It is well known that global biodiversity is being lost at an alarming rate (e.g., Wilson, 1992; Lawton & May, 1995; Pimm et al., 1995). The loss of a species not only impoverishes the community of which the species was once a part, but these losses also affect humans. Biodiversity has economic value to humans and many suggest that of all groups who should be most interested in the preservation of biodiversity, it should be industries leading the way, as biodiversity has provided, and will continue to provide, many raw materials used by industries around the world. This is especially the case for the pharmaceutical industry, as extinction results in the "... loss of raw materials for existing and new weapons in the fight to alleviate human suffering and prevent death" (Grifo et al., 1997). In order for students to fully appreciate the value of biodiversity, especially the value of species as sources of new drugs, it is important to understand the process of drug discovery more completely. This laboratory exercise was designed to help students understand important steps that are part of the actual drug discovery process, while at the same time, help them appreciate the value of biodiversity as a source of new and important drugs to treat a range of human diseases.

Natural Products & The Process of Drug Discovery

How important is biodiversity as a source of prescription drugs? A number of studies reported that from 25% to 57% of prescription drugs sold in the U.S. or worldwide have at least one active compound that now or was once derived or patterned after compounds isolated from natural products (Grifo et al., 1997; Newman et al., 2003; Butler, 2004). Natural products are especially important as sources of new cancer drugs (Mann, 2002; Newman et al., 2003; Cragg & Newman, 2005) and as antiparasitic agents (Tagboto & Townson, 2001; Newman et al., 2003), including antimalarial drugs. One of the first effective antimalarial drugs was quinine, derived from the bark of the cinchona tree, while one of the newest and most effective antimalarial drugs currently available, artemisinin, was first isolated from the herb, Artemisia annua (Hien & White, 1993; Klayman, 1995; Dhingra et al., 2000; Figure 1). Artemisinin has been found to have potential as a cancer drug as well (Singh & Lai, 2001; Lai & Singh, 1995; Lai & Singh, 2006). Although some drug companies have restricted their natural product screening programs, many still believe that the natural world will continue to be our most important source of novel molecules effective in treating a wide array of human diseases.

One of the biggest hurdles we face when considering natural products as sources of new drugs is the question of which species to screen. For example, there are now estimated to be approximately 421,968 species of flowering plants (Bramwell, 2002), but only about 11% of these species have been screened phytochemically (Verpoorte, 1998). This is unfortunate, as many drugs currently being used are derived from plants, including some of the best-selling drugs on the market (Farnsworth et al., 1985; Baker et al., 1995; Newman et al., 2003). Also, the "hit rate," the rate at which the screening of
plant extracts results in bioactivity (has an effect on living cells, tissues, or whole organisms) is higher when screening natural products derived from plants when compared to other approaches (Verpoorte, 1998; Harvey, 2000). And finally, many species are becoming extinct before they are even identified and classified, let alone screened. The race is on to find novel biomolecules and develop new drugs, and at the current rate at which new species are being discovered and screened, we have a long way to go. There is a considerable amount of renewed interest in the use of natural products in the process of drug discovery and development (Harvey, 2000; Strohl, 2000; Tulp & Bohlin, 2002; Vuorela et al., 2004; Cordell & Covard, 2005; Koehn & Carter, 2005), but we must act quickly, as we may be losing as many as one major drug every few years as species become extinct. We need focused and efficient screening programs if we hope to find new drugs. The key question remains: Which organisms do we collect and screen for potential drug development?

There are a number of different screening strategies and approaches that can be used to help determine which species to test for the presence of novel molecules that might prove useful in the process of drug discovery, including random screening, the taxonomic approach, the biorational approach, and the ethnobotanical approach (Grifo et al., 1997; Verpoorte, 1998; Harvey, 2000; Fabricant & Farnsworth, 2001). Although these approaches have all proved useful, many believe that the most valuable approach to help identify species that might contain bioactive compounds involves ethnobotany. The ethnobotanical approach involves looking at organisms used in traditional medicine by various cultures around the world. This approach, and more specifically, ethnobotany (use of plants), has been invaluable in helping to identify species useful in the development of new drugs, many of which are in use today. In general, ethnobotanically-selected plants have a higher hit rate when compared to plants selected by random screening (Balick, 1990; Lewis & Elvin-Lewis, 1995) and there is generally good correlation between ethnobotanical use of various plants and clinical use (Farnsworth et al., 1983; Grifo et al., 1997). Both <i>Cinchona</i> sp. and <i>Artemisia</i> annua, sources of important antimalarial drugs, have an ethnobotanical history. The role of ethnobotany in the process of drug discovery is now well known and accepted (Martin, 1995; Alexiades & Sheldon, 1996). This should not come as a surprise, especially since it has been suggested that up to 80% of people around the world still rely on traditional medicines for all their medical needs. As a result, relying on ethnobotanical information may greatly speed up the search for new and effective drugs derived from natural products.

### Objectives

- Learn about ethnobotany and ethnobotanical research, and how it is conducted.
- Collect basic ethnobotanical data for a plant taxon and consider the importance of ethnobotanical research to the process of drug discovery.
- Evaluate potential medicinal value of some traditional and/or herbal medicines by completing basic methods of extraction and rapid screening procedures to determine the potential bioactivity of compounds extracted from plant material.

### Activities

#### Selection of Plant Species

First, students must choose or be assigned a species to work with for the exercise. I generally assign the species that each student will work with, and provide the material, although there are other approaches. In a perfect world, students could conduct actual ethnobotanical research (i.e., communicate with a shaman or medicine man) to select a species, though this approach is rarely practical. Students could also select a species used by traditional cultures based on research conducted using the literature or the WWW. Regardless of the approach, all species we work with are selected based on ethnobotanical information (i.e., the species has been used by a number of different North American tribes or other traditional cultures), availability of sufficient plant material, and potential bioactivity (Table 1). The material we use is either material I have collected in the wild myself (e.g., sassafras root, skunk

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Part used</th>
<th>% recovery efficiency*</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt; **</th>
<th>Antibiotic effects***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skunk cabbage (&lt;i&gt;Symplocarpus foetidus&lt;/i&gt; (L.) Salisb. Ex Nutt.)</td>
<td>leaves</td>
<td>14%</td>
<td>565 Fg</td>
<td>none</td>
</tr>
<tr>
<td>Cranberry (&lt;i&gt;Vaccinium macrocarpon&lt;/i&gt; Aiton)</td>
<td>fruit</td>
<td>10.4</td>
<td>330.9</td>
<td>1-2 mm zones inhibition</td>
</tr>
<tr>
<td>Slippery elm (&lt;i&gt;Ulmus rubra&lt;/i&gt; Muhl.)</td>
<td>bark</td>
<td>17</td>
<td>771</td>
<td>none</td>
</tr>
<tr>
<td>Horsetail (&lt;i&gt;Equisetum arvense&lt;/i&gt; L.)</td>
<td>whole plant</td>
<td>22.3</td>
<td>457.2</td>
<td>1-2 mm zones inhibition</td>
</tr>
<tr>
<td>Stinging nettle (&lt;i&gt;Urtica dioica&lt;/i&gt; L.)</td>
<td>leaves/stems</td>
<td>4.35</td>
<td>836.5</td>
<td>slight halos</td>
</tr>
<tr>
<td>Juniper (&lt;i&gt;Juniperus communis&lt;/i&gt; L.)</td>
<td>fruit</td>
<td>6.2</td>
<td>318</td>
<td>inconclusive</td>
</tr>
</tbody>
</table>

* Recovery efficiency during extraction from dry plant material, where data are means with n = 2 or 3 replicate extractions

** LD<sub>50</sub> = lethal dose which kills 50% of brine shrimp determined from regression equation, and data are means with n = 2 or 3 replicates

*** effects on <i>E. coli</i>, inhibition of bacterial growth.

Scientific names checked in Foster and Duke (2000).

Table 1. Examples of different species used in ethnobotany and drug discovery lab exercise, along with the part of the plant used, percent recovery efficiency during extraction using 95% ethanol, LD<sub>50</sub> for brine shrimp bioassay, and effects on growth of <i>E. coli</i> over 24 h incubation. More detailed explanation of some information contained here found below.
cabbage, stinging nettle, horsetail, may apple, dandelion root) or plant material purchased from specialty shops selling various herbal medicines, or natural food grocery stores (e.g., cranberry, **Echinacea**, white sage, juniper berries, bilberry, scullcap, birch, and elm bark). Some material has been obtained from herbal teas (i.e., brand name Alvia, including cranberry, horsetail, and scullcap tea bags), which can be purchased at Whole Foods, Wild Oats, or similar stores. All species used are believed to have at least some medicinal value, although this does not mean that they will show bioactivity.

**Ethnobotanical Research**

As part of this exercise, I require students to collect ethnobotanical data on the plant species they are working with for the bioassays. The types of ethnobotanical data that students should try to collect include the following information, outlined in much more detail in Martin (1995), and Alexiades and Sheldon (1996).

- Specific uses of the plant material by specific groups of people should be determined. Which Native American tribes or other traditional cultures are believed to have used this plant and for what specific purposes? This should include documenting historical and traditional, and current uses of plant material, if possible.
- How the product is “administered.” Is the plant material used to make a tea, or ground and formed into capsules?
- All local names and other key terms should be recorded as well. Although it is often best to collect this information by interviewing local people, this is generally not possible.
- Determine the proper scientific name of the plant material, and taxonomy (i.e., family name). For most samples of herbal medicines, this is a relatively easy task, though for some, it will only be possible to determine the name of the genus.
- Collect ecological information on the specimens. What are the native origins and distribution of the plants? What are the preferred habitat and resource requirements of the species?
- Are there any relationships (both complementary and conflicting) between biodiversity conservation and traditional plant uses? Is the species listed by the U.S. Fish and Wildlife Service, or other agency involved with administering the Endangered Species Act, and what is the conservation status of the species?

Most of this information can be collected from various texts and Web pages dedicated to ethnobotany and the use of herbal and traditional medicines. There are extensive databases available online where you can search for relevant information (e.g., Dr. Duke’s Phytochemical and Ethnobotanical Databases, and University of Michigan-Dearborn Native American Ethnobotany). Some Native American tribes also maintain Web pages that could be of value. In addition, there are a number of journals which might be of interest, including the Annals of the Missouri botanical Garden, Economic Botany, Ethnobotany, the Journal of Ethnobiology, and the Journal of Ethnopharmacology, although many of these might be difficult to access.

**Bioassays & The Process of Drug Discovery**

After the selection of appropriate species of plants, we need to consider, in a more formal way, whether these species might contain natural products that could prove useful in the development of new drugs. For the purposes of this lab, we are only interested in the initial stages of the process of drug discovery which involve pre-screening of crude extracts for bioactivity. This process has three basic steps: grinding, extraction, and bioassays for bioactivity. The grinding, which is really part of the extraction process, involves reduction in particle size of the dried or frozen plant material. Extraction involves getting organic molecules into solution, in water and/or in organic solvents. And finally, the process of drug discovery then really begins with the pre-screening bioassays to determine bioactivity.

**Grinding & Extraction**

The first step in the process is to grind the dried plant material in order to reduce the size of particles to less than 2 mm in maximum dimension. Because extraction is really a process of simple diffusion, the size of the particles may affect both the rate and efficiency of extraction. Grinding can usually be accomplished with a coffee grinder, Wiley Mill, or even a mortar and pestle. Although many samples can be ground in the dried state, it may be necessary to work with frozen samples; plant material with a high content of oils can easily “gum up” grinders. The goal is to have close to 5 g or more of dried, ground plant material. All samples should be kept dried and in the dark as much as possible.

The next step is to extract compounds from the ground material. Generally, there are two components: one soluble in organic solvent and one water-soluble extract. Although we generally do not extract the water-soluble component, this can be accomplished using highly-purified distilled or deionized water. Extraction with water has not generally yielded the same number of potentially-useful compounds as extraction with organic solvents (T. McCloud, personal communication). For extraction using an organic solvent, we use 95% ethanol. Although extraction with ethanol is generally not as effective as with other solvents, there are fewer problems with disposal, or problems associated with working with volatile and somewhat hazardous solvents (i.e., 6:3:1 mixture of methanol, ethyl acetate, and methyl t-butyl ether). All samples should be handled with care (wear latex gloves); the amount of material is limited for some types of plant material and some material might be somewhat toxic or cause allergic reactions in some individuals. The specific steps we use are provided below, part of which is based on information provided by Thomas G. McCloud at the SAIC Frederick, under contract to the NCI-Frederick Cancer Research and Development Center (T. McCloud, personal communication).

1. All samples need to be thoroughly dried at 60 °C for at least 48 hours prior to extraction.
2. Dried samples are ground to a size of less than 2 mm.
3. A known amount, weighed to nearest 0.1 mg on weighing paper, not weigh boats (static seems to result in ground material sticking to the boat), is carefully added to a syringe barrel (5, 10, or 25 cc) with a cotton plug at the tip (Figure 2). Be sure to use enough cotton to prevent leakage of solvent out of the syringe barrel. We also wrap a piece of Parafilm around the tip to prevent leakage. Generally, about 0.5, 1.0, or 2.0 g should be added to the 5, 10, and 25 cc syringes, respectively.
4. After the plant material has been added to the syringe, a known volume of 95% ethanol is carefully added to the syringe barrel. Volumes used for the 5 cc syringes should be about 6 to 8 ml of ethanol (the plant material will absorb much of the ethanol), 10 to 11 ml works well for the 10 cc syringes, and at least 17 to 18 ml should be added to 25 cc syringes. Once the ethanol has been added, the top end of the syringe barrel should be tightly sealed with Parafilm (do not replace the syringe plunger at this point) and gently inverted a few times to thoroughly wet the dried sample to allow for efficient extraction. The syringes are then placed in a test tube rack, stored in the dark at room temperature, and allowed to steep for 24 hours.

5. After 24 hours, the extract is removed from the syringe by carefully and slowly replacing the syringe plunger into the barrel. The extract is then drained through the cotton plug at the tip of the syringe and into a pre-weighted (to nearest 0.1 mg) 15 ml centrifuge tube or 20 ml glass scintillation vial. If there is more than 10 ml of extract, it is best to use 20 ml scintillation vials.

6. Next, the solvent is removed. Although there are a number of ways to accomplish this (i.e., rotary evaporation or vacuum drying), we simply allow the ethanol to evaporate passively. Although this may allow more volatile compounds to be lost, passive evaporation seems to work well for the majority of extracts that we have worked with. Centrifuge tubes or scintillation vials containing the extract are left in a fume hood, in the dark, overnight to allow for the solvent to evaporate. Generally, evaporation is more efficient if 20 ml scintillation vials are used as these allow for more surface area for evaporation. Once the solvent has completely evaporated, the tube or vial should be re-weighted. This allows us to determine the percent extraction from the plant material and to determine exact doses of the extract once the dried extract is reconstituted in ethanol. Percent extraction is determined as: (mass of extractable material + initial mass of material used for extraction) x 100%. Do not forget to do this as it is very important; low extraction efficiency may influence future drug development and the processing of raw material.

7. Samples can now be stored dry or frozen in sealed scintillation vials until assays for bioactivity are performed.

Bioactivity Bioassays

The methods used here are pre-screening assays designed to be able to screen extracts in a high through-put fashion that requires little in the way of sophisticated and expensive equipment. There are a number of primary methods being used for the rapid pre-screening of extracts for potential bioactivity, including the crown gall potato disc tumor inhibition assay (Galsky et al., 1981; Ferrigni et al., 1982; Anderson et al., 1991) and the Lemma sp. fond proliferation assay (Einheilig et al., 1985). In addition to these, the two most popular methods are the brine shrimp toxicity bioassay and the disc diffusion assay.

The disc diffusion assay has been around for a number of years (see Bauer et al., 1966) and is generally very effective at detecting compounds that have antibacterial properties and thus have potential as antibiotics. This assay works equally well whether considering antibacterial or antifungal activity (T. McCloud, personal communication). The brine shrimp assay continues to be a popular assay to evaluate bioactivity of natural extracts, and has proved to be successful in many situations since its original development by Meyer et al. (1982). Although some suggest that the shrimp are not highly sensitive to some toxins, others, such as Anderson et al. (1991), continue to find that this is an inexpensive and relatively easy assay that has been very useful as a pre-screen for bioactive compounds, especially those that have antitumor capabilities (Anderson et al., 1991; McLaughlin et al., 1993). This assay has also been used successfully as part of a multi-layer testing regime for evaluating traditional medicines (Kyremenat & Ogunlana, 1987) and in ethnomedical research (Trotter et al., 1983). We use the brine shrimp toxicity bioassay, which measures general bioactivity, and the disc diffusion bioassay, which evaluates antiboctic properties of extracts. Detailed methods for both bioassays are outlined below.

Brine Shrimp Toxicity Bioassay

1. Brine shrimp eggs (Artemia salina Leach) are hatched in artificial sea water ("Instant Ocean") in a "hatchery" overnight. This should produce hundreds of nauplii (immature stage), which are small, but visible without magnification (i.e., tiny pink "dots" swimming around).

2. In the meantime, dried extracts are reconstituted in 95% ethanol. The target, maximum "dose" for this assay is 500 μg per treatment, and the amount of ethanol used to reconstitute the samples depends on the mass of dried extract available after the solvent evaporated. For example, if we have 50 mg of extract, we can add 5 ml of solvent to yield a final concentration of 10 mg mL⁻¹ = (10,000 μg mL⁻¹). Then to add 500 μg of extract to a filter paper disc (used to deliver the extract), with a starting concentration of 10,000 μg mL⁻¹, we would need to add 0.05 mL. It is important not to dilute the extract too much as it then might be impossible to obtain the target maximum dose of 500 μg; it would be necessary to add much more liquid to the filter paper disc than can be absorbed. At the same time, students need to have a sufficient amount of reconstituted extract as they will be conducting two different assays and working at different doses, and thus they will be preparing a number of discs containing extract. Lack of sufficient extract is rarely a problem if students are careful (e.g., for the numbers above, we have 5 ml of concentrated extract but only need to add 0.05 mL of extract with a concentration of 10,000 μg mL⁻¹ in order to add 500 μg of extract). For this assay, three doses are used: 500, 250, and 100 μg, with three replicates at each dose. This allows us to generate a dose-response curve and determine the LD₅₀ (lethal dose which kills 50% of the test population).

3. Once the extract has been reconstituted, a known volume is added to 6 mm discs of filter paper using a micropipettor. To accomplish this, discs are placed into 9-well plates in an organized fashion. Again, discs with 500, 250, and 100 μg of extract should be prepared, with three replicates at each dose level. It is also necessary to prepare three “control” discs that have only a known volume of 95% ethanol added and no extract. All discs are then allowed to dry in the 9-well plates for 20 to 30 minutes before the assay is begun. There will be plenty of time for the discs to dry while students are counting out brine shrimp.

4. To set up the assay, 10 shrimp are transferred using Pasteur pipets to each of 12 tubes containing 5 ml of artificial sea water. Generally, 15 mL centrifuge tubes work well for this assay. This means that if one is going to assay the extract at 3 dose levels, plus controls, it will be necessary to count out a minimum of 120 shrimp. It might also help to add a couple of drops of yeast solution to each of the
5. To begin the assay, each disc is carefully added to each of the tubes using filter forceps. Then after exactly 6 and 24 hours, the number of survivors in each tube are counted in order to determine the percent of deaths at each dose. Nauplii are small, but can easily be counted in the tubes if held up against a lighted background (i.e., a piece of white paper works pretty well). Counts after 6 hours are only an issue when working with especially toxic extracts; this should not be the case for the majority of extracts.

6. After 24 hours, these data can be used to evaluate the bioactivity of the extract and to determine the LD₉₀. When deaths in the control occur, data can be corrected using Abbott’s formula (Abbott, 1925): 

\[
\% \text{ mortality} = \frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}} \times 100.
\]

The LD₉₀ can be determined graphically, or by linearizing the dose-response data if necessary via \( \log_{10} \) or \( \ln \) transformation, and fitting a least-squares linear regression line to the data at the 3 dose levels. Once the data have been graphed (dose versus % mortality) and the regression has line has been fit, it is then a simple matter to visually use the graph to determine the LD₉₀.

Or even better, one can use the regression equation, and the slope of the line to calculate the LD₉₀; enter a value for \( y \) (50 percent mortality), and solve for \( x \) (concentration of extract) (Figure 3).

**Disc Diffusion Assay**

1. First, a number of filter paper discs must be prepared, as was done for the brine shrimp bioassay. It is extremely important that the discs be completely dry and free of ethanol as the solvent will kill any microorganisms. These should have a final dose of 1000 \( \mu \)g and 500 \( \mu \)g, plus ethanol controls, all in triplicate. These discs are again prepared and dried in 9-well plates.

2. Earlier, petri dishes containing nutrient agar need to have been prepared. Before inoculating these with microorganisms, you should label the bottom of the petri dish (i.e., name of student, date, etc.) and include a "map" to identify each of the four discs that will be added to each dish (Figure 4). These dishes are then inoculated with an appropriate microorganism (e.g., *Escherichia coli*, *Bacillus subtilis*, or *Candida albicans*). This assay works equally well whether working with bacteria or yeast. We have used simple "overnight" cultures of *E. coli* in the past; see your resident microbiologist for help with these cultures. It is very important to know which bacteria you are using, as Gram negative and Gram positive bacteria may respond differently.
differently to given extracts. To inoculate the petri dishes, 0.2 mL of microbial culture is carefully pipetted onto the center of each agar plate. An L-shaped rod (“hockey stick”), flame-sterilized, is then used to evenly spread the bacterial sample over the surface of the agar. The plate is now ready for placement of the discs.

4. The discs are carefully placed according to the “map” on the agar plates now containing the “film” of bacteria, using sterile, fine-tipped forceps (see Figure 4). Four discs are placed into each petri dish: one with 1000 µg and one with 500 µg of extract, one with a commercial antibiotic (serves as a type of standard to allow for comparisons in terms of effectiveness), and one control disc (ethanol only).

5. Once the discs have been added to the agar plates, they are incubated for 24 hours at 37 °C.

6. Bioactivity is evaluated based on zones of inhibition of bacterial growth around each of the discs, except the control discs (see Figure 4). The antibiotic standard should have produced a clear zone of a certain and consistent size (i.e., 30 mm diameter).

7. For discs containing extract, there may or may not be a zone of inhibition. If possible, students measure the zone of inhibition using a small ruler (calipers work great, if available). The zone of inhibition can also be evaluated using the following symbols (Bauer et al., 1966; T. McCloud, per. comm.).

- T = trace (less than 8 mm clear)
- H = halo (hazy zone, if you can measure it, do so; if not, just put H)
- C = strong color (strong in that extract compounds seep into the agar and make a halo, not just staying on the disc)
- K = contamination of disc, or surrounding area (more common on aqueous plates; usually bacterial but occasionally fungi)
- A = antagonistic (zone seems pushed in on one side by activity of adjacent extracts if more than one extract evaluated on single plate; very unusual)
- S = symbiotic (zone is increased on one side, seemingly due to interaction with adjacent extracts)

There can also be a zone like a bull’s-eye, where there is a clear inner zone and then a re-grown halo area on the outer ring; these are not common. There are always going to be some subjective variation and judgment calls when reading the plates. Often it is best to have more than one person read plates, and a discussion may then allow plate-readers to come to a consensus on the degree of inhibition. We also always replicate our plates to try to make more consistent evaluations of inhibition, while also protecting against the potential effects of contamination.

**Conclusions & Perspectives**

I have completed this laboratory exercise a number of times and it has always worked very well. The students are generally able to collect a significant amount of ethnobotanical information on the species used. Also, the bioassays are easy to complete; require relatively little in terms of specialized equipment, supplies, or expertise; and generally are effective with unambiguous results. The brine shrimp bioassay is usually more sensitive, but this might be expected, as not all bioactive compounds will by rule also have antimicrobial properties.

The only real problem that occasionally arises occurs when we “experiment” with new plant species. There are often times when certain species do not appear to have any bioactivity, even though there might be extensive ethnobotanical records suggesting the potential utility for drug development. Students always want “positive results,” they do not like it when things “don’t work.” Students do not seem to appreciate the fact that we learn almost as much when we discover that an extract does not appear to have bioactivity as in situations when we do kill lots of brine shrimp.

But, I have had students select their own species to work with, and although these species must have at least some ethnobotanical background, this does not mean that extracts show bioactivity. Our overall “hit rate” is around 46%, at least for the brine shrimp bioassay. In other words, we usually find significant bioactivity for 46% of the plant species we work with, all of which have been used as medicinal plants by traditional cultures. This actually compares well with results reported by Lewis and Elvin-Lewis (1995), where they report results from NCI anti-HIV screens for ethnobotanically-targeted plants showing 30% of plants show activity against HIV. Random screens of extracts from terrestrial plants yielded only 8.5% active in the NCI anti-HIV screens (Lewis & Elvin-Lewis, 1995). Others have reported similar results, with ethnobotanically-selected plants resulting in 25% activity, while random plant collections provided only 6% activity (Balick, 1990). As a result, I try to stick with the tried-and-true species, species that have extensive ethnobotanical records, and that have a higher probability of showing bioactivity.

The most important aspect of this laboratory exercise is that students learn much about the importance of biodiversity to the process of drug discovery. Before completing this laboratory exercise, I think most students imagined that all drugs are “invented” by men and women in white coats, working in windowless rooms filled with beakers and flasks, and lots of expensive instruments. Giving students a more detailed and realistic picture of the process of drug discovery and development, emphasizing the role of ethnobotany, and illustrating the importance of Mother Nature as the best biochemist of all, is what makes this exercise one of the most productive and effective labs I have ever used in any class that I have taught.

**Acknowledgments**

Development of this laboratory exercise would not have been possible without the assistance of Tom McCloud at SAIC Frederick, NCI-Frederick Cancer Research and Development Center. Also, many students provided valuable feedback after completing this
laboratory exercise which has significantly improved this exercise. This effort is dedicated to the memory of my younger sister, Tracy L. Shelley, who lost her courageous battle with cancer. She and many, many others have contributed to the process of drug discovery through participation in clinical trials of experimental drugs. An important, underappreciated, and courageous part of the process of developing new drugs is saving lives.

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