wild plants, or purchase ended from local markets where it is sold as soap (21).

Another major reason for community participation is to have the community, through its Municipal Council, continue the control program on completion of the 5-year experimental work. Involvement of the affected population in the control of the disease on a "community self-help" basis is critical to the long-term success of a program. Projects are all too often discontinued as soon as the outside team leaves.

Detergent and Foaming Properties

The detergent and foaming properties of ended have been tested (Shell Crane, pers. comm.) with a detergiometer and compared with other commercially available detergents in a preliminary trial at the Stanford Research Institute (SRI) in **1971. It was** found to be an effective clothes cleaning agent. Further studies should find ways to make the extract serve as a supplement or substitute for other detergents. Ended has the advantages over some chemical detergents of being harmless to delicate fabrics (such as fine cotton, linen, and wool) and of leaving the clothes noncompressed. Further, it is biodegradable and its use has no apparent deleterious environmental effects. Experimental evidence suggests this as does its centuries of use in streams, but more research should be done in this area.

The high foaming property of ended could be modified for use in lightweight concrete and foam rubber. It may also be possible to use it as a dispersant in perfume manufacture. Limited preliminary studies indicate commercial potential, but a great deal more investment and study is needed.

Larvicidal Properties

Studies at Harvard University on the comparative toxicity of ended and other compounds on stream flora and fauna showed that mosquito larvae are particularly susceptible to the lethal effect of ended (22). This led to other investigations which demonstrated the susceptibility of larvae of the notorious black fly *(Simulium spp.),* which transmits river blindness, or onchocerciasis, and larvae of the domestic house fly, ***Musca domestica*** (23,24). Further development of ended as an insecticide for village use could have public health significance. Since snail and malaria-transmitting mosquitoes may breed in the same type of environment, control of snails with ended may have the added benefit of reducing mosquito populations.

Hirudinicidal Property

The aquatic leech, *Lymnatis nilotica,* a major animal pest of livestock in many tropical countries, is susceptible to ended (5). Ended has been used for centuries in Ethiopia to control this pest. This use should be improved for more effective protection of domestic animals against this debilitating ectoparasite.

Trematodicidal Property

Schistosome cercariae and other trematode larvae are highly susceptible to ended. Infected waters can be rendered safe for several days by application of small quantities of ended. The active ingredient can also be prepared in ointment form for application on exposed skin of workers in irrigation canals as a prophylaxis against cercarial penetration. This has been tried at SRI with some success using the tails of test mice coated with ended ointment and immersed in cercaria-containing water **(20).**

Spermicidal Properties

Systematic biological screening of the butanol extract of ended showed it to be an extremely active biological agent against human sperm, thus suggesting its possible use as a locally produced, vaginal foam birth control agent (9). Ended long has been known and is widely used as an abortifacient in traditional societies in Ethiopia and other parts of East Africa. Recent laboratory studies have shown it to cause strong uterine contractions **(25).** Intrauterine injection of small quantities of ended extract in pregnant mice causes sterile and apparently harmless abortion. In addition to preventing pregnancy, it maybe useful as a "day after" pill **(26),**

Other Snail-Killing Properties

Ended is also effective against snails that transmit other important human and animal diseases besides schistosomiasis. Laboratory and field studies have indicated that *Lymnaea* spp. are extremely susceptible to ended. These are the snail hosts of important cattle and sheep liver fluke that cause fascioliasis. Spraying pastures with relatively low concentrations of ended will kill snails, eggs, and infective larvae of the parasites without affecting the animals or vegetation on which it is sprayed. In

view of the worldwide distribution of fascioliasis, development of ended to control this disease could benefit not only developing but also developed countries.

Lymnaea snails, which multiply rapidly, reach a biomass of 1.5 MT/ha in some ricefields in the Philippines and graze heavily on blue-green algae that are carefully introduced and grown for nitrogen fixation in paddy fields (47), The International Rice Research Institute in Los Banes, the Philippines, is investigating the possibility of using ended to control these snails (P.A. Roger, pers, comm.).

Fungicidal Properties

Biological screening tests have revealed that endod has a selective toxicity to dermatophytes, the fungi that cause a variety of skin conditions, such as athlete's foot and ringworm, The possible use of ended for treatment of these diseases needs further investigation (27,45),

Toxicological Studies

One of the most important criterion for widespread use of any molluscicide is its safety to humans, animals using the treated bodies of water, and local flora and fauna. The possibility of long-term negative effects from using such compounds should also be determined. Preliminary studies on the toxicity of ended to a variety of animal and plant species and tests for carcinogenic properties have been undertaken (4,28). Some of these earlier tests have been recently reconfirmed at a WHO reference laboratory at the University of Illinois in Chicago (Norman Farnsworth, pers. comm.).

A comparative toxicity study in sheep and dogs, as representatives of large ruminant and monogastric animals, both domestic and wild, that might drink endod-treated water, has also been undertaken. Sheep force-fed with the water extract at a dose of 1 gm/kg body weight died within 96 hours, whereas a dose of 200 mg/kg body weight had no apparent effect on kidney and liver function tests done over a period of 4 days (29). Oral administration to dogs at a dose of about 100 to 200 mg/kg body weight caused vomiting within minutes. Intravenous injection at the dose of about 50 mg/kg body weight was lethal in less than 24 hours, but 8 mg/ml of blood did not show any significant changes (29). Thus, although ended is known to be hemolytic to red blood cells if injected intravenously, it generally is well tolerated by animals if administered orally. When taken orally by humans and monkeys, it immediately causes vomiting so most of the product fails to reach the intestine. The emetic property of the berries is so strong that SRI chemists considered using it to prevent possible overdose from potentially dangerous drugs such as sleeping pills. *

Continuous application of high concentrations of ended solution to economically important plants over long periods of time appears to act as a fertilizer, promoting more rapid growth of test plants compared with controls given only water (4).

As with other molluscicides (20,30), small fish and tadpoles are affected by ended at molluscicidal concentrations. However, edible fish rarely breed in the small streams and canals where disease snails normally breed; adult frogs instinctively jump out of treated waters; and large fish swim rapidly from endod-treated sites to avoid its irritant effects. Hence, the product does not appear to have a significant impact on these animal populations. Birds known to feed on berries of wild plants seem unaffected, as do waterbugs in treated streams, During the 5-year experimental period in Adwa, treated streams were monitored for possible toxicity of ended to representative species of the flora and fauna, and no apparent effects on the aquatic ecology were noted. There has been no apparent negative impact from ended use in rural communities where ended has been and still is being used for washing clothes.

Our studies have shown that the active principle in ended biodegrades rapidly. The lethal effects following ended application to streams, canals, or lakeshore persist for 24 to 48 hours, after which time ended's potency rapidly declines.

Ended berries have been used for washing clothes in streams and lakeshores in Ethiopia and other parts of Africa for centuries, with no apparent toxic effect. Also, in Ethiopia and elsewhere in Africa, high concentrations of the ended leaves, roots, or berries are taken orally for various medicinal purposes, such as for purging intestinal parasites and for abortion (2,25). If ended had any harmful effects, surely it would not have survived centuries of human use. As with all natural products used by local people, its dangers would have been recognized and the substance discarded,

These assumptions were in part tested through mutagenic studies done on ***Phytolacca*** *dodecandra*

*Since the active component in ended is a relatively large molecule, it is assumed that it may not be able to easily penetrate the walls of the gut and enter the bloodstream where it could cause much damage. Also, itsemetic properties in regurgitating animals may give it a built-in protective mechanism to safeguard against its possible harmful effects.

by Lemma and Aimes (1974) on in vitro assays using a ***Sahnonella typhimurium*** strain that since has given possible false results in the presence of histidine, a material normally found in most plant extracts including ended. Since traces of histidine from ended could have interfered in those tests, the negative data obtained in the 1974 study were considered equivocal and new tests were undertaken by Dr. N. R. Farnsworth of the WHO Collaborating Centre for Traditional Medicine and College of Pharmacy, University of Illinois, Chicago, using strains of ***Salmonella*** that are not affected by histidine. The results were all negative, confirming that neither ended nor pokeweed, which was tested for comparison, have any mutagenic properties under the experimental conditions employed (Farnsworth, 1983, pers. comm.).

However, conclusive evidence of mutagenic tests and other chronic toxicity of ended or any other similar product should come from carefully designed long-range toxicity studies in different species of animals, rather than from tests in isolated in vitro cell systems. To this end, the author hopes that WHO and/or another concerned organization will finance such a long-range study on ended so that its widespread use in areas where it is most needed could be further promoted and effectively applied.

Chemical Studies

The ended plant provides material of special interest to organic chemists involved in saponin chemistry, partly because of the high percentage by weight of crude saponins in dried berries, and partly because of the chemical complexity of the materials. Most chemical work on ended until now has concentrated on the saponins of dried berries. Although this work has yielded interesting chemical findings and valuable biological discoveries, according to Parkhurst, it seems that this is only "the tip of the iceberg" in developing the chemical potential of ended (31).

Chromatographic separation of crude saponins in ended demonstrates the dozens of compounds present, of which only a few have been characterized. The nonsaponin fraction, amounting to 75 percent of the total weight of the dried berries, may be broken down into petroleum-soluble lipids, water-soluble sugars, starches, pectins and gums, and a water-insoluble fraction. Little is known about the chemistry or potential uses of these fractions. Abundant as the berries are, they represent a small fraction of the plant, the rest of which is ample material for future chemical study (35,36,37),

Valuable byproducts have been obtained through the development and refining of endod. The water-soluble fraction with sugars and various polysaccharides, produced in the isolation of crude ended saponins, remains to be studied. New pectins, starches, thickening agents, material for fermentation to alcohols, and sources of rare sugars with industrial importance all may be found in the plant material left after the berries are cropped. The water-insoluble fraction, also not yet investigated, may be useful as animal feed, a fuel, or a soil additive.

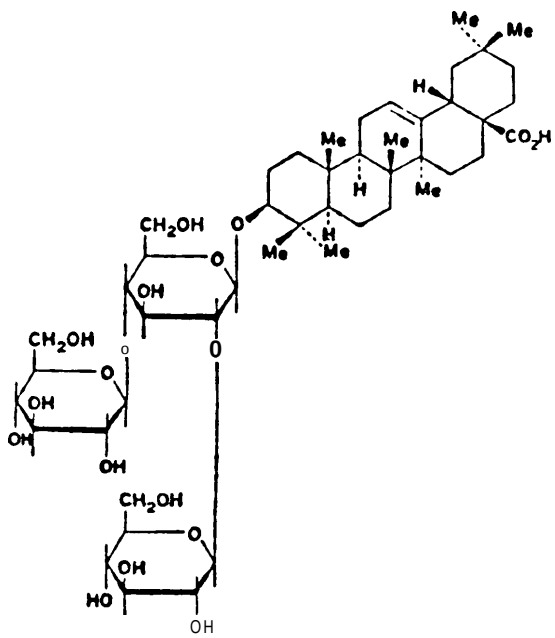
A green lipid material is obtained from the petroleum extraction of ended. While most of this material is composed of palmitic, oleic, and stearic acids, 12,5 percent consists of a nonsaponifiable, bright orange, waxy material containing squalene and a complex mixture of high molecular weight alcohols—i.e., phytosterols and/or triterpenols. As the value of ended saponins is established and production rises, large-scale development and additional uses for the saponin will follow and more byproducts should become available.

Chemical studies to isolate and identify the active principle in the ended berries have led to the discovery of a new compound, oleonolic acid glucoside, which Parkhurst, et al., have named *Lematoxin (32,33,34)* (fig. *4).* Three different procedures have been developed in different laboratories to extract the active ingredient from ended berries. The first method, developed and patented by the Tropical Products Institute in London in 1971, was relatively complicated, based on methanol extraction followed by potassium hydroxide hydrolysis in two steps (7). In 1972, a simplified method involving single-step butanol extraction from an aqueous suspension of dried berries was developed and patented by SRI investigators in California (8,38). Both of these procedures give extracts effective at 2 to 4 ppm, However, both procedures have the disadvantage of depending on importing extraction solvents, thus demanding hard currency. This shortcoming was finally overcome by the third and perhaps most practical and promising extraction procedure which is based on fermentation techniques. It was discovered by the Ethiopian chemist Tesfaye Lemma, working under the author's supervision at the Institute of Pathobiology in Addis Ababa.

Improved Extraction Procedures

Water extracts of powdered ended berries after defatting with benzene were more active in killing snails than were extracts obtained without a defat-

Figure 4.— Lemmatoxin-A



SOURCE Parkhurst, et al (32)

ting procedure. The concentration needed to obtain a 100 percent kill in 24 hours was 2 ppm. The increase in molluscicidal activity was proportional to the degree of defatting. The molluscicidal potency of a suspension of ended left to ferment over a period of time increased gradually for a long time (up to 5 months) if the ended concentrations were 10 to 20 percent. On the other hand, if the concentrations were 0.1 to 1.0 percent, the molluscicidal activity decreased within a few weeks. The best results were obtained when fermentation took place at 22° C (39),

The fermentation-based extraction procedure depends on practical, simple techniques using yeast cells that are part of the normal flora of the berries. When ground ended berries are soaked in water and left in a warm place, they ferment rapidly and eventually separate into a clear supernatant fluid containing the active principle, and a residue, mainly yeast cells and debris, The supernatant is easily separated from the residue and can be dehydrated with small, locally constructed solar drying chambers and ground into a fine powder. The powder then can be dusted directly over the infected water or prepared in different formulations such as flakes that can either sink or float on water depending on the specific snail targets; made into emulsion concentrates for spraying; or compressed into special briquettes of different hardness for slow release in water. In the case of briquettes, farmers and villagers could easily be taught how to prepare them, how much and how often (per week or per month) they should be applied, and at which specifically predetermined spots (based on the volume of water to be treated and the degree of hardness of the briquette) (5).

The simple fermentation chambers required and the drying and processing apparatus for this molluscicide could be constructed by students at neighborhood schools, a local blacksmith, or itinerant "barefoot technologists." The apparatus must be simple enough for easy operation by village-based community-health workers. Although preliminary studies to develop these possibilities have much promise, the packaging of technology and know-how have yet to be developed and standardized.

Yeast cells from the fermentation process can be washed, sundried, and used as high-protein supplements for animal feed, particularly as additives for chicken feed, These prospects, however, have not yet been fully studied.

Another useful tool to be developed is a simple procedure for calorimetric determination of the concentration of the active principle of ended in treated bodies of water. Such a device would allow more effective use of the product in the field (40).

Agronomic Studies

While research on the toxicity, chemistry, extraction, and application of ended has been progressing rapidly since the discovery of its molluscicidal properties in 1964, in general, studies on the agronomic aspects have lagged behind this research. Detailed investigations of the agronomic aspects of ended production were begun by Dr. Legesse W. Yohannes and his colleagues only in 1972. These efforts subsequently were enhanced by Dr. Charles B. Lugt, a Dutch agronomic chemist, sent to work in Ethiopia under a special grant from the Netherlands Government. A major goal of these studies is to select and breed plants for favorable growing characteristics, productivity, and high potency of berries, Ended is a dioecious plant but can be propagated readily from berries and cuttings, Some agrobotanical data on plant ecology, nutrition, germination, spacing, and irrigation have been gathered during the past few years, and studies on the ecology of the plant and its pests have been undertaken in Ethiopia (44).

The great climatic and ecological diversity of Ethiopia apparently has given rise to several geographic strains of Phytolacca *dodecandra* with

varying growth characteristics, yields, insect resistance, and molluscicidal potencies. In a 5-year (1976-81] extensive agronomic study done on endod by Lugt, 65 different strains and varieties of the ended plant collected from different parts of Ethiopia were studied under comparable conditions. Three strains were selected for their exceptional growth characteristics, high molluscicidal potency (two to three times more potent than the originally used varieties), high annual yield (about 1,320 kg/ha), and high resistance to attacks by insect pests and drought conditions. The results of these studies have been published in a book by Lugt (41). The studies in Ethiopia also discovered that the green unripe ended berries contain more active molluscicidal components than the ripe pink berries, thus reducing time before harvest and damage done to ripe berries by birds (42,43).

One major difficulty with mass cultivation of endod is attack by larvae of a Gitoma drosophilid fly which bores through the stem, selectively killing young shoots of the plant (41,43). Although these insects can be controlled effectively by the application of appropriate insecticides, agronomic studies to select plant varieties with particular resistance to this insect have been promising.

In a current study, Dr. Hugh Bollinger of Plant Resources Institute, Salt Lake City, Utah, is undertaking ended tissue culture using the three selected strains of ended supplied from Ethiopia by Dr. Lugt. He is making significant advances on two fronts:

1. Development of tissue culture methods of mass propagation of selected vigorous strains of the plant through cloning. Plantlets developed through Dr. Bollinger's cloning method are being supplied to Ethiopia, Zambia, Brazil, and Swaziland for field evaluation. The new procedure should improve significantly the prospects for mass cultivation of known strains for large-scale field use.

2. Development of tissue culture system for in vitro biosynthesis of Lemmatoxin, the active principle responsible for killing snails. This exciting approach uses ***"calus"*** cultures with proven high molluscicidal potencies, Dr. Bollinger plans to expand these studies to produce the molIuscicide and facilitate studies on nutritional and other biological characteristics of the plant.

Cost Effectiveness of Endod

Questions about the economy and cost effectiveness of ended should be investigated. During early stages of the study, it appeared that ended would be more costly than other molluscicides. However, two sets of questions must be asked before an adequate comparison of molluscicides to be employed in Third World countries can be made:

1. Does the molluscicide have to be imported from abroad? At what cost? For how long? Rapidly rising rates of inflation and the large quantities of molluscicide required on a continuous basis are key considerations. Can developing countries that are debilitated by schistosomiasis but have limited foreign exchange capabilities afford imported molluscicides?

2. Can the molluscicide be produced locally in sufficient quantities for large-scale use? If so, the initial cost of production during the experimental period presumably would be high, but what would be future large-scale production possibilities? At what cost? With what savings in hard currency and buildup of local agricultural and production facilities? The possibilities and cost of introducing modern agricultural techniques, better yielding plant varieties, and more insect resistant varieties should be included in this evaluation.

It seems that since local production and processing of a multiple use plant product for internal consumption and foreign sale is preferable to continued depletion of limited hard currency, the initial developmental costs should be considered as a high priority investment. As improved methods of cultivation and improved plants are introduced, costs should fall and both the usefulness and profit from the products should rise. As nonmolluscicide uses of ended, such as soaps, drugs, insecticides, and foaming agents, are developed, its cost effectiveness will increase proportionately.

The 1969-73 Adwa schistosomiasis control program that reduced disease transmission by about 85 percent used ended berries collected from wild plants or bought from local markets. The cost was Eth. $0.25 (US $0.10) per person per year (21),

Agrobotanical studies on ended in Ethiopia over the last 5 years under a special grant from the Netherlands Government have shown encouraging results. According to Dr. Lugt and his Ethiopian counterpart, Dr. Yohannes, selected strains of the ended plant can produce about 1,500 kg berries/ha/yr at the cost of about US $1,000 in local currency, while the German product, Bayluscide, costs about $25,000 per MT in foreign currency. Ended grown on only about 1 hectare of land would treat 1,200 ha of irrigated sugar cane, for which 91,000 MT of water per year are needed (4 I).

Where high-potency ended varieties (able to kill snails at 5 ppm) can be harvested directly from local

farms, the ground or pressed berries could be applied directly to streams and irrigation canals, without need to concentrate or extract the molluscide. This would greatly reduce product and treatment costs.

Current Status of Research and Projected Activities

Field and laboratory studies on ended are being carried out in Ethiopia at the Institute of Pathobiology, Addis Ababa University. The Ethiopian Ministry of State Farms is collaborating with the Institute because of their interest in possible commercial farming of ended. The Ministry of Public Health is interested in the field applications of endod for schistosomiasis control in selected localities. However, owing to extremely limited financial resources available for such studies in Ethiopia, the level and intensity of research activities are very restricted.

During the period 1964 to 1974, molluscicidal and other properties of ended were intensively studied in Ethiopia, the Institute of Pathobiology in Addis Ababa, the Tropical Products Institute in London (8), the Stanford Research Institute in California (9), the Department of Epidemiology and International Health of the University of California in San Francisco (5), the Harvard School of Public Health in Boston (22), the U.S. Public Health Service laboratory and field stations in Puerto Rico, and the U.S. Naval Medical Research Unit (NAMRU-3) in Cairo, Egypt (5). Investigations conducted in other institutions in the United States, England, St. Lucia, Brazil, Egypt, the Sudan, Tanzania, and Japan on molluscicidal properties of ended under different conditions helped characterize its biological activity (5,46).

In addition to Dr. Bollinger's studies, Dr. John Lambert and colleagues at Carleton University and Ottawa University in Canada plan to undertake long-range toxicological and agronomic field studies on ended. The toxicological studies are intended to develop ended as a Canadian-backed and registered pesticide for possible widespread use as a molluscicide in different parts of the world. The ecological and agricultural studies, particularly of Dr. Lambert, involve the natural ecology of ended and determination of optimal conditions for growth and development of cloned plantlets, berries, and cuttings of selected strains for adaptation to different countries including Ethiopia, Zambia, and Swaziland, As a result of these recent developments, a renewed interest in ended is rapidly spreading among African and other countries. UNICEF has also shown interest in possible large-scale application of ended for schistosomiasis and other snail-borne disease control, especially with regard to its use on a community self-help basis, to be developed as part of UNICEF's integrated basic health services delivery programs in rural areas.

The Zambian National Council for Scientific Research held an "International workshop on Phytolacca dodecandra" in March 1983, in Lusaka, Zambia, to bring these interested individuals and groups together, review work done on ended to date, identify shortcomings, delineate gaps to be filled, and develop specific areas and ideas for future work. A group of about 30 scientists with varying backgrounds from a number of African countries, the United States, England, and Brazil participated. The Lusaka meeting is expected to lead to further collaborative work on ended by participating experts as well as on other plant products and the control of schistosomiasis in general.

The benefits of ended go well beyond the potential applications of its many different properties, however promising they appear to be. It sets an example of local development of a natural product for multiple use that can be adapted to endemic social and cultural systems, raise the level of group participation and confidence, and improve the local and regional economy at minimal international cost and hard currency expenditure. Ended's major importance, however, is in the control of schistosomiasis and other trematode diseases.

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