16 The role of biotechnology: Jamaica

General information

- **Implementing institution**
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- **Implementation period**
  1999-2001 for the grant from the Organization of American States (OAS) (see below). Since the completion of the three-year OAS project, collaborating groups have been able to obtain further funds to continue their activities.
- **Costs**
  US$200,000 from OAS. In-kind input from the laboratories involved in this project, including salaries, is hard to quantify.

Summary

The project was designed to facilitate the development of commercial pharmaceutical products based on indigenous plants of the Caribbean.

Countrywide information on medicinal plants was collected and many local plants were investigated for their biologically active compounds for eventual production of nutraceutical and/or pharmaceutical products. Genebanks were established for the main species and tissue culture was used — often after developing species-specific protocols in the laboratory — for conservation and propagation purposes. Hot pepper was chosen as a suitable crop for promotion within the small island States of the Caribbean as even 0.005 hectare has proven to be an economic unit. This crop is priced well in the market and is growing in demand both as a spice and for its therapeutic properties. Neem (*Azadirachta indica*) was also chosen as a flagship plant for development owing to its potential applications in both medicine and agriculture. Neem oil was tested for its antimicrobial activity and agriculturally useful formulations (biopesticides and a disinfectant) were tested. This project was innovative mainly because it was multifaceted and aimed at strengthening existing or newly established biotechnology-related research teams throughout the Caribbean that could then sustain the initiative beyond the duration of the project.

Background and justification

Since its inception in 1948, the University of the West Indies (UWI) has been extracting, identifying and analysing biologically active ingredients found in Jamaican plants. Many such natural products have been isolated, their structure elucidated and their bioactivity identified. Pharmacological trials have also been carried out on selected compounds and a few patents have been issued. Among these are products developed from ganja (*Cannabis sativum*) and an extract from spirit weed (*Eryngium foetidum*) active against *Strongyloides*, a parasitic worm. However, previous research carried by UWI has not been thoroughly collated and many aspects of research and development required for the sustainable use of local plants were being neglected.

The inherently rich biodiversity of the Caribbean island States presents many opportunities, and governments in the region recognize that the conservation and wise use of environmental resources — including indigenous and locally grown plants — are crucial for sustainable economic development. However, over the years, malpractice, the neglect of the environment and the poor management of natural resources have combined to deplete many indigenous species and several important ecosystems have been degraded.

The wise use of biological diversity must be preceded by gaining a knowledge of the species with commercial potential. However, knowledge about local plants is incomplete. There is also a need to preserve indigenous medicinal plants, many of which are vulnerable to extinction, threatened or rare, and to improve varieties of many cultivated species.

The application of biotechnological tools can provide modern and reliable methods for supporting the exploitation of the Caribbean's rich biodiversity. Therefore, this project was designed by the Biotechnology Centre at UWI to encourage the use of its biotechnology capacity — including molecular biology and tissue culture skills — to enable the collection, identification, conservation, characterization and documentation of the gene pools of locally important endemic and indigenous plants as a basis for the further development of commercially viable products from Caribbean plants.

The project was also geared towards strengthening research capabilities — both at UWI and in collaborating institutions — which was especially needed in the identification of plant extracts and formulations with economic potential.

Description

This experience can be divided into five main areas:

- collation and dissemination of information on indigenous medicinal plants;
- development of *ex situ* and *in vitro* germplasm collections of locally grown medicinal plants;
- development of commercially viable products from medicinal plants;
- improvement of the productivity of hot pepper (*Capsicum chinense*) in the Caribbean; and
- training workshops in molecular and micropropagation techniques, plant taxonomy and negotiation of bioprospecting collaboration agreements.

Collation and dissemination of information on indigenous medicinal plants

Research carried out on natural products and medicinal plants at UWI from 1948-2001 was compiled and reviewed. The review included all available doctoral theses and papers published in regional journals and authored by UWI scientists. From this research, 348 plants (all growing in Jamaica) were identified as having ethnomedical properties. Of these, extracts from 193 plant species were tested at UWI and natural products worthy of further investigation were identified in 44 species. Of these, 29 were found to have biological activity. Plants from this list were located and established in an *ex situ* medicinal plant garden at the Biotechnology Centre, UWI, Mona (see below).

A conference, "Prospects of Biotechnology and Biodiversity for the Caribbean Agro-industry", held at the

end of the first year highlighted the accomplishments of the Caribbean scientists involved in the project and featured 18 papers presented by scientists from five countries (Barbados, Jamaica, Mexico, Trinidad and Tobago and the United States). A seminar presenting the results obtained in the field of ethnopharmacology was held in the second year. A workshop for environmental non-governmental organizations (eNGOs), "Identification of Jamaican Medicinal Plants", was also held in the second year. A seminar, "Trends in Plant Cell Culture and Biotechnologies for Plant Improvement", took place in the third year.

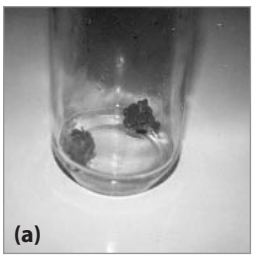
In addition, a series of articles highlighting the various medicinal uses of native plants has become a regular — and popular — feature in a Jamaican newspaper. More than 50 such articles have now been published on such subjects as: "Garlic's antibiotic action", "Getting to know our medicinal plants: leaf-of-life", "Ginger as medicine", "Medicinal plant research and development", "Medicinal plants of Jamaica", "Neem as medicine", "Soursop seeds are toxic", "The healing value of hot peppers" and "Thyme: useful in the pharmacy and kitchen".

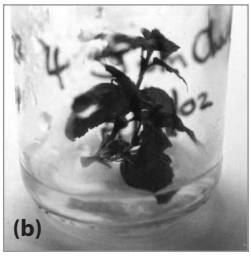
Development of *ex situ* and *in vitro* germplasm collections of locally grown medicinal plants

An *ex situ* plant garden was established that now contains more than 60 species of locally grown medicinal plants. The purpose of this garden was to establish a representative collection of all known Jamaican medicinal plants. Owing to the diversity of the species collected, the garden contains a border area, rockery, vine arbours and raised beds as well as a hardening-off area. Research was also carried out to determine the ease of establishment of the plants, many of which were wild species, in a garden.

In vitro methods for the multiplication and conservation of indigenous medicinal plants were also developed. The plants used for micropropagation were obtained from the established garden. To date, tissue culture lines of 15 medicinal plants have been initiated (figs. 1a and 1b). These are: black mint (*Mentha viridis*), burin-the-bag (*Priva lappulacea*), colic mint (*Lippia alba*), fever grass (*Cymbopogon citratus*), French thyme (*Plectranthus amboinicus*), garlic (*Allium sativum*), guava (*Psidium guajava*), Jack-in-the-bush (*Eupatorium odoratum*), John Charles (*Hyptis verticillata*), jointer (*Piper* spp.), leaf-of-life (*Bryophyllum pinnatum*), peppermint (*Satureja viminea*), quako (*Mikania micrantha*), Spanish needle (*Bidens cyanpiifolia*) and vervine (*Stachytarpheta jamaicensis*). Tissue culture lines of five tree species have also been initiated: neem (*Azadirachta indica*), Honduras mahogany (*Swietenia macrophylla*), Jamaican mahogany (*Swietenia mahogoni*), noni (*Morinda citrifolia*) and Spanish cedar (*Cedrela odorata*).

During the project, tissue culture facilities at Jamaica's Scientific Research Council (SRC) were also improved, and staff there micropropagated several species of medicinal and aromatic plants.





Figures 1a and 1b |
Plantlets of French thyme (a) and John Charles (b) in tissue culture.

Figure 2 |
Neem tree derived from micropropagation planted out in a nursery.

Owing to its widespread use in medicine and agricultural pest control, initial efforts have focused largely on the neem tree. To promote its dissemination and wider use, tissue culture media for the species were developed. Using these tailored solutions, hundreds of plantlets were produced by micropropagation and, in an outdoor nursery, were hardened (by covering with a plastic cup for one week) and grown to field size; 96 per cent of the young trees survived (fig. 2). Three months after transplanting, the trees were large enough to be planted out in the field.

Development of commercially viable products from medicinal plants

Based on information from folklore practices, crude extracts from seven Jamaican-grown plants and two Trinidad-grown plants were screened for specific pharmacological activities.

The following extracts with economic potential were identified:

- unripe tamarind (*Tamarindus indica*) fruit had antibacterial activity against *Bacillus cereus, Escherischia coli, Pseudomonas aeruginosa, Shigella flexeneri* and *Staphylococcus aureus*;
- leaf-of-life (*Bryophyllum pinnatum*) leaves had antibacterial activity against *B. cereus, E. coli, P. aeruginosa, S. flexneri* and *St. aureus*;
- spirit weed (*Eryngium foetidum*) leaves exhibited anti-convulsion (epilepsy) properties;
- breadfruit (*Artocarpus altilis*) leaf was tested for its anti-inflammatory properties;
- freeze-dried noni (*Morinda citrifolia*) fruit extract was tested for its anti-inflammatory potential. Using tests on a rat paw model, inflammation was produced by injections of bradykinin and prostaglandin.

Noni extracts significantly inhibited the induced swelling. This potential may be of therapeutic value in the treatment of rheumatoid arthritis, especially as bradykinins and prostaglandins contribute to the symptoms of this disease;

- chemical analyses of *Abutilon trisulcatum* (family Malvaceae) have identified the presence of choline, a vital compound in the transmission of nerve impulses. An aqueous extract of *A. trisulcatum* was therefore tested for its potential to improve memory. After 14 days of continuous administration to rats, short-term memory was examined using passive avoidance training. Results indicated that this plant species has at least one compound with the potential to improve short-term memory. The extract, therefore, may represent a source of choline supplementation for use in the treatment of memory disorders; and
- ginger (*Zingiber officinale*) oil, extracted by steam distillation from ground ginger, was tested for its analgesic (pain suppression) effect. Synovitis, a major symptom of rheumatoid arthritis, was produced experimentally in the knees of rats. Treatment with ginger oil substantially reduced the swelling, suggesting that ginger oil contains at least one compound that may have therapeutic value in the treatment of rheumatoid arthritis.
  Two plants from Trinidad were also tested, *Pluchea symphytifolia* (family Asteraceae) and Jamaica verbain (*Stachytarpheta jamaicensis*). Ethanol leaf extracts were tested on rats for their anti-inflammatory activity. Acute inflammation was induced in the paw using carrageenan and was measured using the water plethysmography technique, which measures variations in the size of the limb. The extract from *P. symphytifolia* inhibited swelling, an anti-inflammatory property, while the extract from *S. jamaicensis* exhibited analgesic properties as it increased the pain threshold of rats tested with an analgesimeter.

As with the micropropagation aspect of the project, significant efforts were again focused on the neem tree. Neem oil was used to formulate a disinfectant (antibacterial agent), an antifungal agent and an insecticide in accordance with the Jamaica Bureau of Standards guidelines. The bioactivity of neem oil against bacteria (*E. coli, Klebsiella pneumoniae, S. aureus* and *Stretptococcus faecalis*) and fungi (*Aspergillus flavus, A. niger* and *Fusarium oxysporum*) was assessed using standard microbiological methods.

In the tests, 2 per cent neem oil incorporated into potato dextrose agar plates completely prevented fungal growth. A similar concentration of neem showed antibiotic activity against eight pathogenic bacteria. Growth of the gram-negative species (*E. coli, Klebsiella, Proteus, Pseudomonas, Salmonella*) was decreased by three orders of magnitude while growth of the gram-positive species (*Staphylococcus* and *Streptococcus*) was reduced a hundredfold.

Neem disinfectants also decreased the bacterial load on the floor of a food processing plant by 94 per cent and decreased insect attack on callaloo (*Amaranthus viridis*) by 100 per cent. In addition, a neem oil formulation was able to significantly decrease the percentage of contamination and depth of fungal infection of cut yam (*Dioscorea cayenensis*) tubers packed in sawdust or wood shavings. A treatment of 2 per cent neem oil for 5 seconds proved to be the most effective. Stronger neem oil solutions (5 per cent) or longer treatment times (5 minutes) appeared to damage the tubers and allow some fungal growth, although this was still less than in the untreated controls (see table).

IMPROVEMENT OF THE PRODUCTIVITY OF HOT PEPPER

Although there is a high demand for hot pepper and products derived from it, growers in Jamaica and elsewhere in the Caribbean were failing to capitalize on the potentially lucrative market, owing mainly to the susceptibility of the prevailing cultivars to several pathogens. To address this problem, a pepper-breeding programme was established with the aim of developing improved varieties.

Pepper varieties were collected from throughout the region and subjected to molecular analyses to obtain their genetic fingerprints as a starting point for combining both classical plant breeding and

Effect of neem formulations on fungal growth on cut yam tubers packed in wood shavings.

TREATMENT	PERCENTAGE OF FUNGAL INFECTION AFTER STORING FOR 3 WEEKS	MEAN DEPTH OF FUNGAL INFECTION FROM EACH CUT SURFACE OF THE TUBER
2% neem oil in IPA*— tubers dipped for 5 seconds	17	0.4 cm
2% neem oil in IPA— tubers dipped for 5 minutes	50	0.3 cm
5% neem oil in IPA— tubers dipped for 5 seconds	83	1.8 cm
2% neem oil in water made from 20% neem solution in IPA — tubers dipped for 5 seconds	92	3.5 cm
Control — tubers dipped in water for 5 seconds	10	5.4 cm

**IPA = isopropyl alcohol. The experiment used 12 cut tubers per treatment and 6 cut tubers per box.*

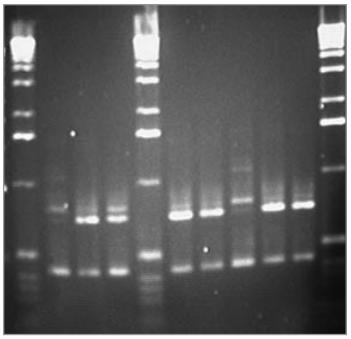


Figure 3 | DNA markers of hot pepper accessions.

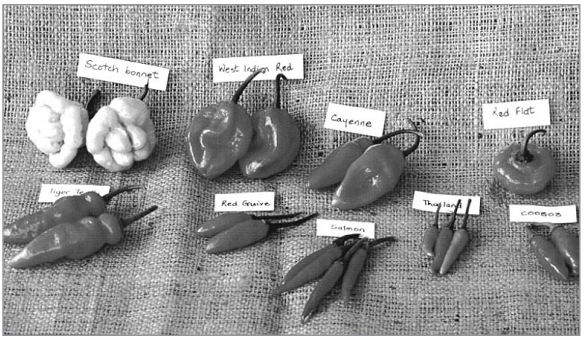


Figure 4 | Examples of the variety of hot pepper accessions in the UWI Biotechnology Centre genebank collected from farmers' fields in the Caribbean region.

biotechnological methods. A genebank has been established that now contains seeds from more than 80 accessions obtained from several Caribbean countries, including Antigua, Barbados, Belize, Jamaica, St. Vincent and Trinidad and Tobago.

Randomly amplified polymorphic DNA sequences (RAPDs) were used to obtain genetic fingerprints and determine the genetic diversity within and between the accessions. Genetic differences within pepper types were detected, with Scotch Bonnet varieties 60 to 100 per cent similar, West Indian Red varieties 53 to 100 per cent similar and Bird Pepper varieties 57 to 89 per cent similar (fig. 3).

DNA molecular markers (such as RAPDs) that can be used to facilitate selection of such useful economic traits as disease resistance were identified and used to assess the progeny of crosses made between Scotch Bonnet and Bird Pepper (fig. 4). From these efforts, six lines of hot peppers resistant to diseases caused by potyviruses, common throughout the Caribbean, were developed and resistant pepper seeds are now being disseminated to farmers.

Training

Training in molecular biology micropropagation and techniques was provided by researchers at UWI to more than 30 people from the Caribbean region, including Barbados, Guyana, Jamaica and Trinidad and Tobago, and a training manual was produced. Another course, "Negotiating Bioprospecting Collaboration Agreements", was taught by staff from the Instituto Nacional de Biodiversidad (INBio), Costa Rica, with participants drawn from across the Caribbean and across disciplines — including scientists, lawyers and government officials. This led to the first draft of the Jamaica Bioprospecting Programme that can be found on the Jamaican National Commission on Science and Technology web site at *www.ncst.gov.jm*. Training in taxonomy was also provided to one scientist from UWI at a Tropical Botany course in Florida, and a graduate student was sent to Mexico for training in genetic fingerprinting techniques.

PARTNERSHIPS

The Biotechnology Centre at UWI, Mona, conceived the project and submitted the proposal to OAS through the Planning Institute of Jamaica. The project was multilateral and included biotechnology research groups from UWI in Barbados, Guyana and Jamaica supported by the Caribbean Agricultural Research and Development Institute (CARDI) and ministries of agriculture. In all, nine research groups in six different institutions from three countries were involved in the project. The funds were administered through the National Commission on Science and Technology (NCST).

Local partners included NCST, the Institute of Jamaica, the Ministry of Agriculture, the National Biodiversity Strategy and Action Plan Steering Committee and the Scientific Research Council (SRC). Through the project, interactions between the Ministry of Agriculture and UWI were strengthened, particularly with regard to the use of molecular techniques that helped to speed up the traditional pepper-breeding programme by enabling the more rapid identification of disease-resistant lines. There was also a strengthening of ties between regional collaborators. Links were also developed with researchers in Costa Rica, India, Mexico and Wisconsin (United States).

REPLICABILITY

This experience is very relevant to other regions and could easily be replicated. Primarily it was not only concerned with the identification of bioactive chemicals but also focused on strengthening the research capability that underpins the development of viable industries based on the use of local medicinal plants. It accomplished this by strengthening existing or newly emerging research groups rather than by creating a new institute or entity.

Other aspects that can be replicated are:

- the emphasis on developing local plants into potential commercial ventures;
- the concentration on a few lead crops;
- the establishment of collaborations and links among research groups at the regional level; and
- the dissemination of collated information in language that is easily understood and in a suitable medium (local newspapers) to both educate the general public and help to sway political opinion towards supporting the use of medicinal plants.

POLICY IMPLICATIONS

While it is unclear if government policy has changed as a result of this project, the initiative has helped to establish the economic potential of exploiting indigenous medicinal plants as an aid to sustainable development, elevating the area to one of national priority. In addition, the work-

shop, "Negotiating Bioprospecting Collaborative Agreements", has aided the development of policies addressing access to Jamaica's biodiversity by those wishing to collect and study it. As such, the project helped to influence an upgrading of Jamaica's intellectual property legislation, although it was not directly involved in it. This will assist in protecting this sector from "biopiracy" — the illegal development, patenting and commercialization of products based on the biodiversity of a country without that country's permission. Similarly, indigenous groups in Guyana were concerned that once the plants they had identified as having useful medicinal or pesticidal properties have been grown in tissue culture, their control over the resource would be lost. This issue needs to be addressed through educational initiatives and strong legislation.

Finally, the project has been able to influence the government to coordinate activity in the medicinal plant sector and this has facilitated a change of legislation that now allows medicinal plant products to be legally registered.

Lessons learned

From the project management point of view, obstacles were faced in procuring equipment and the timely disbursement of funds. In some instances, these problems hampered the full accomplishment of a goal. Also, the main grant was insufficient to cover all the project goals, so funds had to be sourced from elsewhere. For example, a web page was designed but it was not launched as there were inadequate funds to ensure its continued maintenance. There were also questions over who should supply the information, who owned the copyright to the material and where the web page should be hosted.

In the laboratory, high levels of secondary metabolites in plant samples hampered efforts to obtain clean DNA and to establish some species in tissue culture. Methodologies for these plants need to be further developed and refined.

Another obstacle was encountered when project participants attempted to publish various results, especially as many journals will not publish papers based on the effects of crude extracts or formulations.

Impact

The main priority of this project was to use the region's scientific capacity to encourage development and to improve technology exchange and transfer. It is expected that the continued development and growth of economically viable products from Caribbean plants will increase both the health and wealth of the region and will therefore have positive social implications. Indeed, the project has highlighted the need for value-added products and the possibility of developing safe products from medicinal plants and has encouraged the further development of this sector. The business community is now interested in several plant-derived pre-commercial products.

Project results were disseminated at local conferences and seminars, on research days, to local journals and newspapers, and to horticultural and other interest groups through public lectures, radio interviews, regional conferences and international journals. The response to the results on neem and hot pepper has been overwhelming, especially from the agricultural sector. A positive perception of the use of medicinal plants in the health sector has been established as Jamaican medicinal plants are no longer being referred to as "bush medicine" and are now regarded as "herbal remedies" or even "nutraceuticals" — foods or naturally occurring food supplements that have beneficial health effects.

The success of the project has also had a significant impact in the area of scientific development as it used the tools of biotechnology to address existing plant protection problems in the pepper crop. Resistant lines of hot pepper were developed from the pepper genebank and these are being used to rejuvenate the pepper industry. The strengthened research groups have been able to access further funding to allow this initiative to continue and to grow. In addition, they are now collaborating with other research groups, government entities and the business community from research right through to viable business ventures.

Also of note was the technology transfer in the areas of genebank establishment, molecular techniques, parataxonomy (i.e., training technicians to assist taxonomists) and bioprospecting (searching the genebank for potentially useful compounds). Costa Rica, India and Mexico were the contributing countries, with Barbados, Guyana, Jamaica and Trinidad and Tobago being the recipients.

There was also a positive impact on the region's effort towards sustainable development and the environment community as eNGOs were sensitized to the value of biodiversity and the benefits that can accrue from its sustainable use.

Areas in which the project could have more impact include the genebanks, which need to be further expanded to include all the country's rare and endemic medicinal plants and — together with the *in vitro* collections — more fully exploited. Even so, the establishment of the *ex situ* genebanks and the use of *in vitro* techniques to conserve and produce disease-free planting material means that less pressure is placed on the environment, especially with regard to the unregulated harvesting of medicinal plants.

From the standpoint of dissemination of information, the public — with an increased knowledge of its local herbs and medicinal plants — has also benefited. Farmers have benefited by having access to more disease-resistant seeds. As the result of this project, there has also been an increase in the interactions between scientists, the business community and national governments.

While this initiative was unfolding, several related projects were initiated and are continuing. In 1999, UWI inaugurated its Natural Products Institute, which is

mandated to develop commercial products from local medicinal plants by performing the necessary toxicological and clinical trials. In December 2002, the "Caribbean Herbs Business Forum" was held in Jamaica, bringing together business interests from Europe and the Caribbean as well as experts from Australia, Brazil, Canada, Guatemala, New Zealand and South Africa. As a parallel event, several scientific organizations in Jamaica, including the Biotechnology Centre, organized the "Caribbean Herbs Scientific Forum", thus facilitating interaction between scientific and business interests. As a result of these meetings and associated activities, the Caribbean Herbal Business Association (CHBA) was formed. Businesses within CHBA are being supported by the Centre for the Development of Enterprise (an African, Caribbean and Pacific (ACP)-European Union initiative), which is helping to ensure the sustainability of the sector.

FUTURE PLANS

The biotechnology and basic medical science research groups that were developed and strengthened as a result of this programme are joining with other research groups at UWI and other organizations in Jamaica to develop phytopharmaceuticals and improved cultivation methods for medicinal plants. There are also plans to continue to collaborate with similar groups elsewhere in the Caribbean to develop a Caribbean brand of products from the region's medicinal plants. Such Caribbean plant resources have not been fully explored or exploited as the developing island States have been advancing independently owing to differences in language history and because of their geographical isolation. This research is being done in coordination with government and business interests so that legislation, intellectual property, branding, labelling and other issues can be resolved in a coordinated manner, which will, it is hoped, ensure that the benefits from these developments will be widespread.

PUBLICATIONS

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Barbados: UWI: *Leonard O'Garro* (genetic diversity of hot peppers). CARDI: *Herman Adams* (pepper breeding).

Guyana: National Agricultural Research Institute: *N. Ahmad* (collection of medicinal plants, tissue culture).