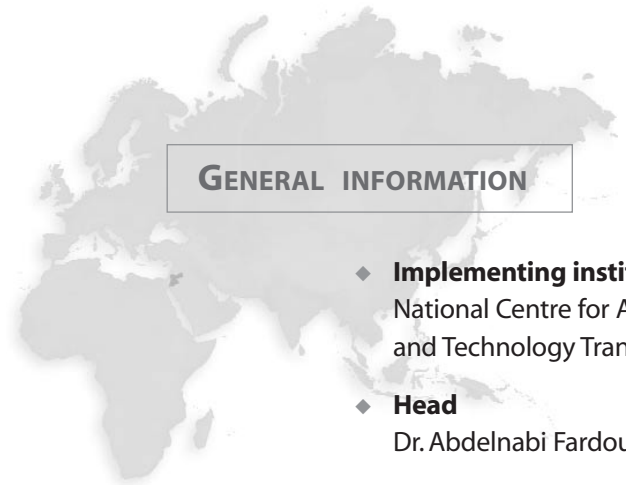
# 17 Focus on local alternatives: Jordan



## General information

- **Implementing institution**
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- **Head**
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- **Implementation period**
  The time needed for this preliminary phase was around 6 months.
- **Costs**
  The project has been a collaborative effort between the National Centre for Agricultural Research and Technology Transfer (NCARTT) and the Department of Medicinal Chemistry and Phamacognosy, Jordan University of Science and Technology (JUST). Local funding sources were used, including US$1,500 from NCARTT and US$500 from JUST. Total to date: US$2,000.

## SUMMARY

Saint John's wort (*Hypericum perforatum*), used to treat mild depression, is the most widely sold herbal remedy in the United States and Europe. However, the active ingredient, hypericin, is present in several other *Hypericum* species. NCARTT has therefore undertaken preliminary work to develop *Hypericum triquetrifolium* — a species found growing wild in Jordan — into a commercial product.

The hypericin content of methanol extracts of dried flowers, leaves, stems and roots of *Hypericum triquetrifolium* was determined. Exposure of the samples to light for 30 minutes immediately before analysis resulted in the conversion of protohypericin to hypericin and helped to improve yields.

The highest content of hypericin was found in the leaves (0.36 per cent by weight). These relatively high values for the hypericin content of *H. triquetrifolium* are encouraging and further studies will focus on the introduction, cultivation and biological evaluation of other *Hypericum* species in Jordan.

## BACKGROUND AND JUSTIFICATION

St. John's wort (*Hypericum perforatum*) is currently listed in the official pharmacopeias of several European countries. Extracts are used as an antidepressant as well as to treat bed-wetting and nightmares in children. In addition, the plant has been shown to have anti-inflammatory, antiviral, and pain-modulating properties. Retroviruses, in particular the HIV/AIDS virus, are susceptible to extracts of St. John's wort. Indeed, the plant's medicinal properties have been known since the time of the ancient Greeks. In the Middle Ages, *H. perforatum* was believed to have the power to ward off evil and protect against disease. Its major use today, however, is in the treatment of depression.

Depression ranks as one of the most common health conditions and affects 80 per cent of people during at least part of their life. Some 17 million people suffer from major depression and hundreds of millions suffer from mild to moderate forms of the affliction. Currently, the leading synthetic anti-depressant drug is Prozac®. In Germany, Prozac® is prescribed to some 300,000 patients a month. In comparison, the leading herbal remedy for depression, Saint John's wort or hypericum, is prescribed to more than 200,000 patients a month.

In fact, St. John's wort is the most widely sold herbal remedy in the United States and Europe and has a market value of more than US$400 million a year. The plant, therefore, can be a profitable crop. In 2000, after a surge in demand, the Ministry of Agriculture, British Columbia, Canada, released a report encouraging farmers to invest in growing St. John's wort and reported that one hectare could produce more than 2,000 kilogrammes and that a kilogramme of dried St. John's wort could fetch around US$15.

According to the report, after deducting major expenses, the net income from this investment would approach US$15,000 per hectare.

Recent analyses attribute the pharmacological activity of *H. perforatum* to a variety of compounds, including pseudohypericin, hyperfolin flavonoids and, in particular, hypericin. One milligramme of purified hypericin costs about US$100.

However, *H. perforatum* is not the only source of hypericin. Some 370 *Hypericum* species (family Hypericaceae) occur naturally in Europe, western Asia, north Africa and Australia. Five species, *H. hyssopifolium, H. languinosum, H. olivieri, H. serpyllifolium* and *H. triquetrifolium*, are found in Jordan. *H. triquetrifolium* (known as Peter's wort, wavy leaf St. John's wort or tangled hypericum and, in Jordan, as Roja) grows in northern parts of the country around Ajlun, Irbid and Ramtha and is often regarded as a weed. It has traditionally been used as a sedative, astringent, antispasmodic, and for intestinal and bile disorders. Anti-inflammatory and pain-relieving properties have also been reported. Given that the hypericin content in both *H. perforatum* and *H. triquetrifolium* is similar, Jordan's wild-growing Roja could emerge as very valuable medicinal crop plant (fig. 1).

Despite its uses and potential as a lucrative crop, little research has been carried out on *H. triquetrifolium*. A long-term project, including a comprehensive phytochemical study to identify the active constituents and their concentration in *H. triquetrifolium*, is required. In addition, studies to determine its safety and efficacy for mild to moderate depression, first in mice and later in controlled clinical trials, will be necessary. Botanical and agronomical investigations also need to be conducted to characterize the plant, study its natural habitat, and develop the best methods for its cultivation.

As a preliminary step, NCARRT scientists have used high performance liquid chromatography (HPLC) to determine the hypericin content of the flowers, leaves, stems and roots of *H. triquetrifolium*.

## Description

The pharmacological activity of *H. triquetrifolium* is attributed to a variety of constituents, the major one of which is hypericin. Hypericin (fig. 2) is a dark green, photosensitive, polycyclic quinone with the molecular formula $C_{30}H_{16}O_8$. It is soluble in methanol and in aqueous alkaline solutions. Below pH11.5, an aqueous solution of hypericin is red, whereas above pH11.5, it is green with red fluorescence.

**Figure 1** | *Hypericum triquetrifolium*, known in Jordan as Roja.

The aim of the project was to determine the hypericin content of the flowers, leaves, stems and roots of *H. triquetrifolium* growing wild in Jordan.

All parts of *H. triquetrifolium* plants were collected from Irbid and Ramtha in northern Jordan in July 2002, when the plants were flowering. The identification of the plants was verified by a qualified taxonomist. In addition, a voucher specimen of *H. triquetrifolium* was registered and deposited at the Herbarium Museum of the Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan. Plant material was divided into four parts — flowers, leaves, stems and roots — and air-dried at room temperature. After drying, exact weights were recorded and the samples ground to a powder.

One gramme of each powdered sample was placed in a 100-millilitre round-bottomed flask fitted with a reflux condenser. After boiling for 20 minutes in 80 millilitres of methanol, the samples were filtered through cotton wool and the filtrates saved. The plant residues and cotton wool filters were subjected to two further extractions in 60 millilitres of methanol and the filtrates combined. The volume was then reduced to about 3 millilitres by rotary evaporation before being accurately measured and transferred to a fresh flask and diluted to a given volume using methanol. Small samples were centrifuged at 4,500 revolutions per minute (rpm) for 5 minutes and the supernatants transferred into HPLC vials. Protohypericin, a light-sensitive precursor of hypericin, was converted to hypericin by exposing the HPLC vials to an 18-watt light source for 30 minutes at a distance of about 10 centimetres immediately before HPLC analysis, a procedure that should help to ensure replicable results between laboratories. Two replicates were prepared for each plant part.

For comparison, a stock solution of commercially available hypericin at 100 parts per million (ppm, or 0.1 milligrammes in 1 millilitre of methanol) was made as a standard and used to prepare five dilutions for a calibration curve and two quality control samples (of 10 and 15 ppm).

HPLC was carried out using 20 microlitre samples and a flow rate of 1.5 millilitres per minute with an ultraviolet/visual light detector set at 590 nanometres. The total run time was 10 minutes.

Duplicates of all plant samples, calibration points and quality control samples were tested.

A linear calibration curve showed that the two quality control samples were accurate to within 2.6 and 8.7 per cent, respectively, of their actual concentrations.

Analysis of the different plant parts of *H. triquetrifolium* showed that leaves contain the highest hypericin content (0.36 per cent by weight) (see table). An HPLC chromatogram of *H. triquetrifolium* leaf extract is shown in fig. 3.

The total hypericin content of the aerial parts of *H. perforatum*, the main commercial source of hypericin, is reported to be in the range of 0.19 to 0.30 per cent.

Hypericin content of flowers, leaves, stems and roots of *H. triquetrifolium*.

| Plant part | Percentage (by weight) of hypericin |
|---|---|
| Flowers | 0.064 |
| Leaves | 0.36 |
| Stems | 0.0085 |
| Roots | 0.0003 |

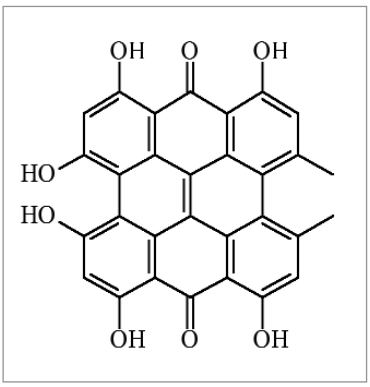


**Figure 2** | The chemical structure of hypericin ($C_{30}H_{16}O_8$).

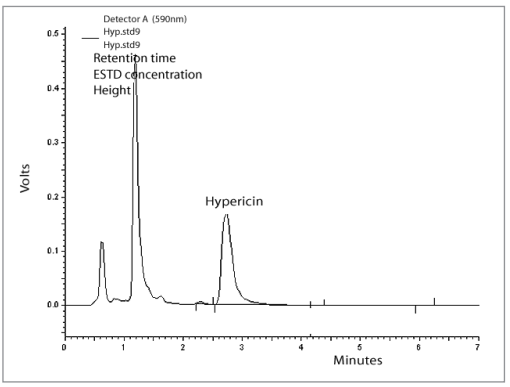


**Figure 3** | Chromatogram of an HPLC run with a sample of hypericin extracted from *H. triquetrifolium* leaves.

The relatively high hypericin content of the *H. triquetrifolium* in this study (0.36 per cent) could be due to the species analysed (*H. triquetrifolium*) or to the climatic conditions (e.g., hours of sunshine) in Jordan. However, the results obtained are encouraging for the introduction and cultivation of *Hypericum* species in Jordan.

Biological and clinical studies to evaluate the safety, cytotoxicity, and antiviral and antidepressant activities of hypericin isolated from *Hypericum* species grown in Jordan will follow.

## Patenting and commercialization

NCARTT is currently exploring the possibility of a commercial partner (i.e., a pharmaceutical company) investing in the next phases of this promising project.

## Partnerships

This phase of the project was a successful achievement between two national and public institutions: the National Centre for Agricultural Research and Technology Transfer (NCARTT), Ministry of Agriculture, Baq'a, and the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology (JUST).

## Replicability

In a sense, this project is a replication of a previous project. In 2001, for example, NCARTT and JUST collaborated on a similar research venture based on assessing the levels of colchicine in two wild crocus (*Colchicum*) species (family Liliaceae). Although it has poisonous properties, colchicine is used as an anti-inflammatory drug to treat gout. It is also used experimentally in tubulin binding studies.

NCARRT scientists and their collaborators reported the presence of colchicine in an appreciable amount in the corms of *C. hierosolymitanum* and *C. tunicatum*. Based on these results, a pharmaceutical company (Dar-Aldwa) is now interested in investing in the further development of these species as a source of colchicine.

With similar results obtained from the *Hypericum* project — based on detailed laboratory analyses of the active ingredients — there is ample reason to believe that other plants used in traditional medicine, in Jordan and elsewhere, could be targeted and are likely to provide useful compounds that can be further developed into commercial products.

## Policy implications

A key factor for the success of this project is that it has opened new avenues of investment for the wise use of Jordan's natural resources.

Taken together, the experiences with *Hypericum* and *Colchicum* outlined above could have a great impact on how the national government views the nation's indigenous biodiversity and, in particular, its medicinal plants.

On a wider scale, the whole of the Middle East hosts a wealth of medicinal plants that have been exploited through the centuries, but their full potential still needs to be explored. Projects such as this should encourage the governments of the region to allocate larger budgets for developing their indigenous medicinal plants into viable commercial products.

## Lessons learned

For such a project to succeed, several parts must come together: a research institution well equipped with modern facilities and qualified personnel; a regulatory body that is flexible and able to cope with rapid changes in the industry; and a public that is aware of the required changes.

In addition, for the project to be completed, funds had to be sourced from organizations convinced of the value of the work.

Looking ahead, the introduction and cultivation of different medicinal and aromatic plants require that both agronomists and pharmacognosists cooperate to develop improved crop varieties with high levels of the desired ingredient. Successful cultivation also needs real collaboration between the producers (farmers) and the buyers (pharmaceutical companies). This could be achieved by the pharmaceutical companies contracting farmers to grow the plants they need in the quantities they need. Such an approach should be supported by government funds and agricultural extension agencies. In conclusion, cultivation of different medicinal plants could be of high economic value for farmers, drug companies and the national economy.

## Impact

With regards to private-sector exports, the pharmaceutical industry is Jordan's second largest sector and thus plays a major role in the national economy. However, it produces and exports mainly generic drugs, and if World Trade Organization regulations are tightened, as is envisaged, the sector may face financial difficulties. Thus, basic research to find and develop new products is a key investment area for the private sector. Jordan's indigenous medicinal plants are obvious targets for these companies.

There could also be valuable returns for the nation's farmers. If the estimates of the Ministry of Agriculture of British Columbia (above) also apply to *H. triquetrifolium*, it could be expected that one kilogramme of dried *H. triquetrifolium* would sell for about US$15, for a net income of US$15,000 per hectare.

## Future plans

A national project on the conservation and wise use of medicinal and herbal plants, funded by a US$2.5 million grant from the Global Environment Facility, will be launched later this year. The goal of the project is to promote the production of herbal and medicinal plants as an integrated and efficient sector in Jordan. NCARRT aims to encourage local industry to invest in medicinal plant production, thereby providing farmers with alternative crops that will, it is hoped, give them higher returns.

NCARRT also aims to look for new plants that have a high value both medicinally and economically. In addition, intellectual property rights and health and safety standards for the medicinal and herbal plant sector will be established, and local plant resources should be better conserved and better utilized.

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